Physiological constraints to seed growth in perennial ryegrass (*Lolium perenne* L.).

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Physiological constraints to seed growth in perennial ryegrass (*Lolium perenne* L.).

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Stellingen

1. Het zaadgewicht in Engels raaigras wordt vooral bepaald door factoren die de ontwikkeling van de aar sturen.

   Dit proefschrift.

2. Groeiende zaden binnen de aar van Engels raaigras beïnvloeden elkaar nauwelijks.

   Dit proefschrift.

3. De ruime hoeveelheid suikerreserves in de stengel van de bloeihalm van Engels raaigras wordt vooral benut voor de groei van nieuwe zijspruiten en niet voor de groei van de zaden.

   Dit proefschrift.

4. Bij bloei ligt de maximum zaadopbrengst van een Engels raaigras gewas reeds vast.

   Dit proefschrift.
   Hebblethwaite et al., 1980. In: Seed production, pp. 71-90. P.D. Hebblethwaite (ed). Butterworths, London.

5. De grote investering van geld en mensuren die de verdeling van onderzoeksgeld nu vergt, tezamen met de vele uren die onderzoekers in acquisitie steken, doet vermoeden dat een lump-sum financiering van onderzoek de efficientie en effectiviteit van dat onderzoek aanzienlijk zal verhogen.

6. De politieke geldingsdrang om de onderzoeksagenda verder te bepalen miskent het verantwoordelijkheidsgevoel en maatschappelijk inzicht van hoogopgeleide onderzoekers en hun gesprekspartners, en zal tot nog meer korte termijn onderzoek leiden.

7. In biologische systemen betekent causaliteit nog geen voorspellend vermogen, hoogstens een statistische voorspelling [1]; kennis van statistiek volstaat daarom bij het maken van gewasgroeimodellen die moeten voorspellen.

   [1] Mayr, 1965. In: Cause and effect, pp. 33-50. D. Lerner (ed.). Collier-MacMillan, Toronto

8. Een goede manier om het cellentekort te verminderen is een verhoging van de begrotingen van onderwijs en sociale zaken.

9. Een positief effect van een sneller verbruik van de makkelijk winbare voorraden aan fossiele brandstoffen zal zijn: een snellere ontwikkeling van energie-zuinige technieken en vormen van alternatieve energiewinning.

10. De milieubelasting is nog niet evenredig aan de belasting van het milieu.

11. Wanneer het aantal uren reality-t.v. nog meer toeneemt kunnen we beter uit het raam kijken.

Stellingen behorend bij het proefschrift getiteld: "Physiological constraints to seed growth in perennial ryegrass (*Lolium perenne* L.)" door Johan Warringa.

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ABSTRACT

Warringa J.W., 1997. Physiological constraints to seed growth in perennial ryegrass (*Lolium perenne* L.). Doctoral thesis, Wageningen Agricultural University, Wageningen, The Netherlands, 139 pp., English and Dutch summaries.

The yield of a seed crop of perennial ryegrass varies between 1 and 2 Mg/ha. This variation is caused by variation in the number of seeds that reach an adequate dry weight. On average 70 % of the seeds present before harvest and cleaning are not recovered because of their low weight. This suggests that seed filling and not seed set determines to a large extent seed yield. Factors influencing seed filling were studied on spaced plants in the greenhouse.

Reducing the light intensity after anthesis from 115 % to 24 % (1.1 MJ/m^2) showed that the amount of carbon assimilates in the reproductive tiller was not limiting to seed filling. Seed yield per ear was reduced by only 14 % and average seed dry weight by only 4 %. Although the amount of water-soluble carbohydrates (WSC) in the stem + rachis was strongly reduced at 24 % light, it could have supported an extra 37 % of seed yield.

New vegetative tillers that developed after anthesis did not reduce seed filling and seed yield per ear because of the large amount of WSC reserves in the stem. Regrowth of tillers cut at anthesis strongly reduced WSC stem reserves.

The large variation of seed dry weight within the ear could for about 60 % be attributed to differences in rate of growth and for about 30 % to differences in duration of growth. The differences in growth rate were determined by the ovule dry weight at anthesis and not by differences in relative growth rate. The availability of assimilates and the accumulation of starch did not differ between seeds within the ear. Differences in the duration of growth were mainly caused by differences in time of anthesis, not ripening. The interaction between seeds in the ear was weak; removal of spikelets or seeds within a spikelet did not strongly affect the remaining seeds. Apparently processes in the seed itself mainly determine seed filling and seed yield of perennial ryegrass. Factors controlling ear development determine the ovule dry weight at anthesis and thus to a large extent the variation in final seed dry weight within the ear.

Key words: anthesis, assimilate availability, assimilate partitioning, ^{13}C-labelling, competition, dry matter distribution, duration of growth, flowering, light intensity, *Lolium perenne* L., nitrogen, perennial ryegrass, rate of growth, ripening, seed filling, seed growth, seed losses, seed set, sink-sink, sink-source, spikelet, starch accumulation, stem reserves, tillering, water-soluble carbohydrates.

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Johan Warringa
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Account

Chapters of this thesis have been included in the following publications:

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Chapter 6	Warringa, J.W., de Visser R. & Kreuzer, A.D.H., 1997. Analysis of sink-sink relations between seeds within the ear of *Lolium perenne* L. - submitted.

CHAPTER 1

GENERAL INTRODUCTION

Grass seed cultivation

The main grass seed producers in the world are the United States, Canada and New Zealand. Denmark is the largest grass seed grower in Europe with 49,261 ha in 1992. In recent years the acreage of grass seed crops in the Netherlands varied around 26,000 ha. Perennial ryegrass (*Lolium perenne* L.) accounts for about half this area, with smooth-stalked meadow grass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.) being the most important of the remaining 13 species in the Netherlands (Anonymous, 1994). Each year about 25,000 tons of grass seed with a value of about 80 million guilders is exported from the Netherlands, mainly to countries within the European Community (Anonymous, 1993).

The importance of grass seed production is illustrated by the fact that each year about 150,000 ha of grassland is sown or resown in the Netherlands. Pastures, lawns or sports-fields are the main types of grassland, though the trend towards more extensive pasture management is bringing about a decline in the use of grass seed for pastures. In contrast, amenity use and the use for road verges and camping grounds are increasing (Anonymous, 1993).

The use of grasses for fodder or turf forces the breeders to focus on vegetative traits. Because grasses are commercially propagated by seed, however, the ability to produce sufficient seed is also important for a cultivar to become a commercial success. Therefore the breeder is looking for that rare combination of excellent vegetative traits and sufficient reproductive capability.

Variable seed yield

The yield of grass seed crops varies greatly between years and locations. In smooth-stalked meadow grass and red fescue seed crops it ranges between 600 - 1200 kg/ha. The yield of a perennial ryegrass seed crop varies between 1000 and 2000 kg/ha (Anonymous, 1980; Anonymous, 1993). The present study focuses on perennial ryegrass because its larger inflorescence and seeds make it easier to handle than red fescue or smooth-stalked meadow grass. Perennial ryegrass is the dominant species in grasslands in North-western Europe. It has a high persistence and dry matter production, and a good digestibility and palatability. Cultivars for turf have also been developed (Elgersma, 1990a). It is probable that results obtained for perennial ryegrass are relevant for other grass species.

In a crop of perennial ryegrass at final harvest only 10 - 20 % of the above-ground dry matter is seed, 50 - 60 % is stem and the rest is (mostly dead) leaves (Elgersma, 1990b). The variation in seed yield of a perennial ryegrass seed crop is mainly (60 - 98 %) attributable to variation in the number of seeds recovered after harvest and cleaning and not to variation in the average seed dry weight of the harvested seed (Hampton &

Hebblethwaite, 1983; Marshall, 1985; Elgersma, 1990b). Within a standing crop, however, there is a large variation in seed dry weight. A considerable gap exists between the number of seeds present just before harvest and after harvest and cleaning. On average, 65 % (range 50 - 80 %) of the florets develop into seeds, but on average only 30 % (range 25 - 70 %) of the seeds are actually recovered. This means that only 20 % of the florets present at anthesis produce a seed that is recovered after harvest and cleaning (Figure 1). Some seeds are lost through shattering but many of the seeds are too light and are therefore lost during harvest or cleaning (Meijer, 1985; Horeman, 1989; Elgersma, 1990b). The small variation in the average seed dry weight after harvest and cleaning can be explained by loss of the lighter seeds (Elgersma, 1990b). Seeds may be too light because they are too young or badly filled. The badly filled seeds have not accumulated enough dry matter possibly because of a lower rate of growth and/or because of a shorter duration of growth. Seed abortion occurs mainly during the first 10 days after anthesis and has cytological and genetic causes associated with outbreeding, not with competition for

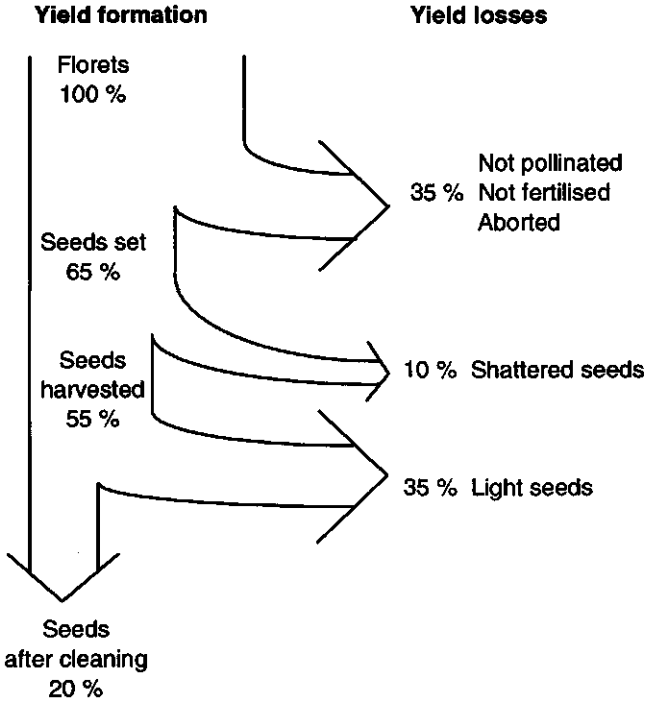

Figure 1. Flow diagram showing the losses of floret sites from anthesis onwards leading to the low realisation of the yield potential in perennial ryegrass seed crops.

assimilates (Elgersma & Sniezko, 1988; Marshall & Ludlam, 1989). The major loss during harvest and cleaning means that in a seed crop of perennial ry grass it is not seed set but seed filling that determines the number of seeds that can be harvested and thus seed yield. The variation in seed yield of a perennial ryegrass seed crop is mainly caused by the variation in the number of seeds that reach an adequate dry weight.

Assimilate production and partitioning

Seed yield is influenced by both the amount of carbon assimilates produced by the green plant parts (source organs) and the partitioning of carbon assimilates between the seeds and other sink organs. Literature data suggest that the amount of carbon assimilates does not control the seed yield of perennial ryegrass seed crops (Spiertz & Ellen, 1972; Ong et al., 1978; Colvill & Marshall, 1984; Marshall & Ludlam, 1989). Spiertz & Ellen (1972) found that the amount of water-soluble carbohydrates in the whole crop increased until maturation, peaking at a concentration of 21.5 %. However, conclusive evidence is lacking. In order to determine the effect of the amount of carbon assimilates on seed filling, plants should be exposed to various irradiances after anthesis.

If it is not the amount of carbon assimilates that controls seed filling and seed yield, then it must be the factors controlling assimilate partitioning that do so. An increasing body of evidence from many species shows that the partitioning of assimilates is regulated by properties of the sink organs themselves (Gifford & Evans, 1981; Gifford et al., 1984; Marcelis, 1996). Sink strength could be defined as the competitive ability of a sink organ to import assimilates (Farrar, 1993; Marcelis, 1996), although there is ongoing debate about what determines sink strength and how to measure it (Farrar, 1993). The potential capacity to accumulate assimilates has been proposed as a measure for sink strength (Wareing & Patrick, 1975; Wolswinkel, 1985). The assimilate partitioning and the resulting distribution of dry matter at final harvest can thus be considered as the result of the sink strengths of the sink organs; sink strengths vary during development. Marcelis (1996) has used the potential growth rate, i.e. the growth rate under conditions of non-limiting assimilate supply, to quantify sink strength of cucumber fruits. He found the potential growth rate to vary with developmental stage and temperature. The vascular system, i.e. the transport system connecting source and sink, is considered not to inhibit or control assimilate fluxes and partitioning (Gifford & Evans, 1981; Wardlaw, 1990).

The question remaining is whether seed filling in perennial ryegrass is determined by the amount of carbon assimilates or by the sink strength of the seeds themselves. This was one of the main questions addressed by the research described in this thesis.

Competing sink organs in perennial ryegrass?

Competition for carbon assimilates can only occur when the amount or rate of supply is limiting. In the literature, however, it has been suggested that other sink organs (e.g. the stem and new vegetative tillers) might compete with the ear for the available assimilates.

The sink strength of the various organs changes during the reproductive development of perennial ryegrass. At first, during elongation the stem of perennial ryegrass is the major sink and assimilate import by the tillers and roots decreases sharply during this phase. The export to the younger tillers decreases from 39% to 20% of the total export and that to the roots decreases from 35% to nearly zero (Ryle, 1970). The latter author suggested that the elongating stem and the developing ear might compete for assimilates. Stem elongation proceeds until after the onset of anthesis and Clemence (1982) found indications that the stem could also be a constraint during early seed growth.

The tillering rate may increase around anthesis, because of the plant's perennial habit (Hebblethwaite et al., 1980). Competition between the seeds and new vegetative tillers has been suggested as a major cause of the low and variable seed yield of perennial ryegrass (Hampton et al., 1983; Clemence & Hebblethwaite, 1984). After anthesis of the ear the partitioning of assimilates towards the ear increases. The partitioning towards new vegetative tillers varies between experiments and is influenced by nitrogen supply (Marshall, 1985) but will increase eventually, ensuring the survival of the plant.

Seed growth might also be determined by processes within the ear and/or seed itself (Figure 2). Final seed dry weight within the ear varies considerably. Mainly within the spikelet it declines from the proximal, older seed to the distal, younger seed (Anslow, 1964). It is possible that the differences in seed dry weight within the ear are determined by differences in the duration of growth and/or the rate of growth. The sink strength of the seeds might also differ within the ear. The duration of growth of the seeds depends on the pattern of flowering and the pattern of ripening along the ear, which differ temporally and spatially (Elgersma & Sniezko, 1988; Anslow, 1964). The ripening pattern along the ear of *L. perenne* has not yet been published. On the other hand, Marshall (1985) suggested that seeds within the ear might compete for assimilates and the presence of neighbouring seeds could inhibit seed set or reduce the dry weight of other seeds, as found in wheat (Evans et al., 1972). The main carbon source in the seeds of perennial ryegrass is sucrose; it is converted into starch, which is the main storage component of ryegrass seeds (Stoddart, 1964; Pollock, 1986).

Figure 2. The ear of perennial ryegrass (*Lolium perenne*) (A), an enlargement of a spikelet (B) and a floret (C). Source: Grass weeds 2. E. Häfliger & H. Scholz (eds). CIBA-GEIGY, Basle, Switzerland. 1981. p 101.

Objectives, experimental approach and outline of this thesis

The central aim of the study described in this thesis was to identify the factors that determine seed filling and seed dry weight in perennial ryegrass. To do so the effect of the amount of carbon assimilates in the flowering tiller on seed yield per ear was investigated in an outdoor experiment. The influence of the possible presence of competing sink organs after anthesis on seed yield per ear, as well as the effect of the sink-sink relations between seeds within the ear on seed dry weight were investigated in several greenhouse experiments.

The experiments were conducted with spaced plants of clones (diploid, late hay type) of perennial ryegrass to minimise inter-plant variation. Source-sink and sink-sink relations were manipulated in various ways. The amount of carbon assimilates in the flowering tiller was varied through shading and supplemental lighting. Sinks (seeds and tillers) were removed or manipulated by nitrogen supply, light intensity and light quality. Stem elongation was not deliberately interfered with.

The experiments were intended to clarify whether seed filling is determined by the amount of carbon assimilates available (Chapter 2) or is impeded by competing sinks such as the elongating stem (Chapter 3), or the development of new vegetative tillers around anthesis (Chapter 4). Processes within the ear itself were investigated to elucidate whether differences in seed dry weight within the ear arise mainly from differences in duration of growth or rate of growth. The capacity to import assimilates might differ between seeds (Chapter 5). The sink-sink relations between seeds within the ear and their mutual influence on seed set and seed dry weight were focused on in the final three experiments (Chapter 6). In Chapter 7 the practical implications for crop growth and breeding are discussed.

REFERENCES

Anonymous, 1980. Teelt van graszaad. Actualiteiten 20. Federatie van Verenigingen voor Bedrijfsvoorlichting voor Zeeland, Goes.

Anonymous, 1993. Meerjarenvisie graszaadteelt 1995-1998. Stichting Nederlands Graan Centrum, Wageningen.

Anonymous, 1994. Stichting Nederlands Graan Centrum. Jaarverslag 1994, Wageningen.

Anslow, R.C., 1964. Seed formation in perennial ryegrass. II Maturation of seed. *Journal of the British Grassland Society* 19: 349-357.

Clemence, T.G.A., 1982. Seed production by amenity ryegrass (*Lolium perenne*). PhD thesis, University of Nottingham, School of Agriculture, Nottingham.

Clemence, T.G.A. & P.D. Hebblethwaite, 1984.
An appraisal of ear, leaf and stem $^{14}CO_2$-assimilation, ^{14}C-assimilate distribution and growth in a reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Biology* 105: 319-327.

Colvill, K.E. & C. Marshall, 1984. Tiller dynamics and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology* 104: 543-557.

Elgersma, A., 1990a. Genetic, cytological and physiological aspects of seed yield in perennial ryegrass (*Lolium perenne* L.). Thesis Agricultural University Wageningen, 91 pp.

Elgersma, A., 1990b. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Elgersma, A. & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S: 59-68.

Evans, L.T., J. Bingham & M.A. Roskams, 1972. The pattern of grain set within the ears of wheat. *Australian Journal of Biological Science* 25:1-8.

Farrar, J.F., 1993 (ed.) Sink strength: What is it and how do we measure it? Forum. *Plant, Cell and Environment* 16: 1013-1046.

Gifford, R.M. & L.T. Evans, 1981. Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology* 32: 485-509.

Gifford, R.M., J.H. Thorne, W.D. Hitz & R.T. Giaquinta, 1984. Crop productivity and photoassimilate partitioning. *Science* 225: 801-808.

Hampton, J.G. & P.D. Hebblethwaite, 1983. Yield components of the perennial ryegrass (*Lolium perenne* L.) seed crop. *Journal of Applied Seed Production* 1: 23-25.

Hampton, J.G., T.G.A. Clemence & P.D. Hebblethwaite, 1983. Nitrogen studies in *Lolium perenne* grown for seed. IV. Response of amenity types and influence of a growth regulator. *Grass and Forage Science* 38: 97-105.

Hebblethwaite, P.D., D. Wright & A. Noble, 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). In: P.D. Hebblethwaite (ed.) *Seed Production*, pp. 71-90. Butterworths, London.

Horeman, G.H., 1989. Effects of fungicides on perennial ryegrass seed production. In: *Proceedings of the XVIth International Grassland Congress*, 1989. Nice, France. p. 667- 668.

Marcelis, L.F.M., 1996. Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany* 47: 1281-1291.

Marshall, C., 1985. Developmental and physiological aspects of seed production in herbage grass. *Journal of Applied Seed Production* 3: 43-49.

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in *Lolium perenne* L. *Annals of Botany* 63: 19-27.

Meijer, W.J.M., 1985. The effect of uneven ripening on floret site utilization in perennial ryegrass seed crops. *Journal of Applied Seed Production* 3: 55-57.

Ong, C.K., K.E. Colvill & C. Marshall, 1978. Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L. *Annals of Botany* 42: 855-862.

Pollock, C.J., 1986. Fructans and the metabolism of sucrose in vascular plants. *New Phytologist* 104: 1-24.

Ryle, G.J.A., 1970. Distribution patterns of assimilated ^{14}C in vegetative and reproductive shoots of *Lolium perenne* and *Lolium temulentum*. *Annals of Applied Biology* 66: 155-167.

Spiertz, J.H.J & J. Ellen, 1972. The effect of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. 'Cropper') and its effects on seed production. *Netherlands Journal of Agricultural Science* 20: 232-246.

Stoddart, J. L., 1964. Seed ripening in grasses I. Changes in carbohydrate content. *Journal of Agricultural Science* 62: 67-72.

Wareing, P.F. & J. Patrick, 1975. Source-sink relations and the partition of assimilates in the plant. In: Cooper J.P. (ed.) *Photosynthesis and productivity in different environments.* Cambridge University Press, pp. 481-499.

Wardlaw I.F., 1990. The control of carbon partitioning in plants. *New Phytologist* 116: 341-381.

Wolswinkel, P., 1985. Phloem unloading and turgor-sensitive transport: factors involved in sink control of assimilate partitioning. *Physiologia Plantarum* 65: 331-339.

THE EFFECT OF LIGHT INTENSITY AFTER ANTHESIS ON DRY MATTER DISTRIBUTION AND SEED YIELD OF *LOLIUM PERENNE* L.

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ABSTRACT

The effect of light intensity after onset of anthesis on dry matter distribution, water-soluble carbohydrates (WSC) concentration and seed filling in *Lolium perenne* L. was investigated in a pot experiment. Spaced plants of five clones were brought to flowering and exposed to four light intensities (24 %, 57 %, 100 %, 115 %) from 7 days after onset of anthesis onwards. The two oldest flowering tillers (i.e. the main and the first tiller) were separated from the rest of the plant and dissected after a seed filling period of 450 °Cd (temperature sum counting from 7 days after onset of anthesis). Their stem dry weight and WSC concentration were reduced at the lowest light intensity. WSC amount was more reduced in the first, younger tiller. Seed yield per ear of the main tiller was reduced by 14 % because seed set and average seed weight were lower at the 24 % light intensity. Seed yield and seed number per ear of the first tiller were lowered by 21 % and 17 % respectively at the 24 % light intensity, but average seed weight was not. The relative contribution of the seeds to total tiller weight was about 20 % and was similar for both tillers and all light intensities. WSC concentration and WSC amount in the stem + rachis were reduced only at 24 % light in the main tiller and at 24 % and 57 % light in the first tiller. It is inferred that seed yield was not limited by assimilate availability, but by the ability of the seeds to fully utilise the abundantly available reserves in the stem. The tiller can support seed yield under a wide range of light intensities.

INTRODUCTION

In crops of perennial ryegrass (*Lolium perenne* L.) there is a substantial gap between potential and actual seed yields. Seed yields also vary between years and locations (Griffiths et al., 1973; Hebblethwaite et al., 1980). Whereas, seed yield is determined by the number of seeds per unit area and the average seed weight, the variability in seed yield depends largely on variation in the number of fertile florets that produce a harvestable seed. There is little variation in average seed weight (Marshall, 1985).

Several workers found that just before harvest 50 to 80 % of perennial ryegrass florets developed into a seed, but that only 25 to 70 % of these seeds were recovered after harvest and cleaning (Meijer, 1985; Horeman, 1989; Elgersma, 1990). On average only 15 to 20 % of the florets produce a harvestable seed (Hampton & Hebblethwaite, 1985; Elgersma, 1990). Some of the non-harvested seed is lost through shedding, but most seeds are too light and are lost during harvest and cleaning. Loss of the lighter seeds can explain the usually small variation in average seed weight (Elgersma, 1990).

Hyde et al. (1959) distinguished three phases in *L. perenne* seed development. The first phase is a phase of rapid growth of ca 10 days after fertilization, in which the seed takes up water rapidly. The second phase is the seed filling phase (ca 14 days) in which the dry weight increases to a maximum and the moisture percentage decreases slowly. In the last phase of about 7 days, dry weight is constant but the seed loses water and ripens. It can be postulated that lighter seeds may not have accumulated enough assimilates during the seed filling phase, because of a limited accumulation rate and/or duration.

Total assimilate amount in the crop might be limiting seed yield. Hebblethwaite et al. (1978) and Burbidge et al. (1978) prevented lodging to improve the penetration of light into the crop and found an increase in seed yield, mainly through a higher seed number per spikelet. Marshall & Ludlam (1989) however, did not record an increase in seed yield after prevention of lodging. In these studies carbohydrate concentrations were not measured nor varied.

Next to the total assimilate availability, assimilate partitioning is an important factor in determining seed yield (Gifford & Evans, 1981). The distance between the source and the sink organ is an important factor in determining the carbon partitioning (Wardlaw, 1990; Farrar, 1992), and because the ear is the main green organ of the flowering tiller (Ong et al., 1978), a sufficient supply of assimilates to the nearby seeds might be assumed.

Spiertz & Ellen (1972) studied the effect of light intensity on a ryegrass seed crop throughout the growing season. They found that light intensity influenced the number of reproductive tillers which was highly correlated to seed yield ($r = 0.95$). Low light intensity (60 % of full light) reduced the final water-soluble carbohydrate (WSC) concentration by 23 % in the seeds and by 40 % in the stem plus leaves. They also found that seed number per ear was not affected before harvest and cleaning. But, calculations

after harvest and cleaning showed a seed set of 32 % in the control treatment and 27 % in the low light intensity treatment. As lighter seeds are lost during harvest and cleaning, this finding suggests that light intensity influences seed filling.

The objectives of the experiment described below were: 1) to vary the assimilate availability in the plant during the seed filling phase through light intensity treatments; 2) to determine the effect of light intensity on dry matter distribution, water-soluble carbohydrate concentration and seed yield of the flowering tiller; 3) to investigate whether assimilate availability in the plant determines seed yield in *L. perenne*.

MATERIALS AND METHODS

The trial was conducted on spaced plants of five clones of perennial ryegrass, selected from a group of 17 clones which varied in tiller number per plant and seed number per ear. The five clones were selected from the Dutch cultivars Barlet (clone B4), Magella (clone Mg4), Edgar (clone E5) and Manhattan (clones Mh3 and Mh6).

Vegetative tillers were propagated on Steiner nutrient solution (Steiner, 1984). To obtain flowering tillers, one tiller per pot (main tiller) was vernalised for 14 weeks at 7 ± 1 °C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. On 13 May 1993 the plants were transferred to a greenhouse at 15°C with the shading screen down and allowed to acclimatise for a week. They were then put in 1.75 l pots on saucers and placed outdoors under a polyethylene hood. Per plant 240 mg N, 110 mg P_2O_5 and 180 mg K_2O were available. Plants were watered weekly by flooding the saucers. A light mildew infection just after the end of vernalization was treated with pyrazofos. Lodging of the flowering tillers was prevented with wire.

Light intensity treatments were arranged according to a non-orthogonal split-plot design in three replicates with four light intensities as the main plots and five clones and two harvest dates as the subplots. The four light intensities were: 24 %, 57 %, 100 % and 115 %. These percentages were calculated after integration of irradiance levels over a few days. The average daily incoming radiation (PAR) under the hood in the unshaded 100 % light intensity was 4.5 MJ/m^2. This was calculated using irradiation measurements from a nearby weather station, taking into account that the polyethylene hood reduced the light intensity by 33 %. The plants were shaded with a double layer of cotton cloth (24 %) or a layer of polyethylene (57 %). Two HLRG lamps (high pressure mercury, Philips 400W) per main plot (1.44 m^2) positioned approximately 60 cm above the plants were used to boost the light intensity for 12 h per day (115 %). This raised the average daytime temperature, measured at ear height by 0.9 °C. For all light treatments the

spectral composition of the light was measured. Phytochrome photoequilibria were calculated according to Lagarias et al. (1987). They were (in order of increasing light intensity): 0.65, 0.66, 0.67 and 0.69. These values show that plant growth was not influenced through effects on the phytochrome equilibrium (Smith & Holmes, 1977). A border row was placed around each main plot, also to ensure pollination. In addition to the five clones investigated three other clones were used for that purpose.

At the start of the treatments, 7 days after onset of anthesis (DAA) of the main tiller, six plants per clone were harvested. The plants were treated individually. At the second harvest (14 DAA) plants from three clones were used. The third harvest date was based on dough ripeness of the main ear under the highest light intensity which occurred at a temperature sum of 450°Cd, counting from the start of the treatment (7 DAA). The mean daily temperature, determined from continuous temperature measurements with a data logger, was used to calculate the temperature sum. Plants under the highest light intensity were harvested at 30 DAA and, according to the temperature sum of 450°Cd, plants under the three lower light intensities at 32 DAA.

At harvest, the two oldest tillers (i.e. the main and the first tiller) were separated from the rest of the plant and dissected into stem, leaf sheaths, leaf blades and ear. The main tiller was marked immediately after vernalisation and the first tiller was selected according to size and development. The ears were stored at - 20 °C before the number of seeds and empty florets per spikelet were counted under a binocular microscope. Any small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret. The dry weight of the different plant parts was determined after drying at 70 °C for 48 h. Roots were not harvested.

In order to determine water-soluble carbohydrates (WSC), plant parts were ground and extracted in water at 100 °C for 15 minutes. Total reducing sugars was measured colorimetrically with an automatic analysis device after hydrolysis in sulphuric acid (0.45 N) (Bran & Lübbe Analyzing Technologies, Inc., Elmsford, N.Y., USA).

Analysis of variance and linear regression were carried out with the GENSTAT statistical package (Payne et al., 1987). Seed set percentage was analysed by a generalised linear regression model, with a logit link function and a binomial distribution (McCullagh & Nelder, 1989). Differences were considered to be statistically significant at the $P<0.05$ level.

RESULTS

Plant growth and development

On average, the main tiller started to flower 54 days after the end of vernalisation. The first tiller flowered 1 - 3 days later than the main tiller. The average total number of tillers and ears per plant differed between clones, but was not influenced by light intensity after anthesis. The average total number of tillers per clone ranged from 48 to 90 per plant and the average number of ears ranged from 19 to 35 per plant. There was no correlation between the numbers of tillers and ears per plant of each clone. After 7 DAA the total number of tillers did not increase, but ear emergence continued. Averaged for all clones the percentage of tillers with an ear increased from 27 to 44 % between 7 DAA and final harvest.

Average tiller dry weight and final plant dry weight were not influenced by the light intensity treatments. When the treatments started at 7 DAA, on average 76 % of the final plant dry weight had already been reached. The average final plant dry weight per clone was 12.5 g and ranged from 9.5 to 15.4 g per plant. Clones differed significantly.

Dry matter distribution

Since there were no interactions between clones and light intensity for dry matter distribution, the average of the five clones is presented.

At final harvest, there was a linear effect of light intensity on total dry weight of the main tiller and seed weight per ear (Table 1A). At 24 % light the stem dry weight was reduced and the dry weight of green leaf blade was higher than at the other three light intensities. Average seed weight was lowest at the 24 % light intensity.

The effects of light intensity were stronger on the first tiller than on the main tiller (Table 1B). Total tiller dry weight was lower at the 24 % light intensity compared to the other light intensities. The effect was primarily caused by changes in stem dry weight. Leaf sheath dry weight decreased at light intensities below the 100 % light intensity. At the 24 % light intensity dry weight of green leaf blade was highest. Ear dry weight was lower in that treatment because the rachis and seed weight per ear were lower. There was no effect of light intensity on the average seed weight in the first tiller.

For both tillers the changes in dry weight resulted in a decrease of the relative contribution of the stem to total tiller dry weight at the 24 % light intensity (Table 2).

Table 1. The effect of light intensity on dry matter distribution (mg) within the main flowering tiller (1A) and the first flowering tiller (1B) in plants of *Lolium perenne*. Values are the averages of five clones harvested at 30 days (115 % light) or 32 days (all other treatments) after onset of anthesis. 100 % light equals 4.5 MJ/m^2/d. Different letters indicate significant differences.

A) Main tiller	Dry weight (mg)				
	Light intensity				Statistical
Tiller part	24 %	57 %	100 %	115 %	significance
Stem	344 a	403 b	412 b	405 b	* *
Green blade	12 b	0 a	0 a	1 a	*
Brown blade	69	78	77	77	N.S.
Leaf sheath	92	93	87	88	N.S.
Leaves	173	171	164	166	N.S.
Rachis	56	60	61	62	N.S.
Seed	174	190	188	202	linear effect
Empty florets + glumes	67	63	64	66	N.S.
Total ear	297	313	313	331	N.S.
Total tiller	813	887	889	901	linear effect
Average seed weight	1.77 a	1.92 c	1.86 bc	1.85 b	* *

B) First tiller	Dry weight (mg)				
	Light intensity				Statistical
Tiller part	24 %	57 %	100 %	115 %	significance
Stem	267 a	326 b	338 b	375 c	* * *
Green blade	7 b	1 a	1 a	1 a	* * *
Brown blade	52	57	54	63	linear effect
Leaf sheath	84 a	84 a	88 b	90 b	* *
Leaves	143	142	143	154	linear effect
Rachis	46 a	54 b	53 b	56 b	*
Seed	124 a	155 b	140 ab	157 b	*
Empty florets + glumes	49	58	53	54	N.S.
Total ear	219 a	267 b	245 ab	267 b	*
Total tiller	628 a	735 bc	727 b	796 c	* *
Average seed weight	1.47	1.50	1.49	1.53	N.S.

* $0.01<P\leq0.05$; * * $0.001<P\leq0.01$; * * * $P\leq0.001$

Table 2. Effect of light intensity on the relative dry matter distribution (%) within the main and the first flowering tiller in plants of *Lolium perenne*. Values are the averages of five clones harvested at 30 days (115 %) or 32 days (all other light treatments) after onset of anthesis. 100 % light equals 4.5 $MJ/m^2/d$. Different letters indicate significant differences.

	Relative dry matter distribution (% of total tiller)				
	Light intensity				Statistical
	24 %	57 %	100 %	115 %	significance
Main tiller					
Stem	42.2 a	45.3 b	46.1 b	45.0 b	*
Leaves	21.6 c	19.5 b	18.8 ab	18.6 a	* * *
Seed	21.4	21.5	21.2	22.4	N.S.
Total ear	36.5	35.3	35.2	36.7	N.S.
First tiller					
Stem	42.4 a	44.2 b	46.2 c	47.1 c	* * *
Leaves	22.9 b	19.4 a	20.1 a	19.7 a	* * *
Seed	19.6 a	21.0 b	19.1 a	19.4 a	*
Total ear	34.7 ab	36.4 b	33.7 a	33.3 a	*

* $0.01<P\leq0.05$; * * $0.001<P\leq0.01$; * * * $P\leq0.001$

The relative contribution of the leaves increased in that treatment. The relative contributions of the seeds and the ear of the main tiller to total tiller weight were not influenced by light intensity. For reasons not clear, within the first tiller the seeds contributed more to total tiller weight at the 57 % light intensity than at the other light intensities. In general, the dry matter partitioning was similar for both the main and the first tiller.

Water-soluble carbohydrates (WSC)

Shading reduced the final WSC concentration of the plants. The final WSC concentration of stem + rachis of the main tiller was lowest at 24 % light intensity. The WSC concentration of stem + rachis of the first tiller and the total plant shoot were also lower at the 57 % light intensity compared to the 100 % and 115 % light intensities (Figure 1). The main tiller and the first tiller had similar WSC concentrations in stem + rachis.

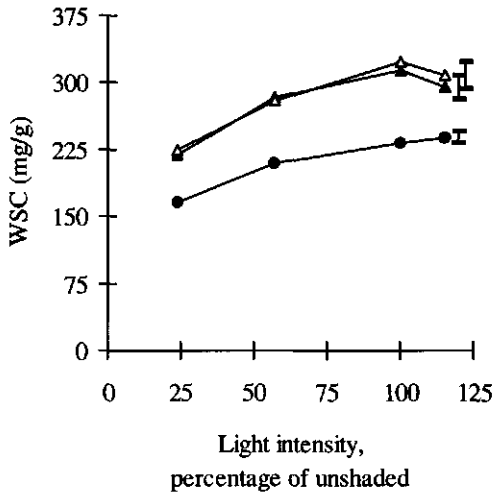

Figure 1. The effect of light intensity on the water-soluble carbohydrate (WSC) concentration of the stem + rachis of the main tiller (▲) and of the first tiller (△) within plants of *Lolium perenne* L. and of the total plant shoot (●). Bars indicate LSD (0.05) values. 100 % light is 4.5 MJ/m^2/d.

Between 14 DAA and 30 - 32 DAA the WSC concentration in the total plant shoot declined by 25 - 74 mg per g dry weight depending on the light intensity (Table 3). Within the main tiller the leaves and the spikelets (one replication) showed an especially strong decrease in WSC concentration (Table 3). In contrast the WSC concentration of the stem + rachis did not decrease at the higher light intensities, but at the lowest light intensity it did decrease between 14 DAA and 30 - 32 DAA. However the interaction between light intensity and DAA was not statistically significant (P=0.076).

The final amount of WSC in the stem + rachis of both the main tiller and the first tiller were reduced at the 24 % light intensity compared to the other light intensities (table 4). In the first tiller the WSC amount at 57 % light was also lower than at the higher light intensities. Between the 115 % and 24 % light intensity the WSC amount in the stem + rachis declined by 37 % in the main tiller and 47 % in the first tiller.

Table 3. The water-soluble carbohydrate (WSC) concentration (mg/g) of the main tiller organs and of the total plant shoot of *Lolium perenne* plants. Measured at 7, 14, and 32 days after onset of anthesis and at four different light intensities.Values are the averages of three clones. 100 % light equals 4.5 MJ/m^2/d. Different letters indicate significant differences.

	Days after onset of anthesis								
	7	14				32			
		Light intensity				Light intensity			
	100 %	24 %	57 %	100 %	115 %	24 %	57 %	100 %	115 %[1]
Stem+rachis	342	305 a	322 ab	330 b	330 b	241 a	294 b	321 c	303 bc
Leaves	182	86	102	102	121	32	29	26	33
Spikelets	139	120	124	131	131	33	37	37	39
Total main tiller	256	213	218	236	229	140	175	193	182
Total plant shoot	285	254 a	261 a	279 b	281 b	180 a	215 b	243 c	256 c

[1] measured at 30 days for this treatment only.

Table 4. The effect of light intensity on the WSC amount (mg) in the stem + rachis of the main and the first tiller in plants of *Lolium perenne*. Values are the averages of five clones harvested 30 days (115 %) or 32 days (all other treatments) after onset of anthesis. 100 % light equals 4.5 MJ/m^2/d. Different letters indicate significant differences.

	WSC content (mg/stem+rachis)				
	Light intensity				Statistical
	24 %	57 %	100 %	115 %	significance
Main tiller	89.4 a	135.0 b	153.6 b	141.0 b	* * *
First tiller	71.4 a	109.7 b	130.3 c	135.1 c	* * *

* * * P≤0.001

Table 5. The effect of light intensity on seed yield components of the main and the first flowering tiller in plants of *Lolium perenne*. Values are the averages of five clones harvested at 30 days (115 %) or 32 days (all other treatments) after onset of anthesis. 100 % light equals 4.5 MJ/m^2/d. Different letters indicate significant differences.

		Number of spikelets per ear	Number of florets per spikelet	Number of florets per ear	Number of seeds per ear	Seed set (%)
	Light intensity					
Main tiller	24 %	23.3	8.8	199.0	97.5	49.3 a
	57 %	23.2	8.2	186.2	98.0	53.5 b
	100 %	22.9	8.6	189.1	100.3	53.5 b
	115 %	23.5	8.6	199.2	107.3	54.4 b
	Average	23.2	8.5	193.4	100.8	52.7
	Statistical significance	N.S.	N.S.	N.S.	N.S.	*
	Light intensity					
First tiller	24 %	21.0 a	8.0	162.6	83.8 a	51.8 a
	57 %	24.1 b	8.1	180.9	96.2 bc	53.4 ab
	100 %	21.0 a	8.1	166.1	93.2 b	56.5 bc
	115 %	22.4 a	8.2	178.0	101.4 c	57.2 c
	Average	22.1	8.1	171.9	93.7	54.7
	Statistical significance	* *	N.S.	N.S.	* *	* * *

* 0.01<P≤0.05; * * 0.001<P≤0.01; * * * P≤0.001

Yield components

In the main ear the number of seeds did not change between 14 DAA (96.2 seeds/ear) and final harvest (95.6 seeds/ear). Seed number per ear in the main tiller was significantly (P=0.002) higher than that in the first tiller (Table 5). The number of florets per ear did not vary significantly among the different light intensities, although the number of spikelets per ear in the first tiller was higher at the 57 % light intensity. In the main tiller seed number per ear was not significantly affected by light intensity, but in

the first tiller seed number per ear was reduced at the 24 % light intensity compared to the other light intensities. In both tillers seed set was lowest at the 24 % light intensity. Seed set was significantly higher in the first tiller than in the main tiller.

DISCUSSION

Reducing the light intensity during seed filling from 115 % to 24 % reduced the seed yield per ear of the main tiller by 14 % and that of the first tiller by 21 % (Table 1). The total dry weight of stem + rachis was reduced to the same extent, i.e. by 14 % in the main tiller and 27 % in the first tiller. But the WSC amount in the stem + rachis declined by 37 % in the main tiller and 47 % in the first tiller (Table 4).

Results show that seed filling was only affected in the main tiller at the lowest light intensity, as average seed weight was reduced and seed number per ear was not. In the first tiller average seed weight was not affected by light intensity (Table 1), but seed number per ear was (Table 5).

Within *L. perenne* the stem is the major storage organ, storing fructans (Pollock & Jones, 1979). The sharp fall in stem reserves under the lowest light intensity might be caused by movement of assimilates to support other tillers or could be an indication that the stem reserves support seed filling under conditions unfavourable for photosynthesis (Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984). However, the finding that the relative contribution of the seeds to total tiller weight is hardly influenced by light intensity (Table 2), shows that seed filling is only partly supported by stem reserves. A constant fraction of the assimilates produced is translocated to the seeds. If seed filling were fully supported by stem reserves, one would expect the relative contribution of the seeds to increase with decreasing tiller dry weight at a lower light intensity.

In wheat, strong effects of shading on the utilisation of stem and leaf reserves by the seeds have been found (Takahashi et al., 1994). Shading wheat during grain filling reduces stem dry weight by depleting stem reserves. These reserves support grain yield under stress conditions (Judel & Mengel, 1982; Kühbauch & Thome, 1989; Kiniry, 1993).

At 7 DAA the main stem + rachis on average contained 142 mg WSC, which is equal to 76 % of the final seed weight. In wheat, Judel & Mengel (1982) found a value of 20 % at anthesis in the stem and leaves. Not all of this is available for seed dry matter, for respiratory losses will occur in the stem and in the developing seeds. Results from Wardlaw & Porter (1967) indicate a respiratory loss of 39 % of the stem reserves during seed filling (Wardlaw & Willenbrink, 1994). Also taking into account the respiratory

costs of protein and starch synthesis in the developing seeds (Penning de Vries et al., 1983) and a protein concentration of about 14 % (Spiertz & Ellen, 1972), stem reserves could support approximately 32 % of the final seed yield in the 115 % light treatment.

At 32 DAA at the 24 % light intensity the WSC amount had decreased by 52 mg in the stem + rachis of the main tiller (Table 4). Taking respiratory losses into account this amount would be sufficient to increase seed yield to that at the 115 % light intensity. Under very low light intensity conditions the seeds of perennial ryegrass are not able to fully utilise the abundant stem reserves.

From the 57 % light intensity onwards the WSC concentration (Table 3) and WSC amount in the stem + rachis of the first tiller was not reduced between 7 DAA and 30 - 32 DAA. The WSC amount in the leaves was reduced by about 88 %. This experiment therefore shows that under a wide range of light intensities the tiller can support seed yield without depleting stem reserves.

In view of the reduced seed set at the 24 % light intensity (Table 5) the abundance of carbohydrate reserves in the stem at anthesis also suggests that the success of fertilisation and early seed growth do not depend on stored carbohydrates. A positive relation between seed number and the light intensity during anthesis has been found in other species such as maize (Andrade et al., 1993), wheat (Wardlaw, 1970; Sofield et al., 1977; Evans, 1978), oilseed rape (Habekotté, 1993), and caraway (Bouwmeester et al., 1995). Whether a low current assimilate production or the light intensity itself reduces seed set, is not clear. Several authors (Struik & Deinum, 1982; Heindl & Brun, 1983) have suggested that as well as affecting photosynthesis, shading directly influences the ability of seeds to import assimilates.

Seed yield per ear, stem dry weight and WSC amount in the stem were reduced more in the first tiller than in the main tiller, probably because the first tiller was in an earlier stage of development when the light intensity treatment started. This shows that when extrapolating the effect of light intensity to crop level, heterogeneity in tiller age is an important factor to bear in mind.

The finding at the 100 % light intensity that seed number per ear did not decrease between 14 DAA and 30 DAA supports the conclusions of Elgersma & Sniezko (1988) and Meijer (1985) that seed abortion after 10 DAA is rare and of no significance to reduced seed yields in perennial ryegrass seed crops.

From the results of this experiment it can be concluded that the amount of carbon assimilate in the tiller does not limit seed yield, but the partitioning of assimilates to the seeds does. Seed yield could be increased by an increased rate and/or duration of carbon assimilate partitioning to the seeds.

Perennial ryegrass is bred for vegetative purposes and is a perennial crop which also produces new vegetative shoots after flowering, that demand assimilates from the

flowering stem (Clemence & Hebblethwaite, 1984). Furthermore it is not clear how the pattern of flowering and ripening across the ear influences the growth of the individual seeds and of the total ear. Seed weight decreases from the basal to the apical seeds in a spikelet (Anslow, 1964). More research into these questions is needed.

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REFERENCES.

Andrade, F.H., S.A. Uhart & M.I. Frugone, 1993. Intercepted radiation at flowering and kernel number in maize: shade versus plant density effects. *Crop Science* 33: 482-485.

Anslow R.C., 1964. Seed formation in perennial ryegrass. II Maturation of seed. *Journal of the British Grassland Society* 19: 349-357.

Bouwmeester H.J., H.G. Smid & E. Loman, 1995. Seed yield in caraway (*Carum carvi*). 2. Role of assimilate availability. *Journal of Agricultural Science, Cambridge* 124: 245-251.

Burbidge, A., P.D. Hebblethwaite & J.D. Ivins, 1978. Lodging studies in *Lolium perenne* grown for seed. 2.Floret site utilization. *Journal of Agricultural Science, Cambridge* 90: 269-274.

Clemence, T.G.A. & P.D. Hebblethwaite, 1984. An appraisal of ear, leaf and stem $^{14}CO_2$-assimilation, ^{14}C-assimilate distribution and growth in a reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Biology* 105: 319-327.

Colvill, K.E. & C. Marshall, 1984. Tiller dynamics and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology* 104: 543-557.

Elgersma, A., 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Elgersma, A., & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S:59-68.

Evans, L.T., 1978. The influence of irradiance before and after anthesis on grain yield and its components in microcrops of wheat grown in a constant day-length and temperature regime. *Field Crops Research* 1: 5-19.

Farrar, J.F., 1992. The whole plant: carbon partitioning during development. In: Pollock C.J., J.F Farrar & A.J.Gordon (eds.) *Carbon partitioning within and between organisms*. Oxford: Bios Scientific Publishers, pp.163-179.

Gifford, R.M. & L.T. Evans, 1981. Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology* 32: 485-509.

Griffiths, D.J., H.M. Roberts & J. Lewis, 1973. The seed yield potential of grasses. Report Welsh Plant Breeding Station 1973, pp. 117-123.

Habekotté, B., 1993. Quantitative analysis of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L.) under field conditions. *Field Crops Research* 35: 21-33.

Hampton, J.G. & P.D. Hebblethwaite, 1985. The effect of growth retardant application on floret site utilization and assimilate distribution in ears of perennial ryegrass cv. S24. *Annals of Applied Biology* 107: 127-136.

Hebblethwaite, P.D., A. Burbidge & D. Wright, 1978. Lodging studies in *Lolium perenne* grown for seed. 1.Seed yield and seed yield components. *Journal of Agricultural Science, Cambridge* 90: 261-267.

Hebblethwaite, P.D., D. Wright & A. Noble, 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). In: Hebblethwaite P.D. (ed.) *Seed Production*. London: Butterworths, pp.71-90.

Heindl, J.C. & W.A. Brun, 1983. Light and shade effects on abscission and ^{14}C-photoassimilate partitioning among reproductive structures in soybean. *Plant Physiology* 73: 434-439.

Horeman, G.H., 1989. Effects of fungicides on perennial ryegrass seed production. In: *Proceedings of the XVIth International Grassland Congress*, 1989. Nice, France. pp. 667-668.

Hyde, E.O.C., M.A. McLeavy & G.S. Harris, 1959. Seed development in ryegrass, and in red and white clover. *New Zealand Journal of Agricultural Research* 2: 947-952.

Judel, G.K. & K. Mengel, 1982. Effect of shading on nonstructural carbohydrates and their turnover in culms and leaves during the grain filling period of spring wheat. *Crop Science* 22: 958-962.

Kiniry, J.R., 1993. Nonstructural carbohydrate utilization by wheat shaded during grain growth. *Agronomy Journal* 85: 844-849.

Kühbauch, W. & U. Thome, 1989. Nonstructural carbohydrates of wheat stems as influenced by sink-source manipulations. *Journal of Plant Physiology* 134: 243-250.

Lagarias, J.C., J.M. Kelly, K.L. Cyr & W.O. Smith, 1987. Comparative photochemical analysis of highly purified 124-kilodalton oat and rye phytochrome in vitro. *Photochemistry and Photobiology* 46: 5-13.

Marshall, C., 1985. Developmental and physiological aspects of seed production in herbage grasses. *Journal of Applied Seed Production* 3: 43-49.

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in *Lolium perenne* L. *Annals of Botany* 63:19-27.

McCullagh, P. & J.A. Nelder, 1989. *Generalized linear models.* 2nd edition Cambridge: Cambridge University Press.

Meijer, W.J.M., 1985. The effect of uneven ripening on floret site utilization in perennial ryegrass seed crops. *Journal of Applied Seed Production* 3: 55-57.

Ong, C.K., K.E. Colvill & C. Marshall, 1978. Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L.. *Annals of Botany* 42: 855-862.

Payne, R.W., P.W. Lane, A.E. Ainsley, K.E. Bicknell, P.G.N. Digby, S.A. Harding, P.K. Leech, H.R. Simpson, A.D. Todd, P.J. Verrier & R.P. White, 1987. *Reference Manual.Genstat 5 Committee Rothamsted Experimental Station.* Oxford: Clarendon Press.

Penning de Vries, F.W.T., H.H. van Laar & M.C.M. Chardon, 1983. Bioenergetics of fruits, and storage organs. In: *Proceedings Symposium Potential Productivity of Field Crops under Different Environments.* Manila, Philippines: International Rice Research Institute, pp. 37-59.

Pollock, C.J. & T. Jones, 1979. Seasonal patterns of fructan metabolism in forage grasses. *New Phytologist* 83: 9-15.

Smith, H. & M.G. Holmes, 1977. The function of phytochrome in the natural environment. III. Measurement and calculation of phytochrome photoequilibrium. *Photochemistry and Photobiology* 25: 547-550.

Sofield, I., L.T. Evans, M.G. Cook & I.F. Wardlaw, 1977. Factors influencing the rate and duration of grain filling in wheat. *Australian Journal of Plant Physiology* 4: 785-797.

Spiertz, J.H.J. & J. Ellen, 1972. The effect of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. 'Cropper') and its effects on seed production. *Netherlands Journal of Agricultural Science* 20: 232-246.

Steiner, A.A., 1984. *The universal nutrient solution.* ISOSC Proceedings of the 6th International Congress on Soilless Culture. Wageningen, the Netherlands, pp. 633-649.

Struik, P.C. & B. Deinum, 1982. Effect of light intensity after flowering on the productivity and quality of silage maize. *Netherlands Journal of Agricultural Science* 30: 297-316.

Takahashi, T., N. Tsuchihashi, T. Takaku & K. Nakaseko,1994. Grain filling mechanisms in spring wheat. III. Effects of shadings on dry matter accumulation in grain and culm. *Japanese Journal of Crop Science* 63: 313-319.

Wardlaw, I.F., 1970. The early stages of grain development in wheat: response to light and temperature in a single variety. *Australian Journal of Biological Sciences* 24: 765-774.

Wardlaw, I.F., 1990. The control of carbon partitioning in plants. *New Phytologist* 116: 341-381.

Wardlaw, I.F. & H.K. Porter, 1967. The redistribution of stem sugars in wheat during grain development. *Australian Journal of Biological Sciences* 20: 309-318.

Wardlaw, I.F. & J. Willenbrink, 1994. Carbohydrate storage and mobilisation by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose- phosphate synthase. *Australian Journal of Plant Physiology* 21: 255-271.

SINK-SOURCE AND SINK-SINK RELATIONS DURING REPRODUCTIVE DEVELOPMENT IN *LOLIUM PERENNE* L.

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ABSTRACT

Spaced plants of *Lolium Perenne* L. were labelled with ^{13}C at regular intervals from main ear emergence onwards in order to identify and measure the activity of source and sink organs during seed formation. The contribution to final seed carbon of current photosynthesis in different stages during reproductive development was estimated. The source activity of the various tiller groups within the plant roughly reflected the relative contributions of these groups to total plant dry weight. After anthesis there was little net exchange of ^{13}C-label between the older and younger tiller groups. From main ear emergence onwards the source activity of the leaves declined sharply, from 95 % of total tiller photosynthesis to 16 % at final harvest. The ear became the main assimilating organ on the flowering tiller as the leaves aged. During anthesis the stem was a stronger sink than the seeds and as a consequence carbon from the leaves was imported by the stem and not by the seeds. At final harvest 70 % of the label was located in the stem, when fixed during anthesis. Water-soluble carbohydrates accumulated in the stem, up to 25 % of dry matter. After anthesis the sink strength of the developing seeds increased and that of the stem decreased. The stem remained a net sink organ up to about mid-seed filling. The contribution of current photosynthates to final seed carbon increased during seed development. Pre-anthesis assimilates contributed 16 % to final seed and spikelet carbon, when correcting for the palea and lemma that are present before anthesis. It is shown that the stem is a temporary storage organ that can support seed filling. Yet only a small amount of the stem reserves was used by the seeds, indicating that yield potential is much higher. In contrast to carbon, nitrogen was largely redistributed from the stem and leaves to the seeds. At final harvest 59 % of the nitrogen in the flowering tiller was located in the seeds.

INTRODUCTION

Seed yields of crops of perennial ryegrass (*Lolium Perenne* L.) are low and variable (Hebblethwaite et al., 1980; Elgersma, 1990), with only 10 - 20 % of the above-ground dry matter being harvested as seed. Competition between the seeds and growth of new tillers is thought to reduce seed yield (Hampton et al., 1983; Clemence & Hebblethwaite, 1984; Griffith, 1992). Also the elongating stem might be a factor reducing seed yield, as it might compete with the developing ear (Ryle, 1970). This may be resolved by monitoring the activity of leaves, stem, seeds and younger tillers as carbon-source or carbon-sink.

The pattern of carbon distribution reported in the literature is consistent up to ear emergence but then becomes less clear cut. Between the start of stem elongation and ear emergence the export of carbon assimilates from the youngest leaf on the main tiller to the stem increases strongly. The export to the younger tillers decreases from 39 % to 20 % of the total export and that to the roots decreases from 35 % to nihil (Ryle 1970; Parsons & Robson, 1981). The fraction received by the developing ear increases, reaching 66 % at ear emergence (Ryle 1970).

In *Lolium temulentum*, in which the same pattern of assimilate distribution until ear emergence has been found, the two or three youngest leaves on the tiller supply most of the carbon to the ear (Ryle, 1972; Ryle & Powell, 1972). On the reproductive tiller the younger, upper leaves mainly supply the ear and the upper stem parts, while the older, lower leaves mainly supply the lower stem parts, younger tillers and roots (Ryle, 1972; Clifford & Langer, 1975; Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984). These differences in carbon distribution appear as stem elongation begins (Ryle, 1970). After ear emergence the ear becomes the main source organ on the flowering tiller. In *L. perenne* the relative importance of the ear as a source organ increases during seed filling when the leaves age (Ong et al., 1978a; Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984). Ong et al. (1978a) found that the ear contributed 50 % to the total amount of ^{14}C-label assimilated by the whole tiller at mid-grain filling.

^{14}C-labelling experiments in the field have shown that the amount exported to either the ear or the younger tillers varies. Clemence & Hebblethwaite (1984) found that the fraction of assimilated ^{14}C exported to the younger tillers increased from 10 % to 24 % during seed development. In their experiment part of the stem was labelled and harvest took place after 24 h. The export to the ear increased from 7 % to 34 %. Colvill & Marshall (1984) however, found much less label leaving the flowering tiller. When label was fixed by the main tiller at anthesis, at ripeness 16 % of the label present was located in younger tillers and over 50 % in the ear. According to Ong et al. (1978b) the flowering tiller exported only traces of ^{14}C-label at anthesis. These reports agree on the increasing export of current assimilates to the ear, but disagree on the fraction exported to the younger tillers. Furthermore, the role of stem reserves is not clear. This could be clarified

by studying one genotype under greenhouse conditions, in order to reduce variation between plants.

Labelling whole plants with $^{13}CO_2$ enables one to follow the distribution of carbon, represented by the ^{13}C-label, through the plant (Yamagata et al., 1987; Svejcar et al., 1990) and thus to quantify the distribution between and within tillers of ^{13}C-label assimilated at regular intervals from ear emergence onwards. The relative amount of ^{13}C-label in an organ after a certain chase period was considered a measure of the sink strength of that organ. In fact this reflects the actual sink strength under the experimental conditions and not that sink organ's potential ability to import carbon. The actual sink strength is also influenced by processes in the source organs and in the pathway between source and sink organ (Farrar, 1993). Carbon input lost through respiration is not measured. ^{13}C-labelling was also used to measure the source activity, by viewing the fraction of ^{13}C-label fixed by a plant part as a measure of the actual carbon flux through that source (Farrar, 1992).

The three objectives of the study were: to measure the relative activity of the source organs from ear emergence onwards, to identify the sinks present in the reproductive plant from ear emergence onwards and measure their sink strength, and to estimate the contribution of photosynthesis at various stages after ear emergence to final seed carbon.

MATERIALS AND METHODS

Plant growing conditions

Vegetative tillers of *L. perenne* were clonally propagated on Steiner nutrient solution (Steiner, 1984). To obtain flowering tillers, one tiller per pot was vernalised for 14 weeks at 7 ± 1°C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. This tiller will be referred to as the main tiller. After vernalisation, on 26 August 1993, the plants were transferred to a greenhouse at 15°C with the shading screen down and allowed to acclimatise for a week. The plants were then transferred to 1.75 l pots in peaty soil. Per plant 240 mg N, 110 mg P_2O_5 and 180 mg K_2O was supplied. In the greenhouse natural daylength was extended to 17 h with incandescent bulbs (approximately 1.7 W/m^2). For 12 h per day supplemental lighting was provided by high pressure sodium lamps (Philips, AGRO SON-T, 400 W). The average daily incoming radiation at plant height was 2.7 MJ/m^2 (400 - 700 nm). The average day and night temperatures were 23°C (12 h, 6.30 -18.30 h) and 17°C respectively. Iron wire was used to prevent the flowering tillers from lodging.

Experimental design

One clone (B1, selected from the Dutch cultivar Barlet) was used and the plants were grown in a randomised block design. Four other clones were used in the border rows and as pollinators. The time schedule of labelling, the number of replicates and number of plants used are shown in Table 1. In the pulse-chase labelling experiments plants were treated in groups of four plants that were pooled at harvest. After each $^{13}CO_2$ feeding four labelled plants were harvested immediately and at each subsequent $^{13}CO_2$ feeding until 42 days after main ear emergence (DAEE), the final harvest. This means that after each $^{13}CO_2$ feeding the label could be chased for various periods until 42 DAEE. At each $^{13}CO_2$ feeding 15 unlabelled control plants (three plants times five replicates) were harvested to determine dry matter accumulation and distribution, water-soluble carbohydrate content, nitrogen content and control values of the ^{13}C stable isotope ratios ($\delta^{13}C$) in the plant organs studied.

$^{13}CO_2$ pulse-chase experiments

The first $^{13}CO_2$ feeding was applied when in 50 % of the plants the top of main ear had emerged from the leaf sheath. Whole plants were labelled in a closed system consisting of a perspex chamber (80 x 80 x 80 cm) connected to a cooling device with a fan that kept the temperature at 20 ± 1 °C and also homogenised the air. The plants for the various harvest dates were randomised inside the labelling chamber. The light intensity during labelling was ± 110 W/m^2 (PAR) provided by metal halide lamps (Philips HPI/T, 400 W). $^{13}CO_2$ was produced by adding a surplus of 1 N sulphuric acid to $Na_2{}^{13}CO_3$ (99 atom %, Campro Scientific, Elst, the Netherlands). The amount of $Na_2{}^{13}CO_3$ used at each $^{13}CO_2$ feeding was aimed at achieving sufficiently enriched plant organs ($\delta^{13}C > 0$) until the end of the desired chase period, hereby taking into account an estimated lowering of the $^{13}C/^{12}C$ isotope ratio through plant growth and respiration. Each $^{13}CO_2$ feeding period was ended when the $^{12}CO_2$ concentration in the chamber, measured by an infrared gas analyser had fallen to 100 - 200 ppm. The feeding usually lasted for 1.5 to 2.5 h depending on the photosynthetic activity of the plants.

Fixation of label by the source organs

In this experiment the fraction of label fixed by a plant part after labelling for ten minutes was considered to be a measure of the source activity of that part. Results from a pilot experiment, in which plants were labelled at mid-anthesis for ten minutes, had shown that

the leaf blades exported 47 % of the label within one hour. This means that labelling for a longer period would underestimate the fixation by the leaves. At the first three $^{13}CO_2$ feedings 0.5 g of $Na_2{}^{13}CO_3$ was used; 1.0 g was used at the last three (Table 1). Immediately after $^{13}CO_2$ feeding the plants were harvested, and the dissected plant parts put on ice to minimise respiratory losses before drying. At the first harvest only the part of the ear that had appeared was separated from the stem. At the other harvests the spikelets and rachis were separated.

Harvest procedure

At each harvest the plants were divided into four groups of tillers (Figure 1): 1) the main tiller (MT), 2) a homogeneous group of younger reproductive tillers, 3) tillers which had at least one fully emerged leaf, and 4) small tillers with a partly emerged first leaf and tillers present inside the leaf sheaths of the tillers in the first two groups. The second group of tillers consisted of all tillers other than the main tiller that were present one week after the end of vernalisation. These tillers were marked at that moment. The group 3 and group 4 tillers were not marked.

Table 1. Growth stage, time schedule of labelling and number of plants used.

		Pulse-chase labelling			
Growth stage main ear	Days after main ear emergence (DAEE)	Number of groups labelled (4 plants/group)	Harvest dates groups (DAEE)	Number of control plants	Number of plants labelled for ten minutes[1]
Main ear emergence	0	6	0, 14, 21, 28, 35, 42	15	5
Onset of anthesis	14	5	14, 21, 28, 35, 42	15	5
Mid-anthesis	21	4	21, 28, 35, 42	15	5
End of anthesis	28	3	28, 35, 42	15	5
3 Weeks after onset of anthesis	35	2	35, 42	15	5
4 Weeks after onset of anthesis	42	-	-	15	5

[1] Plants were labelled one day later than the pulse-chase labelling.

The main tiller and group 2 tillers were divided into stem + rachis, green and yellow leaf and spikelets. At the first harvest the ear was not dissected into rachis and spikelets, and partly emerged ears were taken out of the leaf sheath. At the last two harvests the seeds and empty florets of the main ear were separated. The ears had been stored at - 20 °C until the number of seeds and empty florets were counted under a binocular microscope. Small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret. The dry weight of the various plant parts was determined after drying at 70 °C for 48 h. Roots were not harvested.

Chemical analyses

For the determination of water-soluble carbohydrates (WSC) the plant parts were ground and extracted in water at 100 °C for 15 minutes. Total reducing sugars was measured colorimetrically after hydrolysis in sulphuric acid (0.45 N) with an automatic analysis device (Bran & Lübbe Analyzing Technologies, Inc., Elmsford, N.Y., USA). Starch was determined titrimetrically after enzymatic hydrolysis (AOAC, 1984).

The ^{13}C isotope ratio ($\delta^{13}C$), carbon content and nitrogen content were determined with a continuous-flow isotope-ratio mass spectrometer (Roboprep-CN) and Tracermass (Stable Isotope Analyser) (Europe Scientific, Crewe, UK.). All replicates were pooled before chemical analysis.

Calculations

Dry weight was analysed in an analysis of variance using the GENSTAT statistical package (Payne et al., 1987). To calculate the amount of ^{13}C-label in the different plant organs the dry weight and the ^{13}C atom excess of the labelled plants were used (Svejcar et al., 1990; Boutton, 1991). These results were expressed as a percentage of the total amount present at that harvest, in the whole plant or in a tiller. In this study this relative amount is used as a measure of the sink strength of a certain organ.

The contribution of photosynthesis during the periods between two $^{13}CO_2$ feedings to final seed carbon was estimated as follows. The distribution to the seeds of carbon assimilated during a growth interval was estimated from the distribution of the ^{13}C-label. The amount of ^{13}C-label present at final harvest in the seeds of the main tiller and in the spikelets of the group 2 tillers was expressed as a fraction of the total amount present in the whole shoot just after $^{13}CO_2$ feeding. In this way total respiratory losses were

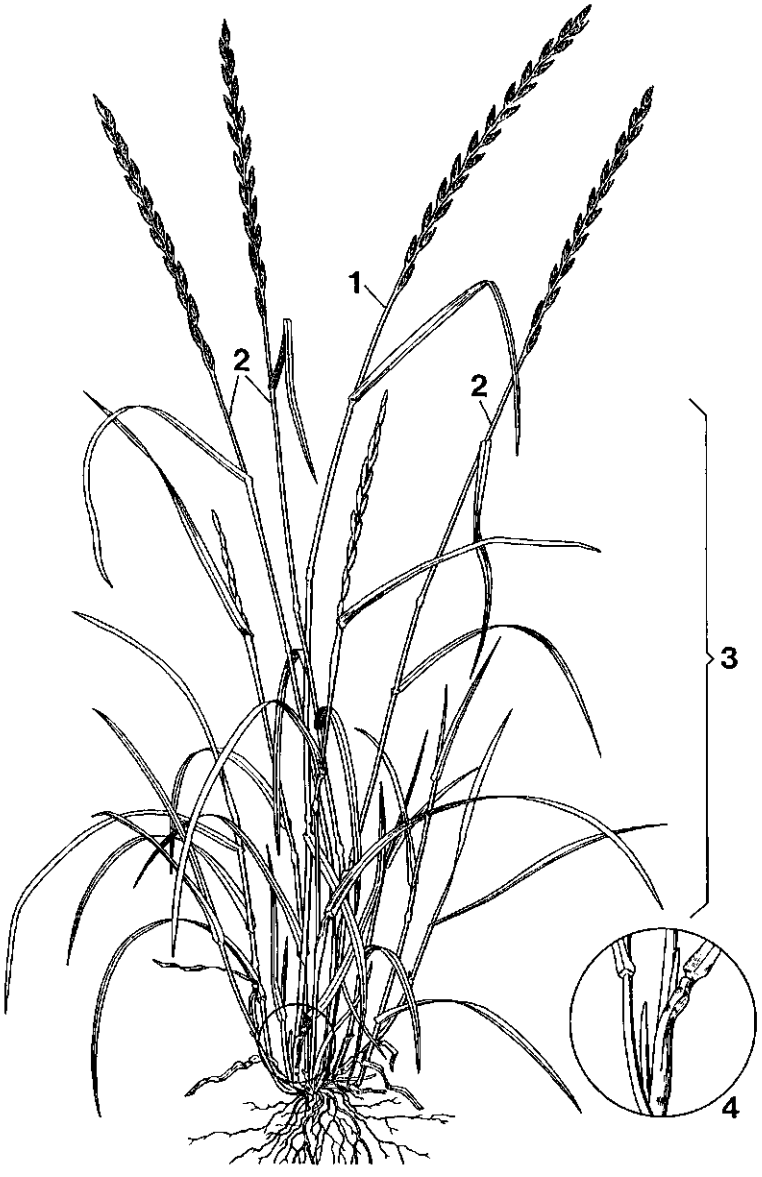

Figure 1. Representation of a flowering *Lolium perenne* plant, divided into four groups of tillers: 1) main tiller, 2) a homogeneous group of younger flowering tillers, 3) tillers with at least one fully emerged first leaf and 4) small tillers present inside the leaf sheaths of the older flowering tillers and tillers with a partly emerged first leaf.

accounted for. To characterise a growth interval the fractions of the two enclosing $^{13}CO_2$ feedings were averaged (FS). The increase in total plant carbon in the growth intervals (C) was determined from the total plant carbon curve fitted with a negative exponential equation. The product of C x FS provided an estimate of the contribution (g) of photosynthesis during that period to final seed carbon (Pate et al., 1980).

RESULTS

Tiller growth and development

Because spaced plants were used, a hierarchy of tillers of different ages developed. Each plant had on average 7.6 ± 2.2 group 2 tillers. The number of group 3 tillers increased from 16 ± 4 at the first harvest to 43 ± 14 at the final harvest. As group 4 tillers developed they were reclassified as group 3 tillers by the next harvest, and therefore the number of tillers decreased from 8 ± 4 to 0.6 ± 0.8 per plant. The main ear started to flower at 41 ± 1 days after the end of vernalisation, the ears in group two started to flower several days later. At final harvest 44 % of the group 3 tillers had an ear.

Figure 2 shows that total plant dry weight reached a plateau at 35 DAEE. The contribution of group 3 tillers to total plant dry weight increased from 25 % at main ear emergence to 64 % at final harvest. The dry weight of the group 4 tillers was negligible and decreased from 50 mg at main ear emergence to 4 mg at final harvest.

The main ear had 22 ± 1 spikelets and 212 ± 20 florets per ear. Seed set was 75 % and the seed yield per ear 223 ± 54 mg with an average seed dry weight of 1.4 ± 0.2 mg. The pattern of dry matter distribution within both the main tiller and the average group 2 tiller was similar, but the main tiller was heavier and some days advanced in development (Figure 3). At final harvest the relative contribution of the spikelets to total tiller dry weight was 31 % for the main tiller and 28 % for the average group 2 tiller. The corresponding figures for the contribution of the stem were 45 % and 46 %.

Water-soluble carbohydrates (WSC), starch and nitrogen

The distribution pattern of WSC and nitrogen amount were similar for both the main tiller and the group 2 tillers. Only the results of the main tiller are shown. From main ear emergence onwards the amount of WSC in the stem increased sharply (Figure 4). At final harvest the WSC concentration had risen to about 26 % in the main stem and 24 % in the

stems of the group 2 tillers. Between main ear emergence and the beginning of anthesis the concentration and amount of WSC in the leaves and the spikelets decreased. After onset of anthesis the amount of WSC in the leaves and the spikelets increased again. As the seeds started to accumulate starch the amount of WSC in the spikelets declined. The seeds in the main ear had a starch concentration of 30 % at 35 DAEE and 36 % at final harvest. The WSC content of the whole plant increased from 11 % at main ear emergence to 17 % at final harvest.

The amount of nitrogen decreased in all the main tiller organs except the spikelets. At final harvest 59 % of the nitrogen in the main tiller was located in the seeds, which had a nitrogen content of 2.1 %. Total plant nitrogen content fell from 2.7 % at main ear emergence to 1.0 % at final harvest.

Nitrogen was also redistributed between tiller groups. From main ear emergence until the end of flowering of the main ear, at 28 DAA, the fraction of nitrogen in the tillers of group 3 increased from 27 % to 67 % on a whole plant basis. The fraction of nitrogen in the main tiller fell from 17 % to 5 % and that in the tillers in group 2 fell from 54 % to 28 %.

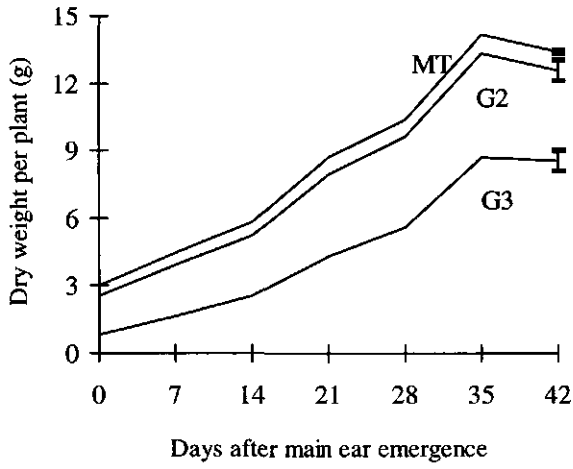

Figure 2. The cumulative dry weight of plants of *Lolium perenne*, divided into main tiller (MT), group 2 and group 3 tillers (see Figure 1). Vertical bars indicate LSD (0.05) values for a tiller group over time.

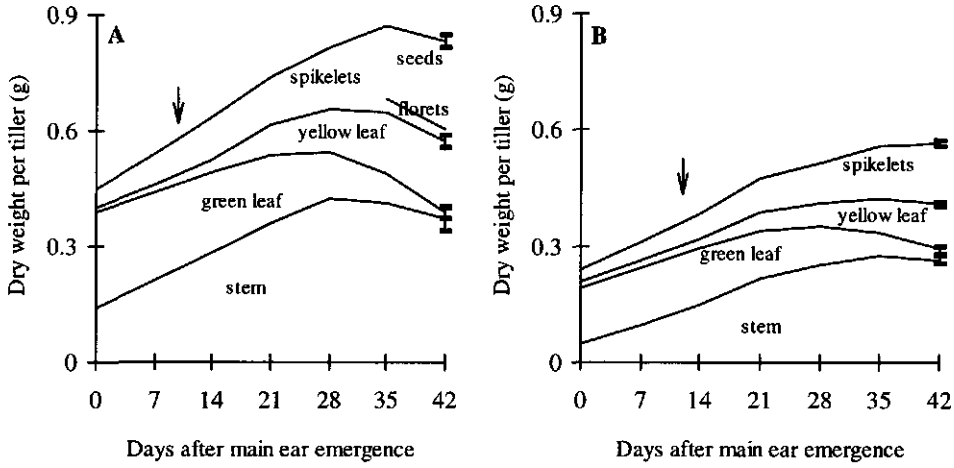

Figure 3. The cumulative dry weight of the main tiller (A) and of the average group 2 tiller (B) in plants of *Lolium perenne*. Onset of anthesis is indicated by the arrows. Vertical bars indicate LSD (0.05) values of the various plant organs over time.

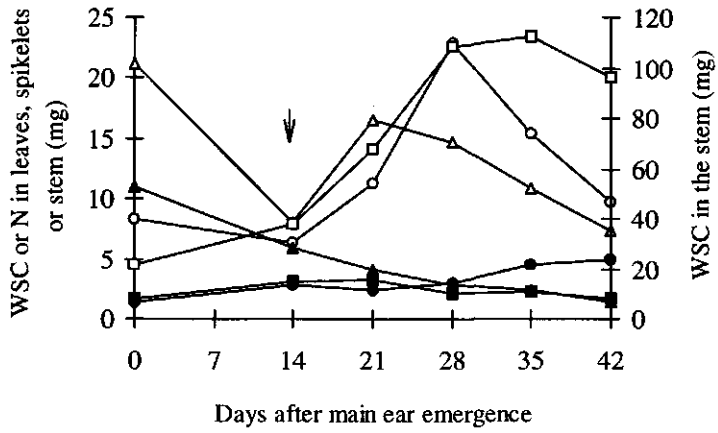

Figure 4. Changes in the amount of water-soluble carbohydrates, WSC (open symbols) and nitrogen (closed symbols) in the spikelets, stem and leaves of the main tiller over time after main ear emergence in plants of *Lolium perenne* (○, ●) spikelets; (□, ■) stem; (△, ▲) leaves. Arrow indicates onset of anthesis.

Fixation and distribution of ^{13}C-label between tiller groups

The absolute amount of ^{13}C-label fixed in ten minutes by the whole plant rose from 0.52 mg at main ear emergence to 0.98 mg at 29 DAEE and then declined to 0.6 mg at 43 DAEE. These values, in the same order, represent 0.9 %, 0.8 % and 0.5 % of the amount of label supplied.

Figure 5 shows the relative ^{13}C-fixation by the different tiller groups immediately after labelling for ten minutes and the relative distribution at final harvest after pulse-chase labelling for 1.5 - 2.5 h. The difference between the two lines gives information about the redistribution of label between the tiller groups. The fraction of total assimilated ^{13}C-label that was fixed by the main tiller and group 2 tillers decreased during plant development, whereas the fraction fixed by the group 3 tillers increased sharply (Figure 5). The small group 4 tillers fixed only traces of ^{13}C-label (< 1 %). These results roughly reflected the pattern of relative contributions to total plant dry weight of those tiller groups (Figure 2).

Net exchange of ^{13}C-label between the tiller groups occurred mainly after labelling at 15 and 22 DAEE, i.e. during anthesis. In the last two $^{13}CO_2$ feedings there was little net exchange of ^{13}C-label between the tiller groups (Figure 5).

Fixation and distribution of ^{13}C-label within the flowering tiller

In both the main tiller and the average group 2 tiller the fraction of ^{13}C-label fixed by the leaves declined sharply from more than 95 % at main ear emergence to 16 % at final harvest. The fraction of ^{13}C-label fixed by the stem increased steadily, while that of the ear rose to a plateau of 30-40 % after anthesis (Figure 6).

The relative distribution of ^{13}C-label within the main tiller and within the group 2 tillers was similar (Figure 7). The ^{13}C-label in the leaves declined sharply between each $^{13}CO_2$ feeding and final harvest both in relative and absolute terms. When the ^{13}C-label was fixed later in development, more was exported to the spikelets and less to the stem. But the distribution of ^{13}C-label fixed during early anthesis by the main tiller (Figure 7 left B) and by the group 2 tillers (Figure 7 right C) was in contrast to this trend. Then the leaves exported to the stem and not to the spikelets. From labelling at 28 DAEE onwards the leaves exported only to the spikelets.

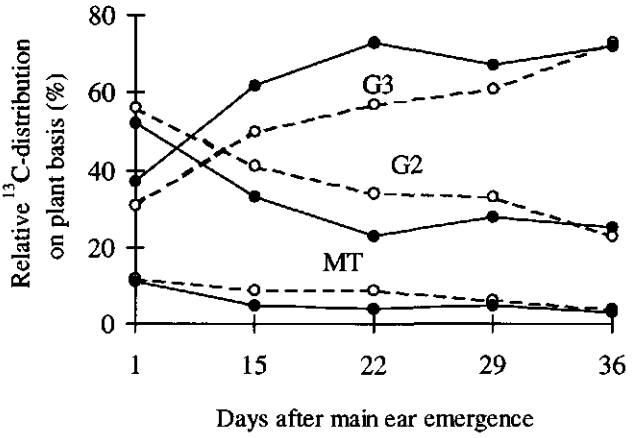

Figure 5. The relative fixation of ^{13}C-label after labelling for ten minutes (○) and the relative distribution at final harvest (42 DAEE) after labelling for 1.5 - 2.5 h (●) between groups of tillers in *Lolium perenne* plants. MT = main tiller, G2 = group 2 tillers, G3 = group 3 tillers. The time of labelling is indicated on the horizontal axis.

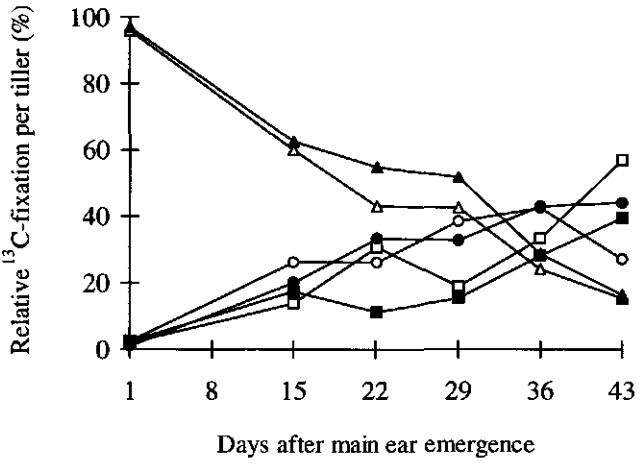

Figure 6. The relative fixation of ^{13}C-label by the ear, stem and leaves of the main tiller (open symbols) and the average group 2 tiller (closed symbols) in plants of *Lolium perenne* after labelling for 10 minutes. Moment of labelling in days after main ear emergence is indicated on the horizontal axis. (○, ●) spikelets; (□, ■) stem; (△, ▲)leaves.

Until about 28 DAEE the stem imported ^{13}C-label from the leaves. An increasing fraction of the ^{13}C-label fixed by the stem after 28 DAEE was exported to the spikelets. In the first $^{13}CO_2$ feeding some of the ^{13}C-label imported by the stem was redistributed after 28 DAEE to the spikelets. The fraction of ^{13}C-label in the stem decreased by about 10 % (Figure 7A).

Towards later stages of development label fixed by the stem and leaves was rapidly distributed to the spikelets. After labelling at 35 DAEE the fraction of label in the spikelets of the main tiller increased from 27 % to 83 % at final harvest one week later and in the group 2 tillers it increased from 35 % to 84 % (Figure 7E).

The distribution of ^{13}C-label at final harvest within the spikelets of the main tiller between empty florets and the seeds shifted towards the seeds when fixed later during development. The fraction of ^{13}C-label in the seeds, as a percentage of the total amount in the spikelets, increased from 72 % after labelling at main ear emergence to more than 96 % in the last $^{13}CO_2$ feeding.

Estimation of the contribution to seed carbon of photosynthesis at different growth stages

Table 2 shows the estimated contributions of current photosynthesis per week to final seed carbon. For the group 2 tillers the contribution to final carbon weight of the spikelets is presented, because in these ears seeds and empty florets were not separated. The contribution to final seed and spikelet carbon decreased slightly from approximately 9 % - 10 % to 7 % - 8 % during anthesis. After anthesis the contribution of photosynthesis to the seeds of the main ear and to the group 2 spikelets increased sharply. During the experiment photosynthesis contributed more to total carbon in the group 2 spikelets than to total carbon in the seeds of the main ear. This difference can largely be explained by taking into account that the contribution to the empty florets is similar to that found in the main ear.

Whole plant photosynthesis after main ear emergence contributed approximately 78 % to seed and spikelet carbon (Table 2). This means that about 22 % is accounted for by redistribution from carbon reserves accumulated before main ear emergence. The estimated pre-anthesis contribution was 42 %. This is, however, an over-estimation because the palea and lemma are present already at anthesis. They contribute about 26 % to total seed dry weight in the genotype used (Chapter 5). This means that only 16 % of the final seed and spikelet carbon was redistributed after anthesis.

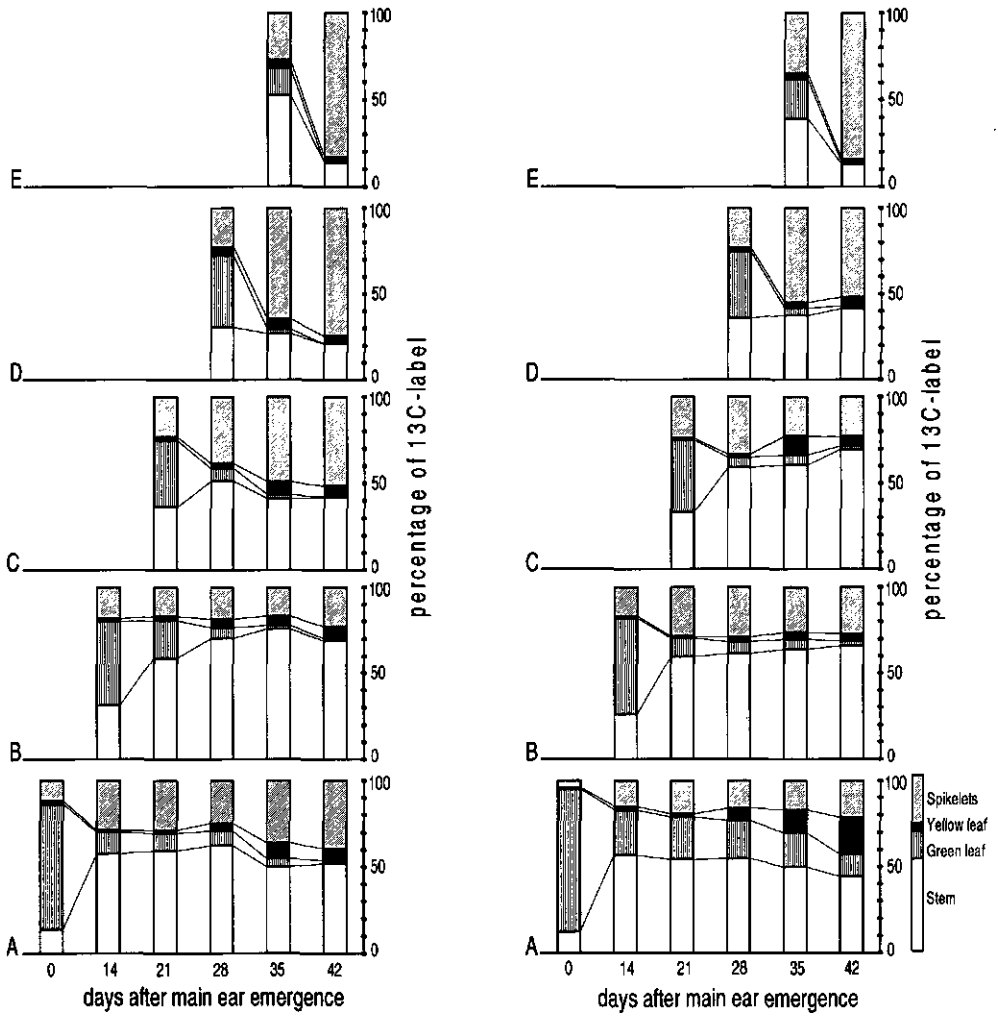

Figure 7. The relative distribution of ^{13}C-label within the main tiller (left) and the average group 2 tiller (right) of plants of *Lolium perenne*, between spikelets, green and yellow leaf and stem. Plants were pulse-labelled at 0 (A), 14 (B), 21 (C), 28 (D) and 35 (E) days after main ear emergence (DAEE) and harvested regularly up to the final harvest (42 DAEE), as indicated on the horizontal axis.

Table 2. Estimated contributions (%) to final seed carbon from current photosynthesis during different growth stages after main ear emergence, in plants of *Lolium perenne*. Contributions are estimated for the seeds in the main ear, for the group 2 spikelets (see Figure 1) and for the total of both.

	Contribution of photosynthesis to seed carbon (%)		
Growth stage, Days after main ear emergence	Seeds main ear	Spikelets group 2 tillers	Total
0-7	9	10	10
8-14	9	10	10
15-21	7	8	8
22-28	12	10	11
29-35	15	19	18
36-42	13	22	21
Pre-anthesis	53	41	42
Total after main ear emergence	65	79	78

DISCUSSION

Fixation of ^{13}C-label and its distribution between tiller groups

The increase in plant dry weight from main ear emergence onwards was mainly due to the increase in number and dry weight of the group 3 tillers (Figure 2). This pattern was reflected by the increasing source activity of these tillers as development proceeded (Figure 5).

During anthesis of the main tiller the group 3 tillers were a net sink (Figure 5), because of their increasing number, as the relative amount of ^{13}C-label per individual tiller hardly changed (data not shown). In the whole plant the fraction of ^{13}C-label in the group 3 tillers had increased by 12 - 16 % at final harvest compared with the percentage of label present immediately after labelling when the ^{13}C-label was fixed during anthesis. During the seed filling phase, after 28 DAEE, there was only a minor net exchange of ^{13}C-label between the tiller groups and tillers seemed mutually independent. The small, not fully developed group 4 tillers represented a negligible sink.

These results agree with Colvill & Marshall (1984), who found 16 % of the label to be located in the younger tillers at ripeness when fixed during anthesis. Clemence & Hebblethwaite (1984) however, found that the fraction of the assimilated label exported from the main tiller to the younger tillers within 24 h increased up to 24 % during seed development. And according to Ong et al. (1978b) the flowering tiller exports only traces of label at anthesis. Clemence & Hebblethwaite (1984) grew plants under high nitrogen availability compared with the other studies mentioned and lodging was severe (Marshall, 1985). This demonstrates that sink strength of the younger tillers is affected by external conditions.

Several authors (Hampton et al., 1983; Clemence & Hebblethwaite, 1984; Griffith, 1992) have suggested that younger tillers compete with the seeds for assimilates. This seems unlikely, given the large amount of carbohydrates in the stem (Figure 4; Spiertz & Ellen, 1972). Indeed, stem carbohydrate reserves even increased concomitantly with the large increase in number of group 3 tillers (Figures 2, 4).

Not only ^{13}C-label but also some nitrogen was redistributed from the flowering tillers to the group 3 tillers. The nitrogen content of the seeds (2.1 %) and of the whole shoot at ripeness (1.0 %) were comparable with results from field experiments (Spiertz & Ellen, 1972). Whether or not an increased sink strength of younger tillers (carbohydrates and/or nitrogen) can depress seed yields remains to be seen. Tillering and growing conditions need to be manipulated in order to investigate this.

Fixation and distribution of ^{13}C-label within the flowering tiller

The source activity of the leaves of both the main tiller and average group 2 tiller declined sharply after main ear emergence, while that of the ear and stem increased (Figure 6). The reduction in source activity of the leaves was due to an 80 - 90 % reduction in green leaf weight (Figure 3) that was accompanied by the nitrogen content of the remaining green leaf falling from 3.5 % to 1.1 %. Generally a positive correlation between leaf nitrogen content and carbon-assimilation rate has been found (Evans, 1989; Sinclair & Horie, 1989).

Compared to measurements in the field (Ong et al., 1978a) the source activity of the ear in these spaced plants was about 10 % units lower, while that of the stem was about 20 % units higher, probably because the stem parts experienced less mutual shading compared to a crop situation. In accordance with our results Ong et al. (1978a) and Clemence & Hebblethwaite (1984) found an increasing source activity of the ear during seed development as the leaves aged. The former found that the source activity of the ear decreases quickly at ripeness (Ong et al., 1978a); this agrees with results for the main tiller

shown in Figure 6. Our results confirm that the ear is the main source organ on the flowering tiller (Ong et al., 1978a; Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984).

Up to about 28 DAEE the stem remained a net sink organ (Figs 7 and 8). During anthesis the stem was a stronger sink than the spikelets. And as a consequence carbon from the leaves was imported by the stem and not by the spikelets. After anthesis the sink strength of the developing seeds increased and exceeded that of the stem. The stem then became a net source organ.

Assimilates exported from the leaves enter the stem, which will then either store them, use them for growth or export them. The increase in amount of WSC in the stem between ear emergence and anthesis, together with the decrease in the leaves and spikelets (Figure 4), indicates that the elongating stem used more assimilates than it produced. At that time the stem was still elongating and increasing in dry weight (Figure 3). During stem elongation the stem is the dominant sink on the flowering tiller (Ryle, 1970), whereas young seeds are a very weak sink.

Hendrix (1985) found a negative correlation between fructan content in the stem prior to anthesis and grain number in different wheat cultivars, and suggested that the stem and the developing ear compete for sucrose. The results reported here show that in *L. perenne* the elongating stem is a much stronger sink than the flowering ear. But it is not clear if this pattern of assimilate distribution reduces the number of seeds. Under reduced light intensity during stem elongation, and limited assimilate availability, the number of florets per spikelet is reduced (Ryle, 1967). However, abortion under non-stress conditions is thought to have genetic and cytological causes associated with outbreeding and not to be caused by competition for assimilates (Elgersma & Sniezko, 1988; Marshall & Ludlam, 1989).

From 28 DAEE onwards the sink strength of the stem decreased compared with that of the spikelets and some ^{13}C-label fixed at main ear emergence was redistributed to the spikelets (Figure 7A). Using a chase period of 24 h, Clemence & Hebblethwaite (1984) also found that the stem changed from a net sink to a net source organ during the seed filling phase. Our results confirm the role of the stem as a temporary storage organ (Colvill & Marshall, 1984; Griffith, 1992) that can support seed filling.

In contrast to WSC, nitrogen did not accumulate in the stem or the leaves but was translocated to the seeds in such a way that at final harvest most of the nitrogen in the flowering tiller was located in the seeds (Figure 4). Accumulation of nitrogen in seeds is thought to be regulated independently from that of carbon (Jenner, 1980; Swank et al., 1982).

The contribution of photosynthesis at different growth stages to seed carbon

The pattern of ^{13}C-distribution described above is reflected in the estimated contribution to seed carbon of photosynthesis at different growth stages. The contribution to final seed carbon of current photosynthates increased sharply during seed development. The exception being the contribution of photosynthates fixed during anthesis, when the stem was a stronger sink than the seeds (Table 2). ^{13}C-label fixed in the earlier stages of reproductive development was probably used more for structural material required in stem and leaf elongation, and not redistributed easily to the filling seeds.

Assimilates fixed before anthesis contributed about 16 % to final seed and spikelet carbon, when correcting for the palea and lemma that develop before anthesis and contribute 26 % of the final seed dry weight. Expressed on the basis of the caryopsis itself (seed without palea and lemma) this would mean a contribution of assimilates fixed before anthesis of about 22 %. In wheat and barley about 5 - 15 % is accounted for by carbohydrates stored prior to anthesis under non-stressed conditions. Wheat seeds are harvested without the palea and lemma but in barley the husk constitutes about 8 % of the final seed dry weight (Bidinger et al., 1977). The contribution of pre-anthesis assimilates to seed carbon seems somewhat higher in *L. perenne* than in wheat and barley.

Under conditions unfavourable for photosynthesis such as drought this contribution increases to 27 - 44 % (Bidinger, Musgrave & Fischer, 1977; Austin et al., 1980; Schnyder, 1993). Reducing the assimilate availability by shading the plant during seed filling led to a sharp fall in stem reserves in *L. perenne* but had a small effect on seed yield (Chapter 2). This indicates support from stem reserves to seed yield.

The relatively low utilisation of available stem reserves during seed filling indicates that seed yield potential is high and not limited by the availability of assimilates. The seeds do not seem to be able to use these reserves fully.

The results presented here have shown that the stem of the flowering tiller does not compete with the filling seeds, but contains large amounts of soluble sugars and exports assimilates as seed filling proceeds. The stem acts as a temporary storage organ. Whether or not increased development of younger tillers after anthesis is detrimental to seed filling needs to be investigated further.

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REFERENCES

AOAC, 1984.Williams S., (ed). Official methods of analyses of the association of official analytical chemists, The William Byrd Press Inc, Richmond, Virginia, USA.

Austin, R.B., C.L. Morgan, W.A. Ford & R.D. Blackwell, 1980.Contributions to grain yield from pre-anthesis assimilation in tall and dwarf barley phenotypes in two contrasting seasons. *Annals of Botany* 45: 309-319.

Bidinger, F., R.B. Musgrave & R.A. Fischer, 1977. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature* 270: 431-433.

Boutton, T.W., 1991. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In: Coleman D.C. & B. Fry (eds.) *Carbon isotope techniques*. San Diego, USA: Academic Press Inc, pp.155-171.

Clemence, T.G.A. & P.D. Hebblethwaite, 1984. An appraisal of ear, leaf and stem $^{14}CO_2$-assimilation, ^{14}C-assimilate distribution and growth in a reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Biology* 105: 319-327.

Clifford, P.E. & R.H.M. Langer, 1975. Pattern of control of distribution of ^{14}C-assimilates in reproductive plants of *Lolium multiflorum* Lam. var. Westerwoldicum. *Annals of Botany* 39: 403-411.

Colvill, K.E. & C. Marshall, 1984. Tiller dynamics and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology* 104: 543-557.

Elgersma, A., 1990. Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). *Plant Breeding* 105: 117-125.

Elgersma, A., & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S:59-68.

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9-19.

Farrar, J.F., 1992. The whole plant: carbon partitioning during development. In: Pollock C.J., J.F Farrar & A.J.Gordon (eds.) *Carbon partitioning within and between organisms*. Oxford: Bios Scientific Publishers, pp.163-179.

Farrar, J.F., 1993 (ed.). Sink strength: What is it and how do we measure it? Forum. *Plant, Cell and Environment* 16: 1013-1046.

Griffith, S.M., 1992. Changes in post-anthesis assimilates in stem and spike components of Italian ryegrass (*Lolium multiflorum* Lam.). I. Water soluble carbohydrates. *Annals of Botany* 69: 243-248.

Hampton, J.G., T.G.A.Clemence & P.D. Hebblethwaite, 1983. Nitrogen studies in *Lolium perenne* grown for seed. IV. Response of amenity types and influence of a growth regulator. *Grass and Forage Science* 38: 97-105.

Hebblethwaite, P.D., D. Wright & A. Noble, 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). In: Hebblethwaite P.D. (ed.) *Seed Production*. London: Butterworths, pp.71-90.

Hendrix, J.E., 1985. Partitioning of sucrose into fructans in winter wheat stems. In: Heath R.L.& J. Preiss (eds.) *Regulation of carbon partitioning in photosynthetic tissue. Proceedings of the Eight Annual Symposium in Plant Physiology*. Baltimore: Waverly Press, pp.273-288.

Ho, L.C., 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 355-378.

Jenner, C.F., 1980. Effects of shading or removing spikelets in wheat: testing assumptions. *Australian Journal of Plant Physiology* 7: 113-121.

Marshall, C., 1985. Developmental and physiological aspects of seed production in herbage grasses. *Journal of Applied Seed Production* 3: 43-49.

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in *Lolium perenne* L. *Annals of Botany* 63:19-27.

Ong, C.K., K.E. Colvill & C. Marshall, 1978a. Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L.. *Annals of Botany* 42: 855-862.

Ong, C.K., C. Marshall & G.R. Sagar, 1978b. The physiology of tiller death in grasses. 2.Causes of tiller death in a grass sward. *Journal of the British Grassland Society* 33: 205-211.

Parsons, A.J. & M.J. Robson, 1981. Seasonal changes in the physiology of S.24 perennial ryegrass (*Lolium perenne* L.). 3. Partitioning of assimilates between root and shoot during the transition from vegetative to reproductive growth. *Annals of Botany* 48: 733-744.

Pate, J.S., C.A. Atkins & M.W. Perry, 1980. Significance of photosynthate produced at different stages of growth as carbon source for fruit filling and seed reserve accumulation in *Lupinus angustifolius* L. *Australian Journal of Plant Physiology* 7: 283-297.

Payne, R.W., P.W. Lane, A.E. Ainsley, K.E. Bicknell, P.G.N. Digby, S.A. Harding, P.K. Leech, H.R. Simpson, A.D. Todd, P.J. Verrier & R.P. White, 1987. *Reference Manual.Genstat 5 Committee Rothamsted Experimental Station.* Oxford: Clarendon Press.

Ryle, G.J.A., 1967. Effects of shading on inflorescence size and development in temperate perennial grasses. *Annals of Applied Biology* 59: 297-308.

Ryle, G.J.A., 1970. Distribution patterns of assimilated ^{14}C in vegetative and reproductive shoots of *Lolium perenne* and *Lolium temulentum. Annals of Applied Biology* 66: 155-167.

Ryle, G.J.A., 1972. A quantitative analysis of the uptake of carbon and of the supply of ^{14}C-labelled assimilates to areas of meristematic growth in *Lolium temulentum. Annals of Botany* 36: 497-512.

Ryle, G.J.A. & C.E. Powell, 1972. The export and distribution of ^{14}C-labelled assimilates from each leaf on the shoot of *Lolium temulentum* during reproductive and vegetative growth. *Annals of Applied Biology* 36: 363-375.

Schnyder, H., 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling - a review. *New Phytologist* 123: 233-245.

Sinclair, T.R. & T. Horie, 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: A review. *Crop Science* 29: 90-98.

Spiertz, J.H.J. & J. Ellen, 1972. The effect of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. 'Cropper') and its effects on seed production. *Netherlands Journal of Agricultural Science* 20: 232-246.

Steiner, A.A., 1984. *The universal nutrient solution.* ISOSC Proceedings of the 6th International Congress on Soilless Culture. Wageningen, the Netherlands, pp. 633-649.

Svejcar, T.J., T.W. Boutton & J.D. Trent, 1990. Assessment of carbon allocation with stable carbon isotope labeling. *Agronomy Journal* 82: 18-21.

Swank, J.C., F.E. Below, R.J. Lambert & R.H. Hageman, 1982. Interaction of carbon and nitrogen metabolism in the productivity of maize. *Plant Physiology* 70: 1185-1190.

Yamagata, M., H. Kouchi & T. Yoneyama, 1987. Partitioning and utilization of photosynthate produced at different growth stages after anthesis in soybean (*Glycine max* L. Merr.): Analysis by long-term ^{13}C-labelling experiments. *Journal of Experimental Botany* 38: 1247-1259.

THE EFFECT OF NEW TILLER GROWTH ON CARBOHYDRATES, NITROGEN AND SEED YIELD PER EAR IN *LOLIUM PERENNE* L.

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ABSTRACT

To clarify whether new vegetative tillers that develop around anthesis in a *Lolium perenne* L. seed crop can depress seed yields, the possible competition for carbohydrates or nitrogen between the seeds and new tillers that develop after the onset of anthesis was investigated. In two greenhouse experiments the number of tillers per plant was varied by a combination of cutting, nitrogen supply, light quality and light intensity treatments. Two genotypes with different tillering rates were used. Seed yield per ear was largely independent of the number of tillers and regrowth of cut tillers after the onset of anthesis. It increased in one genotype, but only under low light and a reduced nutrient availability, and no new tillers were produced. The amount of water-soluble carbohydrates in the reproductive tillers increased in both clones under these conditions. Under more favourable conditions the increased tillering rate and regrowth of tillers after cutting did not adversely affect seed yield per ear in either clone, although carbohydrate reserves in the flowering tillers were sharply reduced. Tiller removal increased the concentration and amount of nitrogen in the remaining flowering tillers, irrespective of the amount of regrowth. It is concluded that competition for carbohydrates or nitrogen between the seeds and new vegetative tillers that develop after the onset of anthesis, is not a major cause of the low and variable seed yields in *L. perenne* seed crops. Processes within the ear itself are probably limiting.

INTRODUCTION

Competition for assimilates between seeds and new vegetative tillers has been suggested as a major cause of the low and variable seed yield in *Lolium perenne* L. seed crops (Hampton et al., 1983; Clemence & Hebblethwaite, 1984). Around the time of anthesis the tillering rate in *L. perenne* seed crops may increase, because of the plant's perennial habit (Hebblethwaite et al., 1980; Parsons & Robson, 1981; Elgersma, 1990). Growth of new vegetative tillers is enhanced by abundant water or nitrogen (Hampton & Hebblethwaite, 1984; Meijer & Vreeke, 1988). At supra-optimal nitrogen fertilisation rates the number of tillers increases and lodging is more severe (Hebblethwaite & Ivins, 1977,1978), whereas seed yield does not increase and may even fall (Hampton et al., 1983; Hampton & Hebblethwaite, 1984).

In field crops the increase in number of new vegetative tillers is associated with lodging, and this makes it difficult to separate the effect of both factors on seed yield. Hampton et al. (1987) found that the seed yield of a field crop, supported mechanically to prevent lodging, was higher than that of a lodged crop. The carbon export from the flag leaf to the ear increased in the supported crop, but the number of vegetative tillers and the carbon export from the other leaves to these tillers was not reduced. In general, field experiments (Spiertz & Ellen, 1972; Hebblethwaite & Ivins, 1977; Hebblethwaite & Burbidge, 1976; Wright & Hebblethwaite, 1979; Hampton et al., 1983; Hampton & Hebblethwaite, 1984; Hampton & Hebblethwaite, 1985; Elgersma, 1990) show no correlation or only very weak negative correlation between the number of vegetative tillers at final harvest and seed yield. It is unclear whether there is a causal relationship between increased new tiller growth and reduced seed growth. To investigate this, tillering has to be manipulated.

If there is competition between new vegetative tillers and seed growth, both carbohydrates and nitrogen may be competed for. Every new tiller emerging from the leaf sheath is initially wholly dependent on carbohydrate from the main tiller. This dependence declines as it starts to increase its leaf area and rate of photosynthesis (Colvill & Marshall, 1981; Clemence & Hebblethwaite, 1984; Marshall, 1990). The stem of *L. perenne* contains a large amount of water-soluble carbohydrates (Spiertz & Ellen, 1972; Wilman & Altimimi, 1982), sufficient to support seed growth (Chapter 2). Competition for carbohydrates therefore seems less likely in this species.

A new tiller will remain dependent on older rooted tillers for water and elements such as nitrogen, until it has developed sufficient roots itself. But it is not known at what moment rooted tillers become independent from older tillers for nitrogen (Marshall, 1990). In a previous experiment with spaced plants (Chapter 3) it was found that during anthesis the relative amount of nitrogen on plant basis decreased in the older flowering

tillers and increased in the younger tillers. This suggests nitrogen is exported from the older tillers to the younger tillers.

The objectives of the study described in this paper were to investigate the effect of the number of tillers produced after onset of anthesis on seed yield and dry matter distribution within the flowering tiller, and also to find out whether or not competition for carbohydrates or nitrogen occurs between tiller growth and seeds.

MATERIALS AND METHODS

Because it is difficult to manipulate tiller numbers in the field, two greenhouse experiments were conducted in which tiller number was varied at the onset of anthesis. Clones of *Lolium perenne* L. were used, to reduce the variability between plants. In a preliminary experiment two clones, differing in tillering rate and seed number per ear, were selected. The first was selected from the Dutch cultivar Barlet (clone B1) and the second from the Dutch cultivar Magella (clone Mg2). Clone B1 had a higher tillering rate and more seeds per ear than clone Mg2. Vegetative tillers of these clones were propagated on Steiner nutrient solution (Steiner, 1984) in the greenhouse at 17 h daylength and at a day temperature of 20 °C and a night temperature of 17 °C.

To obtain flowering tillers, one tiller per pot (main tiller) was vernalised for 14 weeks at 7 ± 1°C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. In an earlier experiment (unpublished) it was established that this procedure ensured complete vernalisation. After vernalisation plants were transferred to a greenhouse at 15°C with the shading screen down and allowed to acclimatise for a week. They were then put in 1.75 l pots in peaty soil. Natural daylength was extended to 17 h with incandescent light (approximately 1.7 W/m^2, PAR).

Experiment 1

On 1 October 1993 vernalisation was completed and plants were transferred to the greenhouse. Per plant 180 mg N, 110 mg P_2O_5 and 180 mg K_2O was supplied. For 12 h per day lighting was supplemented with high pressure sodium lamps (Philips, AGRO SON-T, 400 W). The average daily incoming radiation, including supplemental lighting, was 1.5 MJ/m^2 (400 - 700 nm) and decreased towards final harvest. Average day and night temperatures were respectively 20°C (12 h, 6.30 -18.30 h) and 17°C.

The following two treatments, starting at the onset of anthesis, were combined. In the first treatment all "younger" tillers on a plant were either cut off or left untreated. These

younger tillers comprised all tillers categorised as group three and group four tillers (Chapter 3 figure 1). The second treatment consisted of either: 1) stimulating tillering by placing diodes emitting red light around the base of the plant, 2) inhibiting tillering by placing a blue and amber filter around the base of the plant and 3) no treatment.

The diodes emitting red light (Nijssen, Leiden, the Netherlands) can stimulate tillering by increasing the red : far red ratio of the light (Deregibus et al., 1985). The diodes were kept on 15 minutes longer than the daylight extension. The 15 cm high blue and amber filter (Cinelux, Compact sales, Amsterdam, the Netherlands) was expected to inhibit tillering by reducing the light intensity and the red : far red ratio around the plant base. Combinations of treatments 1 and 2 resulted in six treatments. The plants were grown in a randomised block design with eight replicates.

Three other genotypes were placed in the border rows and used as pollinators. The clones were grown as spaced plants to increase tillering and possible competition between seeds and tillers. Excluding the border rows plant density was approximately 21 per m^2. Iron wire was used to prevent the flowering tillers from lodging.

Experiment 2

The vernalised plants were placed in the greenhouse on 22 March 1994. Because incoming radiation and subsequent plant growth were expected to be higher in experiment 2 than in experiment 1, nutrient supply was increased to: 290 mg N, 240 mg P_2O_5 and 580 mg K_2O per plant, by adding Steiner nutrient solution. In order to further enhance tillering, 100 mg N per plant was added as NH_4NO_3 solution to half of the plants at onset of anthesis. Thus two nitrogen levels (290 mg N and 390 mg N) were established. Treatment 1 and treatment 2 were applied as in experiment 1 and the experiment was carried out in four replicates. The average daily incoming radiation was 2.8 MJ/m^2 (400 - 700 nm) without supplemental lighting. The average day temperature was 21°C (12 h, 6.30 -18.30 h) and the average night temperatures was 17°C.

In both experiments, at the onset of anthesis of the main tiller a homogeneous group (in terms of size and development) of younger flowering tillers, the group 2 tillers (Chapter 3 figure 1) was selected. The main tiller and group 2 tillers were harvested at 30 days after onset of anthesis of the main tiller and dissected into: stem, leaves, rachis, seeds and empty florets + glumes. Before the number of seeds and empty florets were counted under a binocular microscope, the ears had been stored at - 20 °C. Small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret. The number of younger tillers (groups 3 and 4) per plant was counted. The dry

weight of the various plant parts was determined after drying at 70 °C for 48 h. Roots were not harvested.

All plant material of the same organ was pooled to two replicates prior to conducting chemical analyses. For the determination of water-soluble carbohydrates (WSC) the plant parts were ground and extracted in water at 100 °C for 15 minutes. The total amount of reducing sugars was measured colorimetrically after hydrolysis at 90°C in sulphuric acid (0.45 N) with an automatic analysis device (Bran & Lübbe Analyzing Technologies, Inc., Elmsford, N.Y., USA). This method, which was validated for *L. perenne* tissue, ensured complete extraction and hydrolysis of the water-soluble carbohydrates. Nitrogen content was determined according to the Dumas method (Macro N, Heraus, Hanau, Germany).

Analyses of variance and linear regression were carried out with the GENSTAT statistical package (Payne et al., 1987). Regression analysis was used to analyse the effect of the number of younger tillers and of their total dry weight on the seed yield per ear, stem and leaf dry weight of the main tiller and of the group 2 tillers. Seed set percentage was analysed by a generalised linear regression model, with a logit link function and a binomial distribution (McCullagh and Nelder, 1989). Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Plant development and tillering

The number of group 2 tillers selected varied between clones and experiments. In clone B1 6.7 group 2 tillers were selected on average in the first experiment and 3.6 in the second experiment. The equivalent numbers for clone Mg2 were 4.3 and 0.4. In experiment 2 there was a larger difference in development rate between the main tiller and the group 2 tillers in clone Mg2. In both experiments regression analysis revealed that the number of selected group 2 tillers was not related to seed yield per ear or to dry matter distribution within these tillers, indicating homogeneity within this group.

There were large differences in tillering rate and final dry weight of the younger tillers between the two experiments (Table 1). The plants in experiment 1 were grown under low light and lower nutrient availability and had produced 74 % fewer tillers with a 70 % lower total dry weight at final harvest than in experiment 2. As expected clone B1 had produced more younger tillers and more dry weight in younger tillers before anthesis than clone Mg2 in both experiments. But after onset of anthesis the tillering rate of clone Mg2

Table 1. The total number and dry weight of younger tillers of a *Lolium perenne* plant at the onset of anthesis and at final harvest. The number and dry weight at final harvest are expressed as a percentage of the number and dry weight at the onset of anthesis. Measurements were performed on control plants and on plants whose younger tillers were cut off at the onset of anthesis of the main flowering tiller. Different letters indicate significant differences within each column and experiment ($P<0.05$).

				Younger tillers at the onset of anthesis		Younger tillers at final harvest, % of number and dry weight at the onset of anthesis	
Experiment	Tiller treatment	Nitrogen (mg/plant)	Clone	Number per plant	Dry weight per plant (g)	Number (%)	Dry weight (%)
1	None	180	B1	31.0 b	3.9 b	94 b	165 c
	Cut	180				30 a	3.6 a
	None	180	Mg2	20.3 a	2.6 a	110 b	233 d
	Cut	180				127 c	33 b
2	None	290	B1	87.4 b	8.4 b	114 b	239 c
		390				124 b	272 c
	Cut	290				89 a	33 a
		390				123 b	68 a
	None	290	Mg2	35.3 a	3.1 a	209 d	486 d
		390				267 e	504 d
	Cut	290				110 ab	122 b
		390				155 c	170 b

was higher than that of clone B1 (Table 1). In the first experiment in clone B1 some younger tillers died after anthesis. On average the nitrogen added at onset of anthesis in experiment 2 increased final number and total dry weight of younger tillers per plant statistically significantly.

As expected, cutting off the younger tillers significantly reduced final dry weight and number of younger tillers per plant in both experiments (Table 1). Exceptions with regard to the final number of younger tillers were clone Mg2 in experiment 1 and clone B1 at high nitrogen in experiment 2.

Table 2. The total number and dry weight of younger tillers of a *Lolium perenne* plant at the onset of anthesis and at final harvest. The number and dry weight at final harvest are expressed as a percentage of the number and dry weight at the onset of anthesis. Cutting off all younger tillers (treatment 1) was combined with either a stimulation of tillering with diodes or an inhibition of tillering with a filter placed around the plant base (treatment 2). Treatments were started at onset of anthesis. Values are the average of two clones and in experiment 2 the average of two nitrogen levels. Different letters indicate significant differences within each column and experiment ($P<0.05$).

			Younger tillers at the onset of anthesis		Younger tillers at final harvest, % of number and dry weight at the onset of anthesis	
Experiment	Tiller treatment 1	Tiller treatment 2	Number per plant	Dry weight per plant (g)	Number (%)	Dry weight (%)
1	None	None	25.7	3.3	104 b	191 b
		Diodes			100 b	196 b
		Filter			103 b	210 b
	Cut	None			68 a	16 a
		Diodes			110 b	24 a
		Filter			57 a	15 a
2	None	None	61.3	5.7	205 d	389 c
		Diodes			189 c	370 c
		Filter			142 b	367 c
	Cut	None			133 b	105 ab
		Diodes			137 b	121 b
		Filter			88 a	75 a

Compared to cutting off the younger tillers, the second tiller treatment had a much smaller effect on tillering (Table 2). In the first experiment the diodes increased tiller number but only after the younger tillers had been cut off first. The final dry weight of the younger tillers was not influenced. In the second experiment the blue and amber filter inhibited the final number of younger tillers. The diodes slightly inhibited tiller number in the plants were the younger tillers were not cut off. The final dry weight of the younger tillers in experiment 2 was reduced by the blue and amber filter after the younger tillers had been cut off first.

Seed yield per ear and dry matter distribution

In the first experiment the seed yield per ear in clone B1 increased with a decreasing number of younger tillers per plant. A reduction from 30 to 4 younger tillers per plant at final harvest increased seed yield by 11 % in the main ear and by 19 % in the ears in group 2 (Figure 2A). The increase in seed yield in the main ear was caused by the average seed dry weight increasing from 1.4 to 1.5 mg and seed set increasing from 75 % to 78 %. In the group 2 ears the average seed dry weight increased from 1.2 to 1.4 mg. Seed set was not determined in the group 2 ears but the 19 % increase in seed yield per ear (Figure 2A) and the 17 % increase in average seed dry weight implies 2 % more seeds per ear.

The seed yield per ear in clone Mg2 was not affected by the number of younger tillers in the first experiment (Figure 2A). Average seed dry weight in clone Mg2 was 2.6 ± 0.1 mg in the main ear and 2.4 ± 0.1 mg in the group 2 ears. Seed set in the main ear was 46 % on average.

Reducing the number of younger tillers increased the stem + rachis dry weight in clone B1 by 11 % in the main tiller and by 16 % in the group 2 tillers. In contrast, clone Mg2 was unaffected by tiller removal (Figure 2B). The total leaf dry weight of a flowering tiller was not influenced by the number of younger tillers per plant. Similar relations to those mentioned above were found between the total dry weight of the younger tillers and seed yield per ear, stem + rachis and total leaf dry weight (data not shown).

In the second experiment no relationship was found between the number of younger tillers per plant and seed yield per ear in both clones (Figure 3A). Table 3 shows the yield components for both clones in the second experiment. The differences between the two clones were statistically significant. The extra nitrogen added at the onset of anthesis in the second experiment did not influence seed yield per ear, average seed weight or seed set in either clone.

Stem + rachis dry weight in clone B1 was not influenced by a decreasing number of younger tillers per plant in experiment 2. But in clone Mg2 the stem + rachis dry weight fell by 26 % in the main tiller and by 21 % in the tillers in group 2 (Figure 3B). Similar relations with stem + rachis dry weight were found for total dry weight of younger tillers (data not shown). In both clones the total leaf dry weight per flowering tiller did not relate to the number of younger tillers, but increased with decreasing total dry weight of the younger tillers (data not shown).

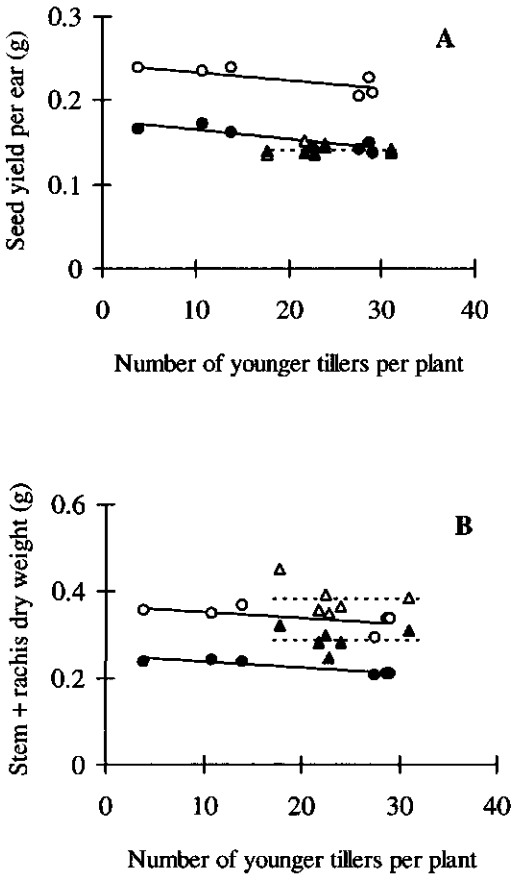

Figure 2. The effect of the number of younger tillers per plant on A) seed yield per ear and B) stem + rachis dry weight in spaced plants of *Lolium perenne* in experiment 1. If the number of tillers has a statistically significant effect the line obtained through linear regression is indicated by a solid line. Otherwise the average is indicated by a dotted line. In clone Mg2 the average seed yield per ear in the main tiller and the group 2 tillers was the same. Points are the averages of the treatments (n=8). (○) clone B1, main tiller; (●) clone B1, group 2 tillers; (△) clone Mg2, main tiller; (▲) clone Mg2, group 2 tillers.

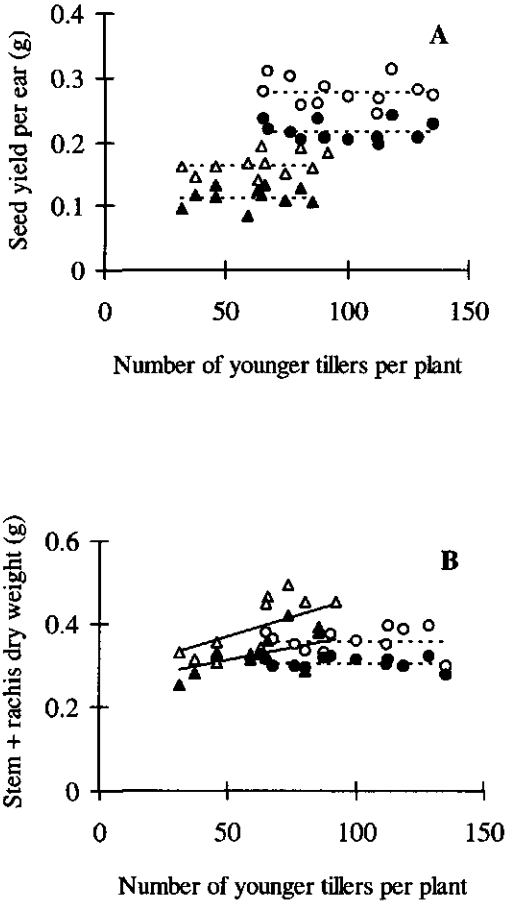

Figure 3. The effect of the number of younger tillers per plant on A) seed yield per ear and B) stem + rachis dry weight in spaced plants of *Lolium perenne* in experiment 2. If the number of tillers has a statistically significant effect the line obtained through linear regression is indicated by a solid line. Otherwise the average is indicated by a dotted line. Points are the averages of the treatments (n=4). (○) clone B1, main tiller; (●) clone B1, group 2 tillers; (△) clone Mg2, main tiller; (▲) clone Mg2, group 2 tillers.

In experiment 2 the more favourable growing conditions resulted in the seed yield per ear in clone B1 being 32 % higher on average than in experiment 1. In clone Mg2 the seed yield per ear was 15 % higher in the main ear but it was 18 % lower in the group 2 tillers, as the group 2 tillers selected in the second experiment were younger and flowered relatively late compared to the main tiller. Nevertheless the similar reaction to the treatments found in the seed yield per ear and dry matter distribution in the main tiller and the tillers in group 2 is striking.

Table 3. Seed yield and seed yield components of the main flowering tiller and a group of younger flowering tillers in the two *Lolium perenne* clones. Experiment 2.

	Seed yield per ear (mg)	Average seed dry weight (mg)	Seed number per ear	Floret number per ear	Seed set (%)
Main tiller					
Clone B1	278.1	1.6	172.3	246.4	69.8
Clone Mg2	165.4	2.6	61.4	152.0	40.3
Statistical significance	***	***	***	***	***
Group 2 tiller					
Clone B1	217.0	1.5	144.4	212.6	68.0
Clone Mg2	114.8	2.4	46.7	145.9	32.0
Statistical significance	***	***	***	***	***

*** $P \leq 0.001$

Water-soluble carbohydrates (WSC)

Cutting off the younger tillers at the onset of anthesis resulted in diametrically opposite effects in the two experiments. In the first experiment (low light and lower nutrient availability) the amount of WSC at final harvest in the remaining flowering tillers increased (Table 4). In the second experiment, in which conditions were more favourable, the final WSC amount in the remaining flowering tillers was reduced, although this was not statistically significant in clone B1. As expected, removing the younger tillers reduced the WSC amount present in younger tillers and in the total plant shoot at final harvest.

In both experiments the changes in the amount of WSC in the flowering tillers were mainly due to changes in the amount of WSC in the stem (Table 5). In the first experiment the increase in WSC amount after tiller removal was larger in clone B1 than in clone Mg2.

The concentration of WSC in the stem of the main tiller of clone B1 increased by 63 % after tiller removal and by as much as 76 % in the stem of the group 2 tillers. The WSC amount in the seeds increased in clone B1, but not in clone Mg2.

Table 4. The amount (mg) of water-soluble carbohydrates (WSC) and nitrogen in different groups of tillers of *Lolium perenne* plants. Measurements were performed at 30 days after the onset of anthesis of the main tiller. Tiller treatment consisted of cutting off the younger tillers at the onset of anthesis of the main tiller. Different letters indicate significant differences within each column within an experiment ($P<0.05$).

			Water-soluble carbohydrates (mg)				
Experiment	Tiller treatment	Clone	Main tiller	Average group 2 tiller	Younger tillers	Total plant shoot	Total plant shoot plus removed younger tillers
1	None	B1	60 a	37 a	618 b	891 a	891 a
		Mg2	118 c	72 b	1241 c	1696 b	1696 b
	Cut	B1	90 b	59 b	22 a	618 a	1007 a
		Mg2	145 d	109 c	192 a	812 a	1150 ab
2	None	B1	119 b	106 a	3574 b	4047 b	4047 c
		Mg2	193 c	-	3495 b	3714 b	3714 c
	Cut	B1	106 b	90 a	832 a	1269 a	2206 b
		Mg2	69 a	-	524 a	619 a	859 a
			Nitrogen (mg)				
Experiment	Tiller treatment	Clone	Main tiller	Average group 2 tiller	Younger tillers	Total plant shoot	Total plant shoot plus removed younger tillers
1	None	B1	7.7 a	4.9 a	63 b	107 b	107 ab
		Mg2	7.7 a	6.5 b	52 b	89 b	89 a
	Cut	B1	8.9 b	6.7 bc	1 a	45 a	120 b
		Mg2	8.8 b	7.1 c	12 a	53 a	104 a
2	None	B1	11.2 a	8.8 a	249 c	290 b	290 ab
		Mg2	13.1 b	-	248 c	263 b	263 a
	Cut	B1	13.3 bc	10.4 b	70 a	122 a	330 b
		Mg2	14.2 c	-	121 b	139 a	256 a

Table 5. The effect of cutting off the younger tillers at the onset of anthesis in spaced plants of *Lolium perenne* on the distribution of water-soluble carbohydrates (mg) in the main reproductive tiller. Experiment 1 was conducted under low light and nutrient availability, experiment 2 under more optimal conditions. Asterisks indicate a statistically significant effect of cutting ($P<0.05$).

		Water-soluble carbohydrates (mg)			
		Clone B1		Clone Mg2	
Experiment	Tiller organ	None	Cut	None	Cut
1	Seeds	5.7	7.2 *	3.9	4.0
	Empty florets + glumes	0.8	0.9	1.7	2.3
	Stem + rachis	41.6	74.6 *	104.7	128.5 *
	Leaves	11.7	7.6	7.9	10.0
2	Seeds	8.5	9.1	6.6	5.5 *
	Empty florets + glumes	2.9	3.0	3.3	3.0
	Stem + rachis	95.5	79.6	167.0	40.3 *
	Leaves	13.7	15.5	15.0	19.1

In the second experiment the WSC amount fell more sharply after tiller removal in clone Mg2 than in clone B1 (Table 4, Table 5). The WSC amount in the stem decreased by 77 % in clone Mg2, because the WSC concentration decreased by 69 %. The WSC amount in the seeds in clone Mg2 also decreased, although the WSC concentration remained 3.6 %. In clone B1, the changes in the WSC amount in the stem + rachis and leaves of the main tiller brought about by tiller removal were not statistically significant, but similar changes in the group 2 tillers were (data not shown).

Linear regression indicated that the significant changes in stem + rachis dry weight of the main tiller (Figure 2B & 3B) could largely be explained by changes in WSC concentration in the stem (Exp. 1, R^2adj. = 53 %; Exp. 2, R^2adj.= 76 %).

Nitrogen

As the effects of tiller removal were similar for both nitrogen levels in the second experiment, the average of both levels is presented in Table 4. In both experiments the amount of nitrogen in the flowering tillers at final harvest increased after the younger tillers were cut off at onset of anthesis. Because of the strongly reduced final dry weight of

younger tillers after cutting them at anthesis (Table 1, Table 2), also the nitrogen amount in these tillers was reduced. The difference in nitrogen amount in the total plant shoot between control and cut plants disappeared when the younger tillers removed were taken into account. This shows that total nitrogen uptake by the plant shoot did not differ between cut and control plants.

In the first experiment tiller removal significantly increased nitrogen amount at final harvest in the stem + rachis and seeds in both clones (Table 6). Nitrogen concentration increased from 0.35 % to 0.39 % in the stem + rachis and from 2.4 % to 2.7 % in the seeds, averaged for the two clones. In the second experiment nitrogen concentration increased from 0.61 % to 0.95 % in the stem + rachis and from 1.37 % to 1.74 % in the leaves. In the seeds in clone B1 the nitrogen amount increased, but in clone Mg2 it decreased after tiller removal (Table 6). The concentration of nitrogen in the seeds of clone B1 remained 2.2 %, but in clone Mg2 decreased from 2.8 % to 2.6 %.

Table 6. The effect of cutting off the younger tillers at the onset of anthesis in spaced plants of *Lolium perenne* L. on the distribution of nitrogen (mg) in the main reproductive tiller. Experiment 1 was conducted under low light and nutrient availability, experiment 2 under more optimal conditions. Asterisks indicate a statistically significant effect of cutting ($P<0.05$).

		Nitrogen (mg)			
		Clone B1		Clone Mg2	
Experiment	Tiller organ	None	Cut	None	Cut
1	Seeds	4.3	5.4 *	3.9	4.3 *
	Empty florets + glumes	0.3	0.2	0.4	0.5
	Stem + rachis	1.2	1.6 *	1.7	1.9 *
	Leaves	2	1.8	1.9	2.1 *
2	Seeds	6.0	6.8 *	5.2	4.1 *
	Empty florets + glumes	0.7	0.8	0.7	0.7
	Stem + rachis	2.0	2.7 *	3.6	4.4 *
	Leaves	2.2	3.0 *	3.5	4.9 *

DISCUSSION

The different experimental conditions in light and nutrient status led to large differences in tillering between the two experiments (Table 1, Figure 2, Figure 3). Growth of new tillers (Colvill & Marshall, 1981; Marshall, 1990) and regrowth of cut tillers (Davies, 1988) requires carbohydrate support from the older tillers. If nitrogen availability and light intensity are reduced, the export of carbohydrates from the main tiller to new tillers declines (Ryle & Powell, 1976; Powell & Ryle, 1978). A low nitrogen supply also reduces regrowth of the leaf laminae of cut tillers (Millard et al., 1990). This would explain why the tillering rate and regrowth of cut younger tillers were less in experiment 1 than in experiment 2.

Seed yield per ear was largely independent of the number and regrowth of younger tillers after the onset of anthesis. Under field conditions far fewer younger tillers develop per flowering tiller than in spaced plants (Spiertz & Ellen, 1972; Hebblethwaite & Ivins, 1977; Hebblethwaite & Burbidge, 1976; Wright & Hebblethwaite, 1979; Hampton et al., 1983; Hampton & Hebblethwaite, 1984; Hampton & Hebblethwaite, 1985; Elgersma, 1990). It seems that growth of new vegetative tillers after onset of anthesis cannot be a major cause of the low and variable seed yield of *L. perenne* seed crops. Only under the low light and reduced nutrient conditions in the first experiment in clone B1 was seed yield per ear increased (by 11 % - 19 %) after cutting off the younger tillers at anthesis (Figure 2).

In the first experiment the final WSC amount in the remaining flowering tillers was also increased after the cutting treatment (Table 4). The demand for WSC was reduced after cutting, because regrowth of younger tillers was very weak in experiment 1. But cutting might also boost the production of WSC. It increased the amount and concentration of nitrogen in the remaining flowering tillers in both experiments (Table 4, Table 6) and, as nitrogen concentration is strongly related to photosynthetic rate (Evans, 1989; Sinclair & Horie, 1989), this could have caused the increase in the amount of WSC. Furthermore, removal of the younger tillers will have reduced shading of the lower stem and leaves of the flowering tillers. However, these parts have a low nitrogen content (Wilman & Altimimi, 1982) and low photosynthetic activity.

From experiment 1 it is clear that the two clones distributed the increase in WSC amount differently between their organs. Clone B1 distributed more carbohydrates to the seeds, thereby increasing seed yield per ear (Figure 2A). Tillering in clone B1 stopped after anthesis (Table 2) and regrowth of cut younger tillers was very weak. However, only part of the increment in WSC amount was used for seed growth. The WSC reserves in the stem also increased (Table 5), resulting in a higher stem + rachis dry weight (Figure 2B). In clone Mg2 tillering and regrowth of younger tillers after anthesis demanded more

carbohydrates than in clone B1 and seed yield per ear and stem + rachis dry weight did not increase.

Under the more favourable conditions in experiment 2 the regrowth of younger tillers was stronger (Table 1) and demanded more assimilates from the remaining flowering tillers. Again, tillering and regrowth after anthesis were stronger in clone Mg2, associated with a sharper decrease in WSC reserves compared to clone B1 (Table 4, Table 5). Stem + rachis dry weight declined strongly after tiller removal in clone Mg2. Yet seed yield per ear was not affected in either clone (Figure 3).

In the second experiment the amount of WSC at final harvest present in the stem, rachis and leaves of the main tiller after cutting (Table 5) could have supported 24 % extra seed yield per ear in clone B1 and 28 % extra seed yield per ear in clone Mg2, after correcting for growth respiration of the seeds (Penning de Vries et al., 1983). Although seeds and younger tillers draw on the same WSC reserves, under more favourable conditions seed yield per ear is not limited by competition for carbohydrates as WSC reserves are sufficiently available. Both seed yield per ear and tillering rate were enhanced in experiment 2 compared to experiment 1.

The results further show that the increased new tiller growth in experiment 2 depleted the reserves of WSC, whereas the increase in seed yield per ear in experiment 1 coincided with an increase in WSC reserves. The ability to make use of the available WSC reserves is greater in the tillers than in the seeds. The potential increase in tiller number and dry weight is far greater than the potential increase in seed yield per ear.

In contrast to the results in experiment 2 (i.e. under more favourable conditions) Griffith (1992) found that seed set in *Lolium multiflorum* was reduced after tiller removal at anthesis. The amount of WSC increased in the upper stem below the ear, although the total amount in the stem remained unaffected. He suggested that tiller removal reversed the WSC gradient from the seeds to the regrowing tillers. But species differences or differences between genotypes within a species cannot be ruled out. Such a gradient could reflect the balance between generative and vegetative reproduction, which does differ between species and genotypes within a species, as the present experiment shows for *L. perenne*. A stronger tendency towards generative reproduction would then mean that tiller growth has a greater detrimental effect on seed yield. In wheat, which has been selected solely for seed production, tiller removal sharply increases seed yield (Mohamed & Marshall, 1979; Kemp & Whingwiri, 1980). Within *L. perenne*, clone B1 tends more towards generative reproduction than clone Mg2 and in the first experiment seed yield per ear was increased in the former after the younger tillers had been removed.

Cutting off the younger tillers increased the amount of nitrogen in the remaining flowering tillers (Table 4), irrespective of the extent to which the younger tillers regrew. Nitrogen uptake by the total plant shoot including the tillers removed at onset of anthesis

was not altered. In Chapter 3 it was found that during anthesis the relative amount of nitrogen on plant basis decreased in the older flowering tillers and increased in the younger tillers, which indicates nitrogen support to the younger tillers. Removing the younger tillers then would have reduced the nitrogen export from the flowering tillers. Exporting nitrogen from the flowering tillers to the younger tillers could well be a strategy in the perennial *L. perenne*, to assure new vegetative growth.

The changes in nitrogen amount in the seeds (Table 6) were not related to changes in seed yield per ear. Although strong regrowth can lower the nitrogen concentration in the seeds (i.e. clone Mg2 in experiment 2), competition for nitrogen between new vegetative tillers and the seeds seems of little significance to reduced seed yields in *L. perenne* seed crops. The nitrogen status partly determines the export of carbohydrates to new tillers (Powell & Ryle, 1978) and tillering rate (Table 1). Adjusting tillering rate to the nitrogen status could enable the plant to reduce competition for nitrogen between the seeds and new tiller growth.

We conclude that competition between growth of new vegetative tillers after onset of anthesis and the seeds for either carbohydrates or nitrogen is not a major cause of reduced and variable seed yield in *L. perenne* seed crops. Although shading within the canopy can reduce the WSC reserves in the flowering tiller, in a crop far fewer younger tillers develop per flowering tiller than in spaced plants. Reduced seed yield and an increased growth of new vegetative tillers could well be the consequence of a common cause, such as lodging, and need not be causally related. As the present work has confirmed the seeds are not able to fully use the available reserves (Chapter 2). Processes within the ear itself seem to limit seed yield. More research is needed to clarify which processes control the carbon flux towards the seeds (i.e. sink strength) in *L. perenne*.

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REFERENCES

Clemence, T.G.A. & P.D. Hebblethwaite, 1984. An appraisal of ear, leaf and stem $^{14}CO_2$-assimilation, ^{14}C-assimilate distribution and growth in a reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Biology* 105: 319-327.

Colvill, K.E. & C. Marshall, 1981. The patterns of growth, assimilation of $^{14}CO_2$ and distribution of ^{14}C-assimilate within vegetative plants of *Lolium perenne* grown at low and high density. *Annals of Applied Biology* 99: 179-190.

Davies, A., 1988. The regrowth of grass swards. In: Jones M.B. and A. Lazenby (eds.) *The grass crop. The physiological basis of production.* London: Chapman and Hall, pp. 85-127.

Deregibus, V.A., R.A. Sanchez, J.J. Casal & M.J. Trlica, 1985. Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology* 22: 199-206.

Elgersma, A., 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9-19.

Griffith, S.M., 1992. Changes in post-anthesis assimilates in stem and spike components of Italian ryegrass (*Lolium multiflorum* Lam.). I. Water soluble carbohydrates. *Annals of Botany* 69: 243-248.

Hampton, J.G. & P.D. Hebblethwaite, 1984. Experiments with vegetative tiller manipulation in the perennial ryegrass (*Lolium perenne* L.) seed crop by the application of growth regulators. *Journal of Applied Seed Production* 2: 1-7.

Hampton, J.G. & P.D. Hebblethwaite, 1985. A comparison of the effects of the growth regulator paclobutrazol (PP333) and flurprimidol (EL500) on the growth, development and yield of *Lolium perenne* grown for seed. *Journal of Applied Seed Production* 3: 19-23.

Hampton, J.G., T.G.A.Clemence & P.D. Hebblethwaite, 1983. Nitrogen studies in *Lolium perenne* grown for seed. IV. Response of amenity types and influence of a growth regulator. *Grass and Forage Science* 38: 97-105.

Hampton, J.G., T.G.A.Clemence & P.D. Hebblethwaite, 1987. The effect of lodging on ^{14}C-assimilate distribution after anthesis in *Lolium perenne* cv S24, grown for seed. *Grass and Forage Science* 42: 121-127.

Hebblethwaite, P.D. & A. Burbidge, 1976. The effect of maleic hydrazide and chlorocholine chloride on the growth, seed yield components and seed yield of S.23 ryegrass. *Journal of Agricultural Science, Cambridge* 86: 343-353.

Hebblethwaite, P.D. & J.D. Ivins, 1977. Nitrogen studies in *Lolium perenne* grown for seed. 1. Level of application. *Journal of the British Grassland Society* 32: 195-204.

Hebblethwaite, P.D. & J.D. Ivins, 1978. Nitrogen studies in *Lolium perenne* grown for seed. 2. Timing of nitrogen application. *Journal of the British Grassland Society* 33: 159-166.

Hebblethwaite, P.D., D. Wright & A. Noble, 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). In: Hebblethwaite P.D. (ed.) *Seed Production*. London: Butterworths, pp.71-90.

Kemp, D.R. & E.E. Whingwiri, 1980. Effect of tiller removal and shading on spikelet development and yield components of the main shoot of wheat and on the sugar concentration of the ear and flag leaf. *Australian Journal of Plant Physiology* 7: 501-510.

Marshall, C., 1990. Source-sink relations of interconnected ramets. In: van Groenendael J. & H. de Kroon (eds.) *Clonal growth in plants: regulation and function*. the Hague, the Netherlands: SPB Academic Publishing, pp. 23-41.

McCullagh, P. & J.A. Nelder, 1989. *Generalized linear models*. 2nd edition Cambridge: Cambridge University Press.

Meijer, W.J.M. & S. Vreeke, 1988. Nitrogen fertilization of grass seed crops as related to soil mineral nitrogen. *Netherlands Journal of Agricultural Science* 36: 375-385.

Millard P, Thomas RJ, Buckland ST. 1990. Nitrogen supply affects the remobilization of nitrogen for the regrowth of defoliated *Lolium perenne* L. *Journal of Experimental Botany* 41: 941-947.

Mohamed, G.E.S. & C. Marshall, 1979. Physiological aspects of tiller removal in spring wheat. *Journal of Agricultural Science, Cambridge* 93: 457-463.

Parsons, A.J. & M.J. Robson, 1981. Seasonal changes in the physiology of S.24 perennial ryegrass (*Lolium perenne* L.). 3. Partitioning of assimilates between root and shoot during the transition from vegetative to reproductive growth. *Annals of Botany* 48: 733-744.

Payne, R.W., P.W. Lane, A.E. Ainsley, K.E. Bicknell, P.G.N. Digby, S.A. Harding, P.K. Leech, H.R. Simpson, A.D. Todd, P.J. Verrier & R.P. White, 1987. *Reference Manual.Genstat 5 Committee Rothamsted Experimental Station*. Oxford: Clarendon Press.

Penning de Vries, F.W.T., H.H. van Laar & M.C.M. Chardon, 1983. Bioenergetics of fruits, and storage organs. In: *Proceedings Symposium Potential Productivity of Field Crops under Different Environments*. Manila, Philippines: International Rice Research Institute, pp. 37-59.

Powell, C.E. & G.J.A. Ryle, 1978. Effect of nitrogen deficiency on photosynthesis and the partitioning of ^{14}C-labelled leaf assimilate in unshaded and partially shaded plants of *Lolium temulentum*. *Annals of Applied Biology* 90: 241-248.

Ryle, G.J.A. & C.E. Powell, 1976. Effect of rate of photosynthesis on the pattern of assimilate distribution in the graminaceous plant. *Journal of Experimental Botany* 27: 189-199.

Sinclair, T.R. & T. Horie, 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: A review. *Crop Science* 29: 90-98.

Spiertz, J.H.J. & J. Ellen, 1972. The effect of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. 'Cropper') and its effects on seed production. *Netherlands Journal of Agricultural Science* 20: 232-246.

Steiner, A.A., 1984. *The universal nutrient solution.* ISOSC Proceedings of the 6th International Congress on Soilless Culture. Wageningen, the Netherlands, pp. 633-649.

Wilman, D. & M.A.K. Altimimi, 1982. The digestibility and chemical composition of plant parts in Italian and perennial ryegras during primary growth. *Journal of the Science of Food and Agriculture* 33: 595-602.

Wright, D. & P.D. Hebblethwaite, 1979. Lodging studies in *Lolium perenne* grown for seed. 3. Chemical control of lodging. *Journal of Agricultural Science, Cambridge* 93: 669 - 679.

THE PATTERN OF FLOWERING, SEED SET, SEED GROWTH AND RIPENING ALONG THE EAR OF *LOLIUM PERENNE* L.

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ABSTRACT

A greenhouse pot experiment was conducted to elucidate the pattern of decreasing seed set and seed dry weight found from the basal to the upper spikelet in *Lolium perenne* L. ears and - very markedly - acropetally within a spikelet. The changes in fresh and dry weight of the seed after anthesis were monitored for 12 positions within the ear. To determine the duration of seed growth the spatial and temporal patterns of flowering and ripening along the ear of *L. perenne* were assessed. The proximal florets of the central spikelets flowered first, and the upper spikelets in the ear flowered before the basal spikelets, with flowering proceeding acropetally within a spikelet. The upper spikelets ripened earlier than the basal ones, and the seeds within a spikelet ripened simultaneously. These patterns of flowering and ripening along the ear resulted in different durations of seed growth along the ear. Thus from the proximal to the distal seed within a spikelet the duration of growth decreased by 26 %, the rate of growth fell by 48 % and also seed set and seed dry weight fell sharply. Four per cent of the total variation in maximum seed dry weight within the ear could be attributed to the difference in seed dry weight between spikelets and 89 % to differences in seed dry weight within spikelets. Differences in duration and rate of seed growth and seed set were also much smaller between spikelets than within spikelets. About 60 % of the differences in seed dry weight along the ear was attributable to differences in growth rate and about 30 % to differences in the duration of seed growth. The relative growth rate did not differ between seeds in different positions. The main factor determining seed growth rate was the dry weight of the ovule at anthesis, which declined sharply from the proximal to the distal floret within a spikelet. Analysis of the fructose, glucose, sucrose and starch concentrations showed that seed growth was not limited by the availability of sucrose nor by the ability of the seeds to convert sucrose into starch.

INTRODUCTION

Previous work has shown that the low and variable seed yield of crops of perennial ryegrass (*Lolium perenne* L.) is not due to a shortage of water-soluble carbohydrates in the flowering tiller (Chapter 2) or to competition for assimilates between the seeds and vegetative tillers that develop after anthesis (Chapter 4). It is therefore hypothesised that processes within the ear determine seed growth and development.

The variation in seed yield between years and locations (Griffiths et al., 1973) can mainly be attributed to the varying number of seeds per unit area that are harvested and not to variation in the average seed weight (Hampton & Hebblethwaite, 1983; Elgersma, 1990). Within a standing crop however, there is large variation in seed weight. Many seeds are too light and are lost during harvest and cleaning. Differences in loss of these lighter seeds can explain the small variation in average seed weight between years (Elgersma, 1990).

The seed set and seed dry weight of individual florets and seeds within the ear of *L. perenne* have been found to vary, decreasing slightly from the basal to the upper spikelets in the ear, but decreasing sharply within a spikelet (Anslow, 1963, 1964; Burbidge et al., 1978; Elgersma, 1990). Anslow (1964) found seed dry weight fell from 2.5 mg to 1.4 mg from the proximal to the penultimate position within the basal spikelet. The ovule dry weight at anthesis, the relative growth rate and the duration of growth determine individual seed dry weight. How these factors differ between seed positions within the ear is unknown.

The temporal and spatial patterns of flowering and ripening along the ear of *L. perenne* differ. The central spikelets flower first; flowering then proceeds rapidly upwards and more slowly downwards. Within a spikelet flowering proceeds acropetally, with one or two florets flowering on the same day (Gregor, 1928; Elgersma & Sniezko, 1988). So distal florets that produce lighter seeds flower later than proximal florets that produce heavier seeds. When the flower opens, anthers and the stigma emerge simultaneously and pollen is shed within minutes (Gregor, 1928; Elgersma & Sniezko, 1988). Pollen tubes reach the embryo sac after 2 to 5 hours (Elgersma & Sniezko, 1988) and Reusch (1959) observed division in endosperm nuclei after 12 hours. The day of anthesis can thus be considered as the start of seed development. Ripening of the seeds starts in the top of the ear. The loss of water starts in the upper spikelets and then proceeds downwards (Anslow, 1964). However, the pattern of water loss within a spikelet is unknown. Thus the position of a seed affects its duration of growth and final dry weight.

The main storage component in the seed of *L. perenne* is carbohydrate stored as starch (Stoddart, 1964, 1968), which is transported to the seed as sucrose (Pollock, 1986). Both the supply of sucrose and the ability to convert sucrose into starch may differ between seeds within the ear, resulting in differences in growth rate between seeds.

Using proximal seeds from central spikelets, Hyde et al. (1959), recognised three phases in the development of *L. perenne* seed. Phase 1, lasting 10 days, is a phase of rapid increase in fresh weight in which the seed takes up water rapidly. In phase 2 (seed filling phase) dry weight increases to a maximum and the amount of water hardly changes. The percentage of water declines steadily. This phase, lasting 10 to 14 days, is followed by phase 3 (lasting 3 to 7 days) in which dry weight remains constant but the seed loses water and ripens. When the seed reaches its maximum dry weight and no more reserves accumulate it is considered physiologically full-grown (Stoddart, 1964; TeKrony et al., 1979).

The aims of the present study were (1) to establish whether differences in final seed dry weight arise from differences in the ovule dry weight at anthesis, or in the relative growth rate or in the duration of growth and (2) to determine whether there are differences in assimilate availability between seed positions that are related to differences in seed growth.

MATERIALS AND METHODS

Plant growing conditions

Vegetative tillers of *L. perenne* were clonally propagated on Steiner nutrient solution (Steiner, 1984). To obtain flowering tillers, one tiller per pot was vernalised for 14 weeks at 7 ± 1°C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. In an earlier experiment (unpublished) it was established that this ensured complete vernalisation. After vernalisation, on 7 September 1994, the plants were transferred to a greenhouse at 15°C with the shading screen down and allowed to acclimatise for a week. The plants were then transferred to 1.75 l pots in peaty soil. Per plant 340 mg N, 240 mg P_2O_5 and 580 mg K_2O were available. In the greenhouse natural daylength was extended to 17 h with incandescent bulbs (approximately 1.7 W/m^2). For 12 h per day supplemental lighting was provided by high pressure sodium lamps (Philips, AGRO SON-T, 400 W). The average daily incoming radiation was 2.6 MJ/m^2 (400 - 700 nm). The average day temperature was 21°C (12 h, 6.30 -18.30 h) and the average night temperature was 17°C. Iron wire was used to prevent the flowering tillers from lodging.

Experimental design and sampling

One clone (B1, selected from the Dutch cultivar Barlet; Chapter 4) was grown in 5 blocks. Four other clones were used in the border rows around each block and as pollinators. Excluding the border rows the plant density was approximately 21 per m^2. A few days before anthesis uniform ears containing 18 spikelets on average were selected. Ears were harvested every two days from 14 until 48 days after onset of anthesis of the ear (DAA). Onset of anthesis of the ear was defined as the first floret to flower in an ear. In view of the expected variability in seed set in the distal florets two ears per block were used per harvest date. All the selected ears started to flower within a five-day period.

At 12 positions in the ear the fresh and dry weights of the seeds were measured. These positions were the proximal, central and distal seeds in spikelet numbers 1, 6, 10 and 15 (numbered from the base upwards) (Figure 1).

Botanically the seed of *L. perenne* is a caryopsis, enclosed by palea and lemma, but will be referred to in this paper as a seed. To be able to calculate the relative growth rate (RGR, d^{-1}) of the caryopsis itself, palea + lemma dry weight was calculated as the difference in dry weight between the seed and the caryopsis. Palea and lemma were removed with a pair of tweezers. Dry weight was determined after drying at 105 °C until constant weight.

Flowering pattern and seed set

In 20 ears the day of anthesis of each individual floret was recorded. Ears flowered between 9.30 h and 12.30 h. The day of anthesis in the proximal floret of spikelets 1, 6, 10 and 15 (Figure 1) selected for the dry weight assessments was also recorded. This allowed a more accurate estimation of the day of anthesis of the other florets in that spikelet, using the flowering pattern observed within spikelets. Seed set (i.e. the presence of a seed) in each individual floret was recorded in 25 ears, using a binocular microscope. Small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret.

Chemical analyses

Prior to chemical analyses the ears had been stored at -20 °C, immediately after harvest. The remaining seeds in the ear not used for fresh and dry weight assessment were divided into three groups - proximal, central and distal - according to their position within the

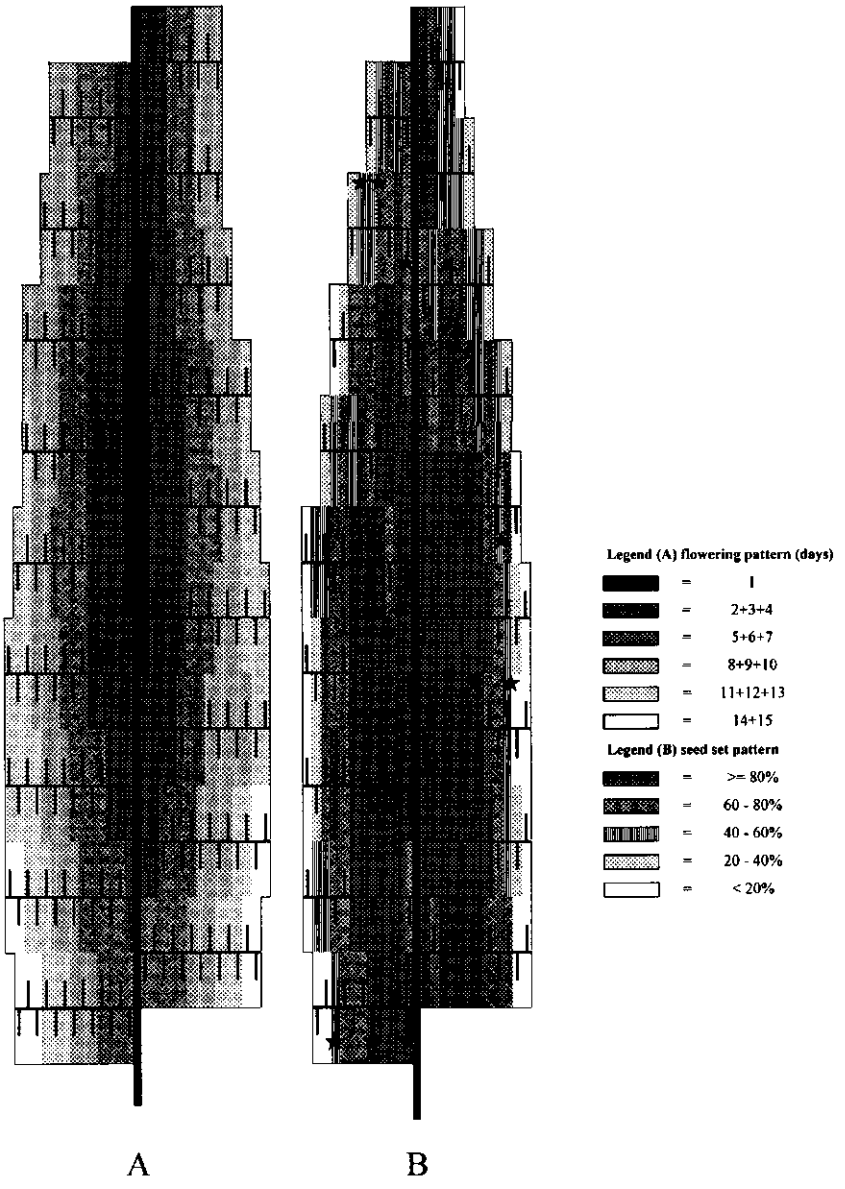

Figure 1. The pattern of (A) flowering and (B) seed set along the ear of *Lolium perenne*. The ears had 18 spikelets and each vertical line represents a floret. The 12 seed positions harvested are indicated with an asterisk (Figure 1B). The spikelets used were numbers 1, 6, 10 and 15 counted from the bottom.

spikelet. All spikelets in the ear were grouped but small top spikelets were discarded. All material was pooled to two replicates. The seeds were dried at 70 °C until constant weight.

To determine water-soluble carbohydrates (WSC), plant parts were ground and extracted in water at 100 °C for 15 minutes. Total reducing sugars was measured colorimetrically with an automatic analysis device after hydrolysis in sulphuric acid (0.45 N) (Bran and Lübbe Analyzing Technologies, Inc., Elmsford, N.Y., USA). Starch was determined titrimetrically after enzymatic hydrolysis (AOAC, 1984).

Glucose, fructose and sucrose were determined using high-performance liquid chromatography (HPLC) using a carbohydrate analysis column (Waters, Millipore Corporation, Milford, UK). Nitrogen concentration was determined with a CHN-analyser (Elementor vario EL, Elementor Analysensysteme GmBH, Hanau, Germany).

Calculations and statistical analyses

The end of seed growth can be characterised by cessation of dry matter accumulation and by the onset of rapid fall in water content of the seeds (Sofield et al., 1977a; Koller & Hadas, 1982). The course of the dry weight of the seeds over time was described with a Gompertz model (Payne et al., 1987) and the dry weight of palea + lemma were used as the value of the lower asymptote. Maximum seed dry weight was considered to be reached at 95 % of the fitted maximum, and duration of growth was calculated as follows:

$$\text{growth duration} = -\ln(-\ln(-0.05 * a/c + 0.95)) / b + m \text{ (days)}$$

where a is the dry weight of palea + lemma, b is a slope parameter, c is the difference between the lower and the upper asymptote and m is the inflexion point. Growth rate (mg/d) of a seed was calculated as an average linear rate from anthesis until the end of seed growth.

The water content over time was fitted using a segmented, piecewise regression model (Montgomery & Peck, 1982), consisting of three phases: a near constant level during the seed filling phase (1), then a linear decrease (2) to a constant level (3). The increase in water content taking place during the first phase of seed development (Hyde et al., 1959) was not included in the measurements or calculations.

The changes in fructose, glucose, sucrose, starch and nitrogen concentration over time were also fitted with a piecewise regression model. These models consisted of two phases: a decrease (fructose, glucose, sucrose) or increase (starch, nitrogen) to a constant level.

The fits were carried out per replicate, and the various model parameters, growth duration and growth rate were tested for effects of spikelet and floret position in an analysis of variance. The RGR was calculated according to Poorter (1989). Linear regression was used to analyse the effect of floret position on the RGR. Curve fitting, linear regression and analysis of variance were performed with the GENSTAT statistical package (Payne et al., 1987).

RESULTS

Flowering and seed set

Flowering started in the proximal florets of the central spikelets. Spikelets close to the top of the ear flowered earlier than the basal spikelets. Within a spikelet, flowering proceeded from the base to the top at a rate of 1.1 - 1.2 florets per day. In 74 % of the spikelets it was observed that on one or two occasions two florets flowered on the same day. The distal florets of the basal spikelets were the last to flower, at 15 days after onset of anthesis of the ear (Figure 1A).

Seed set varied from 53 % to 73 % between spikelets, with the lowest values in the upper spikelets. The variation was much larger within a spikelet. Distal florets rarely produced a seed. The variation in time of flowering and seed set was much larger within spikelets than between spikelets (Figure 1B).

Ripening, duration and rate of seed growth

In the ear ripening proceeded basipetally. The water content in the seeds decreased in the upper spikelets first and then in the basal ones ($P<0.001$) (Figure 2). The rate of water loss was also faster in the upper spikelets than in the basal ones ($P=0.012$).

The growth duration of the average seed in a spikelet (time between anthesis and maximum seed dry weight) increased from the upper spikelet (24.7 days) to the basal spikelet (30.2 days). The average growth rate did not differ between spikelets.

Within the average spikelet the water content of the proximal, central and distal seed started to decline simultaneously and with a similar rate at 30 (LSD = 1.5) days after onset of anthesis of the ear. This coincided with the seeds reaching 95 % of their maximum fitted value, the end of seed filling (Figure 3). Maximum seed dry weight was reached at 31.8 (LSD = 3.8) days after onset of anthesis of the ear. Moisture percentage at the end of seed

filling, i.e. at maximum seed dry weight, was higher in the distal seeds (57 %) than in the central and proximal seeds (46 %) (P<0.001).

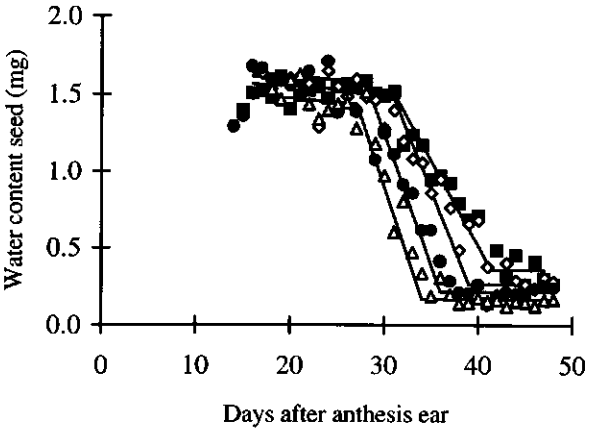

Figure 2. The changes in the average water content in the seeds of different spikelets with time after onset of anthesis of the ear in *Lolium perenne*. Spikelet number 1 (basal) (■), 6 (◇), 10 (●) and 15 (△) were used. The ear had a total of 18 spikelets. Lines are the result of linear regression using a piecewise regression model.

Of the total variation in maximum seed dry weight 4 % could be accounted for by the difference in seed dry weight between spikelets and 89 % by differences in seed dry weight within spikelets. The average seed dry weight in a spikelet ranged from 1.43 mg in spikelet 6 to 1.15 mg in spikelet 15. Within the average spikelet the seed dry weight ranged from 1.86 mg in the proximal seed to 0.71 mg in the distal seed, a decrease of 62 % (Table 1).

As a consequence of the flowering and ripening pattern the greatest difference in the duration of growth was mainly between the central and the distal seeds within a spikelet; the maximum reduction was 26 %. The decrease in the rate of growth within a spikelet was sharper: - 48 % in the distal seeds and - 16 % in the central seeds compared to the proximal seeds (Table 1). About 60 % of the variation in seed dry weight could be accounted for by differences in growth rate and about 30 % by differences in the duration seed growth. This means that lighter, more distal seeds in a spikelet mainly grew more slowly.

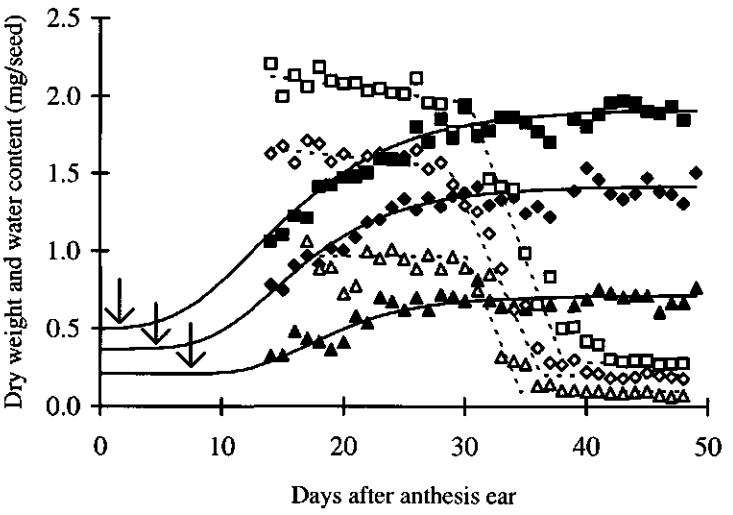

Figure 3. The dry weight (closed symbols) and the water content (open symbols) of the proximal (■,□), central (◆,◇) and distal (▲,△) seeds within a spikelet with time after the onset of anthesis of the ear. Values are the average of four spikelets across the ear of *Lolium perenne*. Dry weight is fitted with a Gompertz curve and amount of water with a segmented, piecewise model. The arrows indicate anthesis of the florets and the y-intercept is the dry weight of the florets at anthesis.

Table 1. Maximum seed dry weight, duration of growth (days after anthesis of the floret) and rate of growth in the proximal, central and distal seeds in the average spikelet of *Lolium perenne*. The relative values are shown in brackets. Letters indicate statistically significant differences (LSD; P<0.05) within a column.

Seed position	Seed dry weight (mg)	Growth duration (d)	Growth rate (mg/d)
Proximal	1.86 c (100)	31 b (100)	0.044 c (100)
Central	1.38 b (74)	28 b (90)	0.037 b (84)
Distal	0.71 a (38)	23 a (74)	0.023 a (52)

Ovule dry weight at anthesis

Measurement of the ovule dry weight at anthesis performed on the same genotype but in another experiment showed a sharp decrease in dry weight from the proximal (0.07 ± 0.01 mg) to the distal floret (0.03 ± 0.002 mg). Ovule dry weight in the central floret was 0.06 ± 0.01 mg on average.

Relative growth rate (RGR)

To determine the RGR of the caryopsis the dry weight of the palea + lemma was calculated. Linear regression showed that the palea + lemma dry weight did not differ between the four selected spikelets, but declined from the proximal (0.48 mg) to the central (0.36 mg) to the distal floret (0.20 mg) within the spikelet. Palea + lemma dry weights did not change significantly during seed growth.

Combining the palea + lemma dry weights with the ovule dry weights revealed a sharp decline in dry weight from the proximal to the distal floret within a spikelet. This is shown by the result of the Gompertz model (Figure 3).

RGR of the caryopsis over time after anthesis showed an interaction (P=0.002) between the position of the seed within the spikelet and days after anthesis (Figure 4A). The RGR of the distal seed declined faster than the RGR of the central and proximal seeds. However, one should compare the seeds on the same developmental scale and correct for differences in duration of growth (Table 1) and seed dry weight. Correcting for differences in duration of growth and seed dry weight by plotting the RGR against the relative dry weight of the caryopsis eliminated the differences between the seed positions within a spikelet (Figure 4B). Differences in RGR between spikelets were not statistically significant.

Chemical composition

The fructose and glucose concentrations declined during the development of the seeds, and also from the proximal to the distal seed group (Figure 5A,B). The sucrose concentration declined less sharply towards the tip of the spikelet (Figure 5C). In contrast to glucose and sucrose the decline of fructose best fitted a curvilinear model. The rate of decline of fructose and glucose differed between the groups of seeds and the minimum concentration was reached earlier towards the distal seed group (P=0.004 for fructose; P=0.02 for glucose). For sucrose the distal seed group reached the minimum level before the proximal

seed group did, but the rates of decline did not differ statistically significantly (Figure 5C). The minimum levels of fructose, glucose and sucrose attained at the end of seed growth did not differ between the three groups of seeds.

The starch concentration increased more slowly in the proximal seed group than in the other seeds (P=0.004) (Figure 5D). Final starch concentration was lower in the distal seed group than in the central and proximal seed groups (300 mg/g versus 360 mg/g).

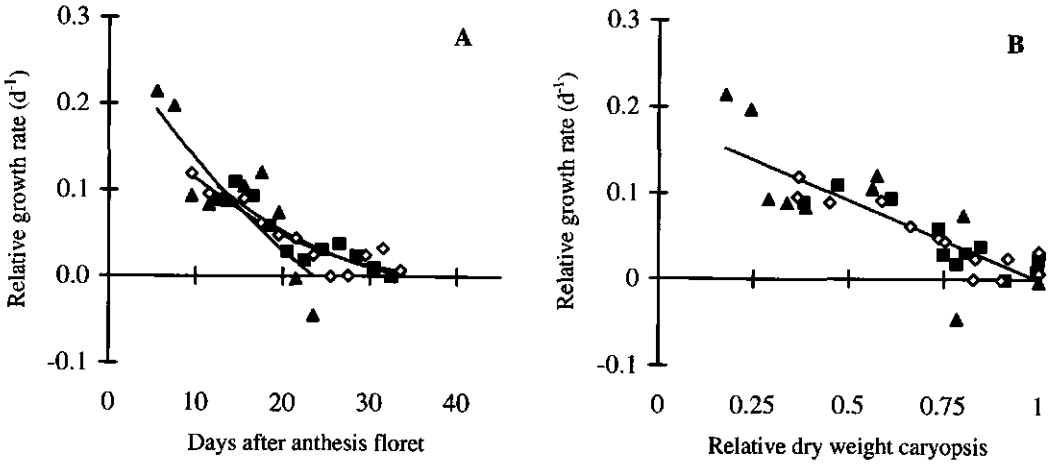

Figure 4. The change in the relative growth rate of the caryopsis with time after anthesis of the floret (A) and with the relative dry weight (B) in the proximal (■), central (◇) and distal (▲) seeds in the basal spikelet in the ear of *Lolium perenne*. Lines are the result of linear regression.

The nitrogen concentration decreased (P<0.001) from the proximal to the distal seed within the spikelet (Figure 5E). In view of the differences in carbohydrate and starch concentrations between the seeds, the nitrogen concentration was corrected for the amount of WSC and starch in the seed. Thus an indication of the nitrogen concentration in the structural dry matter was obtained. The rates of increase in the corrected nitrogen concentration (Nc) did not differ. The final Nc was lower in the distal seed group than in the central and proximal seed groups (Figure 5E). The ratio between the starch and the sucrose concentration increased during seed growth and was higher in the distal seed group than in the other seed groups (P<0.001)(Figure 5F).

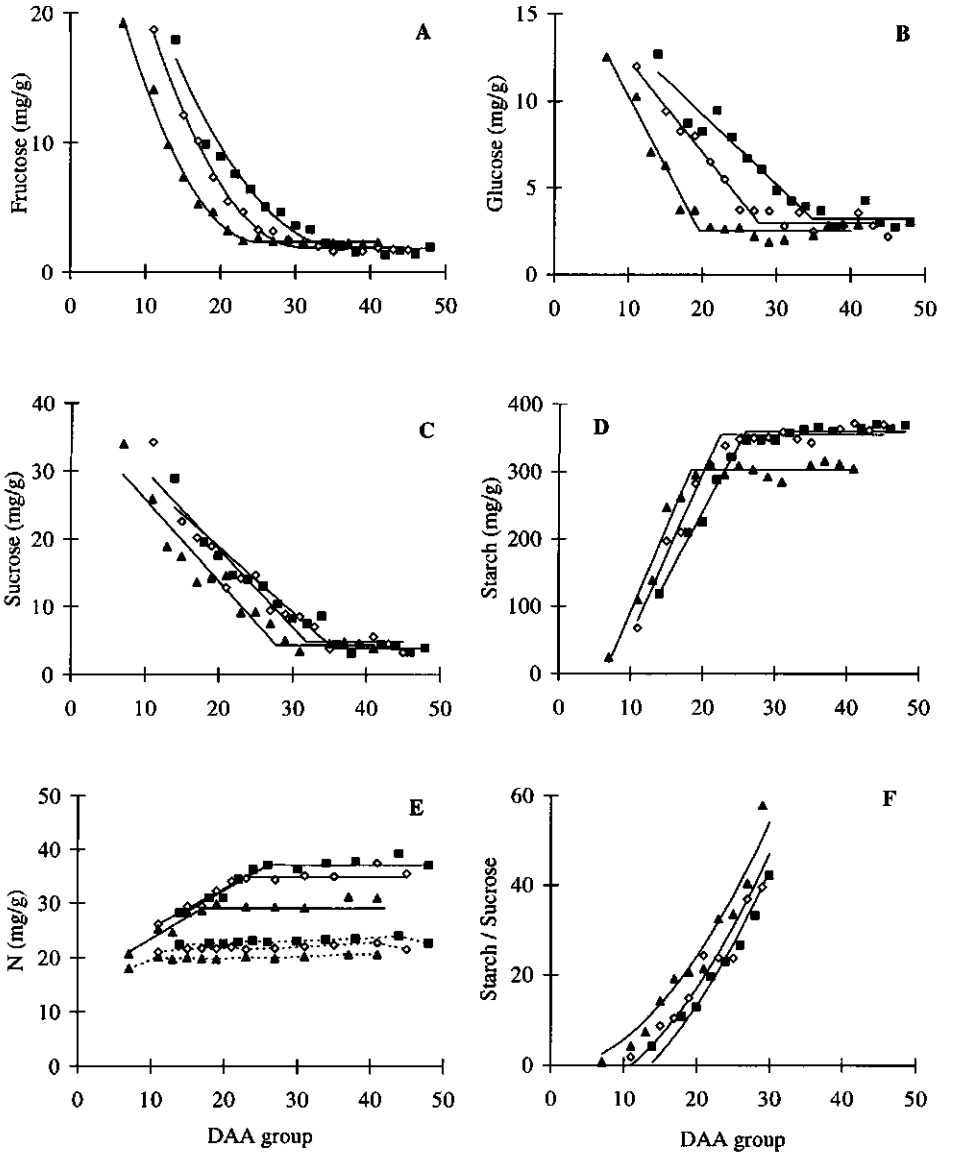

Figure 5. Fructose concentration (A), glucose concentration (B), sucrose concentration (C), starch concentration (D), nitrogen concentration (dotted lines) and the corrected nitrogen concentration, Nc; corrected for the amount of water-soluble carbohydrates and starch (solid lines) (E) and the starch and sucrose ratio (F) with time after anthesis of three different seed groups within the spikelet of *Lolium perenne*. Proximal (■), central (◇) and distal (▲) seed groups. The solid lines are the result of linear regression using a piecewise model.

The differences in the duration of growth of the seeds within a spikelet are also reflected in the time at which the seeds attained their minimum fructose, glucose and sucrose concentrations and maximum starch and Nc concentrations. It is difficult to make a direct comparison with the growth duration obtained from the seed dry weight curves of the individual seed positions (data in Table 1) because the chemical analyses were performed on groups of seeds that contain seeds from several positions within the spikelet.

In all the seed groups the starch stopped accumulating well before the minimum sucrose level was reached (Figure 6). Maximum starch concentrations of the proximal, central and distal seed groups were reached respectively at 26, 23 and 19 days after anthesis and the minimum sucrose concentrations were attained at 36, 31 and 27 days after anthesis. The sucrose concentration at which the accumulation of starch ended was similar (about 14 mg/g) in all three seed groups. Both findings suggest that the cessation of seed filling was not caused by a limited availability of sucrose.

The fructose and glucose concentrations both declined earlier than the sucrose concentration. This time lag increased from the proximal to the distal seed group within the spikelet, and was especially marked for glucose. Maximum Nc concentrations of the proximal, central and distal seed groups were reached at 27, 24 and 18 days after anthesis respectively. This coincided with the cessation of starch accumulation (Figure 6).

DISCUSSION

The flowering pattern recorded along the ear and the progress of flowering within the spikelet agree with the results obtained by Gregor (1928) and Elgersma & Sniezko (1988). The flowering pattern along the ear appears to be regular, at least under greenhouse conditions. Low temperatures in the field may delay or inhibit anthesis (Hill, 1980). Our results show that distal florets within a spikelet rarely produce a seed (Figure 1), confirming data collected by Burbidge et al. (1978) and Elgersma (1990). This sharp fall in seed set within the spikelet however, was stronger than found by Anslow (1963). The differences in genotypes might be responsible for this.

The variation in time of flowering, seed set, seed dry weight (Anslow, 1963, 1964; Burbidge et al., 1978), growth duration and growth rate mainly occur within rather than between spikelets (Figure 1, Table 1). The gradient in seed dry weight within a spikelet is primarily due to a reduced growth rate and to a lesser extent to a reduced duration of growth. The shorter duration of growth is attributable to the distal and central florets in a spikelet flowering later than proximal florets but ripening concomitantly.

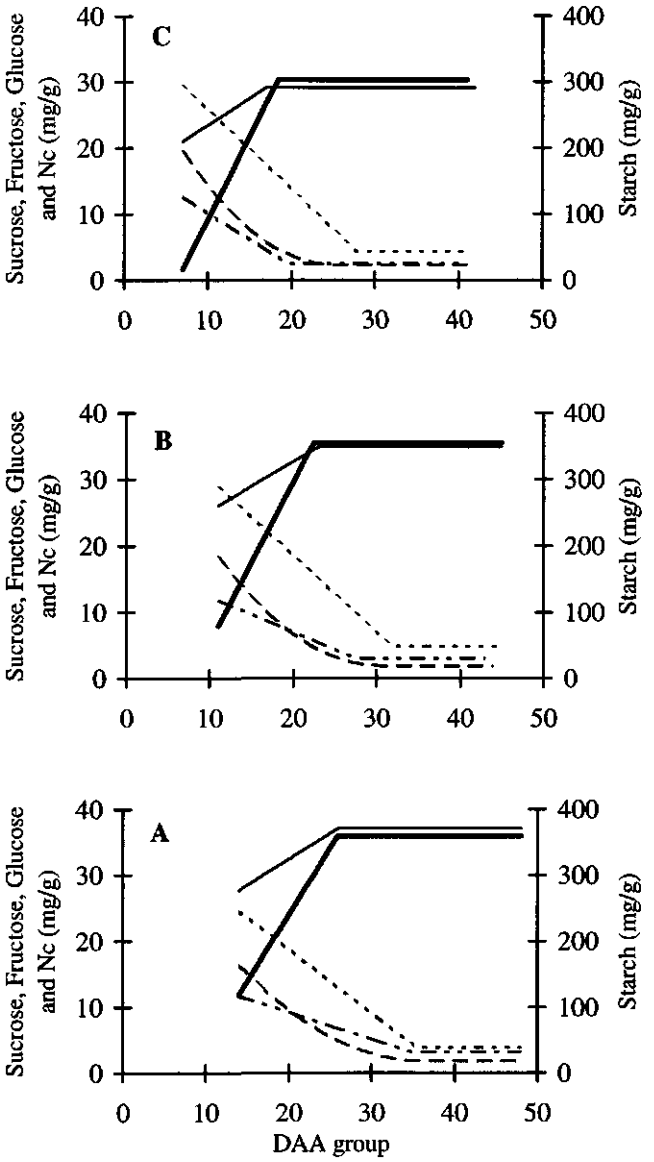

Figure 6. The changes in sucrose (....), fructose (- - - -), glucose (— · · —), starch (———) and in nitrogen concentration (Nc, ———) which was corrected for the amount of water-soluble carbohydrates and starch with time after anthesis of the floret in the proximal (A), central (B) and distal (C) seed groups in the spikelet of *Lolium perenne*. The lines shown are the result of linear regression with a piecewise model, see Figure 5.

In maize (Tollenaar & Daynard, 1978) and wheat (Sofield et al., 1977b; Simmons & Crookston, 1979) too, the lighter, younger seeds in the inflorescence grow more slowly and for a shorter period than the older, heavier seeds. As in *L. perenne*, the lighter seeds have a shorter duration of growth because they start growing (flowering) later but all seeds ripen simultaneous (Tollenaar & Daynard, 1978; Simmons & Crookston, 1979).

Several authors have argued that the duration of seed growth and final seed dry weight are determined by the maximum water content and thus by the volume of the seed. When the seed has reached its maximum volume, the water concentration of the seed will decrease as the accumulation of dry matter continues. This will eventually lead to seed ripening (Egli et al., 1987; Egli, 1990; Schnyder & Baum, 1992). Within a spikelet of *L. perenne* the maximum water content of a seed, growth duration and dry weight decline from the proximal to the distal position (Figure 3). However, it remains unclear why ripening of the individual seeds is synchronised within a spikelet.

The strong decrease in the growth rate towards the tip of a spikelet is caused by the lower ovule dry weight at anthesis, as the RGRs of the seeds were similar. In *L. perenne* the RGR of the distal seeds decreases slightly sharper with time after anthesis than that of the central and proximal seeds (Figure 4A). But after correcting for differences in duration of growth and seed dry weight within the spikelet the differences in RGR disappear. The decrease of the RGR during development is similar for all the seeds in a spikelet (Figure 4B). In this genotype both final seed dry weight (Table 1) and ovule dry weight at anthesis decrease roughly by a factor 2.5 from the proximal to the distal seed.

The sharp decrease in ovule dry weight at anthesis within a spikelet is the result of the developmental pattern of the ear. The florets within a spikelet differentiate acropetally (Bommer, 1959; Latting, 1972). Younger, distal seeds thus have lighter ovules. Akpan & Bean (1977) also found a positive correlation between the size of the ovule and seed dry weight in *L. perenne* and *L. multiflorum*. Both decreased with increasing temperature after ear emergence.

The similar RGR of the seeds indicates that the availability of sucrose per unit of dry weight does not differ between seeds and that the seeds have a similar capacity to convert sucrose into starch. The concentrations of starch increase at a similar rate (Figure 5D), and the demand of the seeds is satisfied. The supply of sucrose to the seeds does differ because seed dry weights differ. The lower final concentration of starch in the distal seeds is due to the shorter duration of growth (Table 1).

Differences in the availability of sucrose to the seeds cannot explain the differences in seed dry weight in *L. perenne*. Although there is a small decline in sucrose concentration from the central to the distal seed of 5 mg/g (Figure 5C), the rate of increase in starch concentration is not lower (Figure 5D). This accounts for the starch to sucrose ratio being highest in the distal seeds (Figure 5F). The cessation of seed growth is not caused by a

limited availability of sucrose either. Starch stops accumulating well before the minimum concentration of sucrose in the seeds is reached (Figure 6). It seems probable that the sucrose concentration continues to decline because of respiration.

The first step in the synthesis of starch from sucrose is the hydrolysis of sucrose into glucose and fructose (Hawker et al., 1991). Our results indicate that in *L. perenne* the rate of sucrose hydrolysis per unit dry weight declines from the proximal to the distal seeds. The falls in fructose and glucose concentrations within the spikelet are much sharper than the fall in sucrose (Figure 5A, B) whereas the rates of increase in the starch concentration are similar (Figure 5D).

In the filling seeds of several species, such as wheat (Dale & Housley, 1986; Riffkin et al., 1995), rice (Patel & Mohapatra, 1996), snap bean (*Phaseolus vulgaris*) (Sung et al., 1994) and lima bean (*Phaseolus lanatus*) (Xu et al., 1989) sucrose is mainly hydrolysed by the enzyme sucrose synthase, not by invertase. Dale & Housley (1986) found that within the ear of wheat the activity of sucrose synthase per gram of fresh weight of endosperm was positively correlated with the growth rate and the final dry weight of the seed.

In *L. perenne* too the activity of the sucrose hydrolysing enzyme, or enzymes, per unit dry weight appears to correlate with the falling growth rate and final seed dry weight within a spikelet. Furthermore, the time lag between the decline of the fructose and glucose concentrations and that of sucrose increases towards the distal seeds (Figure 6). This fact also illustrates the declining activity of the sucrose hydrolysing enzyme within the spikelet. However, the results mentioned above show that the activity of this enzyme is not a limiting factor to *L. perenne* seed growth.

The similar time course of the nitrogen concentration for the three seed positions (Figure 5E) shows that the balance between nitrogen and starch accumulation does not differ between the seeds. Correcting the nitrogen concentration for the amount of WSC and starch in the seeds eliminated the differences in the nitrogen concentration within the spikelet. The rate of accumulation of nitrogen in the structural dry matter (Nc) apparently does not differ between the seeds and is not related to differences in seed dry weight.

The cessation of Nc accumulation coincides with that of starch (Figure 6). Most likely, Nc accumulation is related to the accumulation of storage proteins in the seed (Müntz 1982). Ivanko (1971) also found in barley grain that the increase in total protein N stopped when the maximum dry weight was reached.

From the results presented here it can be concluded that the differences in seed dry weight within the ear of *L. perenne*, under optimal growth conditions (i.e. adequate light and nutrients), mainly arise from differences in growth rate and less so from differences in the duration of seed growth. In turn, growth rate is determined by the dry weight of the ovule at anthesis and not by differences in RGR. Assimilate availability and the ability of seeds to convert sucrose into starch are not important factors in explaining differences in

seed dry weight within the ear of *L. perenne*. Future research should therefore focus on ear development and growth of the ovules.

When breeding for a higher seed yield of *L. perenne* seed crops genotypes with a higher ovule dry weight and a smaller gradient in ovule dry weight within the spikelet would be beneficial. This would give a more homogeneous seed weight distribution at final harvest. Also delaying ripening and thereby increasing the duration of growth of the seeds would increase seed dry weight and thus yield of *L. perenne* seed crops. These traits would benefit seed yield because more seeds would be harvested instead of lost during harvesting and cleaning because of their low weight.

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REFERENCES

Akpan, E.E.J. & E.W. Bean, 1977. The effects of temperature upon seed development in three species of forage grasses. *Annals of Botany* 4: 689-695.

Anslow, R. C., 1963. Seed formation in perennial ryegrass. I Anther exertion and seed set. *Journal of the British Grassland Society* 18: 90-96.

Anslow R.C., 1964. Seed formation in perennial ryegrass. II Maturation of seed. *Journal of the British Grassland Society* 19: 349-357.

AOAC, 1984. Williams S., (ed). Official methods of analyses of the association of official analytical chemists, The William Byrd Press Inc, Richmond, Virginia, USA.

Bommer, D., 1959. Über Zeitpunkt und Verlauf der Blütendifferenzierung bei perennierenden Gräsern. *Zeitschrift für Acker- und Pflanzenbau* 109: 95-118.

Burbidge, A., P.D. Hebblethwaite & J.D. Ivins, 1978. Lodging studies in *Lolium perenne* grown for seed. 2.Floret site utilization. *Journal of Agricultural Science, Cambridge* 90: 269-274.

Dale, E., & T.L. Housley, 1986. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. *Plant Physiology* 82: 7-10.

Egli, D.B., 1990. Seed water relations and the regulation of the duration of seed growth in soybean. *Journal of Experimental Botany* 41: 243-248.

Egli, D.B., W.G. Duncan & S.J. Crafts-Brandner, 1987. Effect of physical restraint on seed growth in soybean. *Crop Science* 27: 289-294.

Elgersma, A., 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Elgersma, A., & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S:59-68.

Gregor, J.W., 1928. Pollination and seed production in the rye-grasses (*Lolium perenne* and *Lolium italicum*). *Transactions of the Royal Society of Edinburgh*, Vol. LV, part III. pp. 773-794.

Griffiths, D.J., H.M. Roberts & J. Lewis, 1973. The seed yield potential of grasses. Report Welsh Plant Breeding Station 1973, pp. 117-123.

Hampton, J.G. & P.D. Hebblethwaite, 1983. Yield components of the perennial ryegrass (*Lolium perenne* L.) seed crop. *Journal of Applied Seed Production* 1: 23-25,

Hawker, J. S. & C.F. Jenner & C.M. Niemietz, 1991. Sugar metabolism and compartmentation. *Australian Journal of Plant Physiology* 18: 227-237.

Hill, M.J., 1980. Temperate pasture grass-seed crops: formative factors. In: Hebblethwaite P.D. (ed.) *Seed Production*. London: Butterworths, pp. 137-149.

Hyde, E.O.C., M.A. McLeavy & G.S. Harris, 1959. Seed development in ryegrass, and in red and white clover. *New Zealand Journal of Agricultural Research* 2: 947-952.

Ivanko, S., 1971. Changeability of protein fractions and their amino acid composition during maturation of barley grain. *Biologia Plantarum* 13: 155-164.

Koller, D. & A. Hadas, 1982. Water relations in the germination of seeds. In: O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler (eds.) *Encyclopedia of Plant Physiology*. New Series, vol. 12B. Springer Verlag, Berlin, pp. 402-431.

Latting, J., 1972. Differentiation in the grass inflorescence. In: V.B. Younger & C.M. McKell (eds.). *The biology and utilization of grasses*. Academic Press, London.

Montgomery, D.C.& E.A. Peck, 1982. *Introduction to linear regression analysis*. John Wiley & Sons, New York, pp.196-198.

Müntz, K., 1982. Seed development. In: D. Boulter & B. Parthier (eds.) *Encyclopedia of Plant Physiology*. New Series, vol. 14A. Springer Verlag, Berlin, pp. 505-558.

Patel, R. & P.K. Mohapatra, 1996. Assimilate partitioning within floret components of contrasting rice spikelets producing qualitatively different types of grains. *Australian Journal of Plant Physiology* 23: 85-92.

Payne, R.W., P.W. Lane, A.E. Ainsley, K.E. Bicknell, P.G.N. Digby, S.A. Harding, P.K. Leech, H.R. Simpson, A.D. Todd, P.J. Verrier & R.P. White, 1987. *Reference Manual.Genstat 5 Committee Rothamsted Experimental Station.* Oxford: Clarendon Press.

Pollock, C.J., 1986. Fructans and the metabolism of sucrose in vascular plants. *New Phytologist* 104: 1-24.

Poorter, H., 1989. Plant growth analysis: towards a synthesis of the classical and the functional approach. *Physiologia Plantarum* 75: 237-244.

Reusch, J.D.H., 1959. Embryological studies on seed development in reciprocal crosses between *Lolium perenne* and *Festuca pratensis. South African Journal of Agriculrural Science* 2: 426-449.

Riffkin, H. L., C.M. Duffus & I.C. Bridges, 1995. Sucrose metabolism during endosperm development in wheat (*Triticum aestivum*). *Physiologia Plantarum* 93: 123-131.

Schnyder, H. & U. Baum, 1992. Growth of the grain of wheat (*Triticum aestivum* L.). The relationship between water content and dry matter accumulation. *European Journal of Agronomy* 1: 51-57.

Simmons, S. R. & R.K. Crookston, 1979. Rate and growth of kernels formed at specific florets in spikelets of spring wheat. *Crop Science* 19: 690-693.

Sofield, I., I.F. Wardlaw, L.T. Evans & S.Y. Zee, 1977a. Nitrogen, phosphorus and water contents during grain development and maturation in wheat. *Australian Journal of Plant Physiology* 4: 799-810.

Sofield, I., L.T. Evans, M.G. Cook & I.F. Wardlaw, 1977b. Factors influencing the rate and duration of grain filling in wheat. *Australian Journal of Plant Physiology* 4: 785-797.

Steiner, A.A., 1984. *The universal nutrient solution.* ISOSC Proceedings of the 6th International Congress on Soilless Culture. Wageningen, the Netherlands, pp. 633-649.

Stoddart, J.L., 1964. Seed ripening in grasses I. Changes in carbohydrate content. *Journal of Agricultural Science* 62: 67-72.

Stoddart, J.L., 1968. Biochemical aspects of seed ripening in grasses. *Journal of the National Institute of Agricultural Botany* 11: 370-377.

Sung, S.S., W.J. Sheih, D.R. Geiger & C.C. Black, 1994. Growth, sucrose synthase, and invertase activities of developing *Phaseolus vulgaris* L. fruits. *Plant, Cell and Environment* 17: 419-426.

TeKrony, D.M., D.B. Egli, J. Balles, T. Pfeiffer & R.J. Fellows, 1979. Physiological maturity in soybean. *Agronomy Journal* 71: 771-775.

Tollenaar, M. & T.B. Daynard, 1978. Kernel growth and development at two positions on the ear of maize (*Zea mays*). *Canadian Journal of Plant Science* 58: 189-197.

Xu, D.P., S.S. Sung & C.C. Black, 1989. Sucrose metabolism in lima bean seeds. *Plant Physiology* 89: 1106-1116.

ANALYSIS OF SINK-SINK RELATIONS BETWEEN SEEDS WITHIN THE EAR OF *LOLIUM PERENNE* L.

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ABSTRACT

Little is known about sink-sink relations between seeds within the ear of perennial ryegrass (*Lolium perenne* L.). Therefore in three greenhouse experiments with two genotypes these relations were investigated by reducing the number of seeds in an ear. In two of the experiments entire spikelets were removed, to study the relations between seeds in different spikelets. The effects on seed dry weight were not proportional to the number of spikelets removed. In one genotype removal of 2/3 of the spikelets increased seed dry weight of the remaining seeds by 15 %. In another genotype such a treatment did not increase seed dry weight. In the third experiment, aiming at elucidating the sink-sink relations within a spikelet, either two proximal seeds or two central seeds were maintained in a spikelet by removing the other ovules in combination with no or 75 % shading. Shading by 75 % reduced seed dry weight by about 10 %. In the unshaded treatment seed dry weight was not affected by ovule removal. Under shading the central seeds in a spikelet became about 12 % heavier if they grew alone, in contrast to the proximal seeds. The effects of a reduction of seed number on seed dry weight were not related to the final nitrogen concentration in the seeds. These experiments show that the assimilate partitioning and the relations between seeds in the ear of perennial ryegrass are already largely determined at anthesis; increasing seed yield by manipulations after anthesis is not feasible.

INTRODUCTION

The two major yield components of a seed crop of perennial ryegrass (*Lolium perenne* L.) are the number of seeds per unit area and the average seed dry weight. The variability of the seed yield can be accounted for by the varying number of seeds recovered after harvest and cleaning (Hampton & Hebblethwaite, 1983; Elgersma, 1990a). This is not because too few florets set seed but because many seeds (25% to 70%) are too light and are therefore lost during harvest and cleaning or are removed to meet certain quality standards (Meijer, 1985; Horeman, 1989; Elgersma, 1990b).

Within the ear of *L. perenne* seed dry weight declines sharply from the older, proximal seed to the younger, distal seed within the spikelet. It varies much less between similar positions in different spikelets (Anslow, 1964; Chapter 5). The main factor, accounting for about 60 % of the variation in seed dry weight within the ear, has been found to be the ovule dry weight at anthesis, which declines from the proximal to the distal position within the spikelet (Chapter 5). In that research it was found that the second most important factor was the duration of growth, which accounted for about 30 % of the variation in seed dry weight within the ear. It was also shown that under optimal conditions, with adequate light and nutrient supply, neither the availability of carbohydrate and nitrogen nor the ability to convert sucrose into starch limit the growth of each seed in the ear of *L. perenne*. It seems possible that a reduction of seed number in the ear might affect these processes.

In several graminaceous species it has been found that seeds within the inflorescence influence each other. Removing the spikelets or the proximal seeds in a spikelet, or delaying the pollination of the proximal florets can increase seed set and seed dry weight in the remaining ear part. This has been demonstrated in wheat (Evans et al., 1972; Rawson & Evans, 1970; Walpole & Morgan, 1973; Bremner & Rawson, 1978; Simmons & Moss, 1978), in oats (Klinck & Slim 1976; Peterson, 1983), in barley (Aspinall, 1963) and in maize (Struik & Makonnen, 1992).

In some studies in wheat, Slafer & Savin (1994) and Miralles & Slafer (1995) found no effect of spikelet removal on the seed dry weight of the remaining seeds. Summarising the literature data Slafer & Savin (1994) concluded that the effect of spikelet removal on the dry weight of the seeds remaining in wheat is strongly affected by an interaction between genotype and environment.

Removal of seeds is assumed to increase the assimilate availability to the remaining seeds (Jenner, 1980). From an increase in seed dry weight after spikelet removal it can be inferred that more carbohydrate will have accumulated in the case of graminaceous seeds, as these store starch mainly. Remarkably, the nitrogen concentration has been found to increase in the seeds remaining after spikelet removal (Jenner, 1980; Simmons & Moss, 1978; Peterson, 1983), suggesting an increased availability of nitrogen assimilates relative

to that of carbon assimilates. In wheat a reduction of the carbohydrate availability by shading reduced the dry weight of the distal seeds more than that of the proximal seeds (Sofield et al., 1977; Bremner & Rawson, 1978). Bremner & Rawson (1978) concluded that the resistance of the vascular connection within the spikelet was the major reason for this. However, at a reduced assimilate supply the resistance of the vascular bundles will be less important, as the assimilate flux will be reduced. Thus it seems more likely that seeds within the spikelet compete directly for the available assimilates.

The relations between seeds in different spikelets or seeds within the same spikelet may differ. Information on the sink-sink relations between the seeds in the ear of *L. perenne* is scarce. In a field experiment Marshall & Ludlam (1989) found no increase in seed dry weight after reducing the number of spikelets to four. Our experiments aimed to investigate (1) whether seeds in the ear positioned in different or in the same spikelet do affect each other's growth and (2) whether such sink-sink relations between seeds can be a factor determining the seed yield in perennial ryegrass.

MATERIALS AND METHODS

Plant growing conditions

Three greenhouse experiments were conducted with spaced plants of *Lolium perenne* L. Clones were used to reduce the variability between plants. In a preliminary experiment two clones were selected. Clone B1 had a higher tillering rate and more seeds per ear than clone Mg2. Vegetative tillers of these clones were propagated on Steiner nutrient solution (Steiner, 1984) in the greenhouse at 17 h daylength and at a day temperature of 20 °C and a night temperature of 17 °C.

To obtain flowering tillers, one tiller per pot (main tiller) was vernalised for 14 weeks at 7 ± 1°C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. A preliminary experiment had established that this procedure ensured complete vernalisation. After vernalisation the plants were transferred to a greenhouse at 15°C with the shading screen down, and allowed to acclimatise for a week. They were then put in 1.75 l pots in peaty soil. Natural daylength was extended to 17 h with incandescent light (approximately 1.7 W/m^2, PAR). The growth conditions in the three experiments are shown in Table 1. The fertiliser rates differ somewhat because of variation between the batches of peaty soil used. Steiner nutrient solution was added to increase the nutrient supply to a sufficiently high level (Chapter 4).

Table 1. The growing conditions and clones used.

Experiment	End of vernalisation	Nutrient supply (mg/plant)	Average day/night temperature (°C)	Average daily incoming radiation, MJ/m^2 (400-700 nm)	Clones used
1	11 July 1994	160 or 360 mg N 180 mg P_2O_5 205 mg K_2O	22/17	not measured	B1
2	7 September 1994	340 mg N 240 mg P_2O_5 580 mg K_2O	21/17	2.6	B1 and Mg2
3	20 April 1995	290 mg N 210 mg P_2O_5 305 mg K_2O	21/17	3.6	B1 and Mg2

Experimental design and sampling

The plants were grown in a randomised block design and four other clones were used in the border rows and as pollinators. Excluding the border rows the plant density was approximately 21 per m^2. At onset of anthesis of the main tiller a homogeneous group of younger flowering tillers, called the group 2 tillers was selected for uniform size and development (Chapter 3). Iron wire was used to prevent the flowering tillers from lodging.

In experiments 1 and 2 the relations between seeds in different spikelets was investigated. In experiment 1 two levels of ovule removal were established at onset of anthesis and combined with two levels of nitrogen supply. In either none or 50 % of the spikelets all ovules were removed with a dissecting needle with a lancet tip. Because of the gradient in seed dry weight across the ear (Chapter 5) spikelets were treated alternately, two by two, in the 50% treatment. On average there were 4 ± 0.9 group 2 tillers per plant.

At onset of anthesis 200 mg N as NH_4NO_3 was added to half of the plants. Thus two nitrogen levels of 160 mg N per plant and 360 mg N per plant were established. The experiment was carried out in 6 replicates.

In experiment 2 entire spikelets were removed at onset of anthesis of the ear. Either no spikelets, 1/3 or 2/3 of the spikelets were removed. In the 1/3 and 2/3 treatments every third spikelet or every second and third spikelet, counting from the bottom of the ear, were

removed. Clone Mg2 had on average 2.5 ± 0.3 group 2 tillers per plant and clone B1 3.2 ± 0.5 group 2 tillers per plant. The experiment contained 4 replicates.

In experiment 3 the relations between seeds within a spikelet were investigated in combination with shading. Four central spikelets in the ear were treated and studied, the other spikelets were left intact. The lower and upper spikelet of these four were not treated. The two spikelets in the middle were either 1) untreated, 2) only the two proximal seeds were maintained or 3) only the two seeds in positions 6 and 7 were maintained. Seed growth in the other florets in the two middle spikelets was prevented by taking away the ovules at anthesis. Because some seeds escaped the treatment on average 2.7 seeds developed in a treated spikelet, compared with 7.8 seeds in an untreated spikelet.

The shading treatment consisted of a double layer of cotton cloth that reduced the light intensity by 75% (Chapter 2). Plants were placed under the cotton cloth when all florets in the selected four spikelets of the oldest selected ear in that plant had flowered. On average the youngest selected ear started to flower 1.7 ± 1.3 days later. In clone Mg2 the main ear was not used as it flowered well before the group 2 tillers did. The experiment was carried out in 7 replicates. Each selected ear was harvested at 30 days after onset of anthesis, at maturation.

In experiments 1 and 2 the main tiller and group 2 tillers were harvested at 30 days after onset of anthesis of the main tiller and dissected into stem, leaves, rachis, seeds and empty florets + glumes. In experiment 3 all seeds were weighed individually. The ears had been stored at - 20 °C before the number of seeds and empty florets were counted under a binocular microscope. Small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret. Dry weight of the different plant parts was determined after drying at 70 °C for 48 h. Roots were not harvested.

Although the seed of *L. perenne* is botanically a caryopsis, enclosed by palea and lemma, in this paper it will be referred to as a seed. The dry weight of the palea + lemma was calculated as the difference in dry weight between the caryopsis and the seed after the palea and lemma had been removed with a pair of tweezers. Measurements were conducted on 48 seeds per treatment from group 2 ears.

Statistical and chemical analyses

To determine whether mainly the lighter, younger or the heavier, older seeds within the same spikelet change in seed dry weight after removal of the ovules from neighbouring spikelets or removal of the entire spikelets, the frequency distribution of the seed dry weight of the main ear was determined in experiments 1 and 2. If it was mainly the lighter seeds that increased in dry weight one would expect a decreasing standard deviation, an

increasing mean seed dry weight and lower quartile, and an unchanged upper quartile. If the heavier seeds become heavier one would expect an increasing mean seed dry weight, standard deviation and upper quartile, and an unchanged lower quartile. The characteristics of the frequency distribution were determined per replicate and the effects of spikelet removal and nitrogen were tested in an analysis of variance.

All seeds in the main ear from experiment 1 were divided into three groups on the basis of the frequency distribution of seed dry weight: first a group of light seeds with an average dry weight close to the lower quartile, a second group with a seed dry weight close to the upper quartile and a third group containing the rest of the seeds. In each of these groups the nitrogen concentration was determined after reducing the number of replicates from six to three. Nitrogen concentration was determined with a CHN analyser (Elementor vario EL, Elementor Analysensysteme GmBH, Hanau, Germany). Starch was determined titrimetrically after enzymatic hydrolysis (AOAC 1984).

The effects of the several treatments on seed dry weight in experiment 3 were analysed with the method of residual maximum likelihood (REML) (Payne et al., 1987 and references therein), as the design was unbalanced and more than one source of variation was present. Analyses of variance, linear regression and REML were carried out with the GENSTAT statistical package (Payne et al., 1987). Seed set percentage was analysed by a generalised linear regression model, with a logit link function and a binomial distribution (McCullagh & Nelder, 1989).

RESULTS

Seed dry weight and dry matter distribution

Experiment 1 was carried out at a low nitrogen supply, therefore increasing the supply of nitrogen by 125 % at anthesis increased the dry weight of the various parts of the flowering tiller (Table 2). The seed yield per ear was increased by 28% through a 12% increase in seed number per ear and a 16% increase in seed dry weight.

Removing the ovules in 50% of the spikelets did not affect seed set in the remaining spikelets. Seed dry weight of the remaining seeds increased by 15% on average in the main tiller (Table 2) and by 19% in the group 2 tillers. The removal of ovules at anthesis had no effect on the dry weight of stem + rachis and the leaves.

In the second experiment spikelet removal increased the seed dry weight of the remaining seeds in the main tiller more in clone B1 than in clone Mg2 (P=0.063) (Table 3). In clone B1 removal of 1/3 of the spikelets increased seed dry weight by 9% and removal

of 2/3 of the spikelets increased seed dry weight by 15%. In clone Mg2 removal of 1/3 of the spikelets increased seed dry weight by 6%, but 2/3 removal did not significantly increase seed dry weight. The seed set in the remaining spikelets was not affected by spikelet removal. Obviously, the removal of spikelets sharply reduced seed yield and seed number per ear in both clones. Spikelet removal did not influence the dry weight of stem + rachis and leaves (Table 3).

In experiments 1 and 2 the effects on the yield components and the dry matter distribution within the main tiller were similar to those on the group 2 tillers. Because the effects of the removal of all ovules from part of the spikelets in experiment 1 were similar to the effects of the removal of entire spikelets in experiment 2, both treatments will henceforth be referred to as spikelet removal.

In both experiments the dry weight of the palea + lemma was not affected by spikelet removal. In experiment 1 the addition of nitrogen increased palea + lemma dry weight from 433 µg at low nitrogen to 481 µg at high nitrogen. In experiment 2 palea + lemma dry weight was 452 µg in clone B1 and 599 µg in clone Mg2.

Table 2. The yield components and dry matter distribution within the main tiller of spaced plants of *Lolium perenne* as influenced by the addition of nitrogen or the removal of the ovules from 50% of the spikelets at anthesis (exp. 1). The ears had 23 spikelets. Asterisks indicate a statistically significant effect ($P<0.05$).

	Nitrogen level (mg/plant)		
Tiller parameter	160	360	Relative change (%)
Seed yield per ear (mg)	156	200	28 *
Seed number per ear (mg)	97.9	109.8	12 *
Seed weight (mg)	1.59	1.83	16 *
Seed set (%)	63.4	68.9	9 *
Leaves (mg)	240	270	13 *
Stem + rachis (mg)	376	424	13 *
	Ovule removal		
	None	50 %	
Seed set in remaining spikelets (%)	65.6	66.7	2
Seed weight (mg)	1.59	1.83	15 *

Table 3. The yield components and dry matter distribution within the main tiller of spaced plants of *Lolium perenne* as influenced by the removal of 1/3 or 2/3 of the spikelets at anthesis. Clone B1 had 20 spikelets per ear and clone Mg2 16 spikelets per ear (exp. 2). Different letters indicate a statistically significant effect of spikelet removal ($P<0.05$).

Clone	Tiller parameter	Spikelet removal		
		None	1/3	2/3
B1	Seed weight (mg)	1.37 a	1.49 b	1.57 b
	Seed set in remaining spikelets (%)	68.2	69.3	68.7
	Seed yield per ear (mg)	196 c	149 b	80 a
	Seed number per ear (mg)	142.8 c	100.4 b	50.0 a
	Leaves (mg)	280	174	210
	Stem + rachis (mg)	301	295	249
Mg2	Seed weight (mg)	2.30 a	2.44 b	2.37 ab
	Seed set in remaining spikelets (%)	51.5	49.9	55.8
	Seed yield per ear (mg)	135 c	85 b	54 a
	Seed number per ear (mg)	59.4 c	35.9 b	22.8 a
	Leaves (mg)	133	121	126
	Stem + rachis (mg)	258	261	254

Frequency distribution of seed dry weight

Removing 50% of the spikelets in experiment 1 had a stronger effect on the frequency distribution of the dry weight of the remaining seeds at the low nitrogen level than at the higher nitrogen level, although the means were affected similarly (Table 4; Figure 1). Spikelet removal at the low nitrogen level induced a similar relative increase of the lower and upper quartiles. The absolute increase of the upper quartile, however, was larger. Together with the increased standard deviation this indicates that at the low nitrogen level the heavier, older seeds increased stronger in dry weight after spikelet removal at anthesis than the lighter, younger seeds.

At the higher nitrogen level the response was less clear and seemed less differentiated between seeds of different weight within a spikelet (Figure 1). Spikelet removal increased the lower quartile and not the upper quartile but the standard deviation did not change (Table 4).

The results in experiment 1 obtained at the higher nitrogen level agree with the results from clone B1 in experiment 2 (Table 5). Clone Mg2 hardly reacted to spikelet removal. The shape of the frequency distribution of the seed dry weight of Clone Mg2 did not change significantly after a reduction in spikelet number.

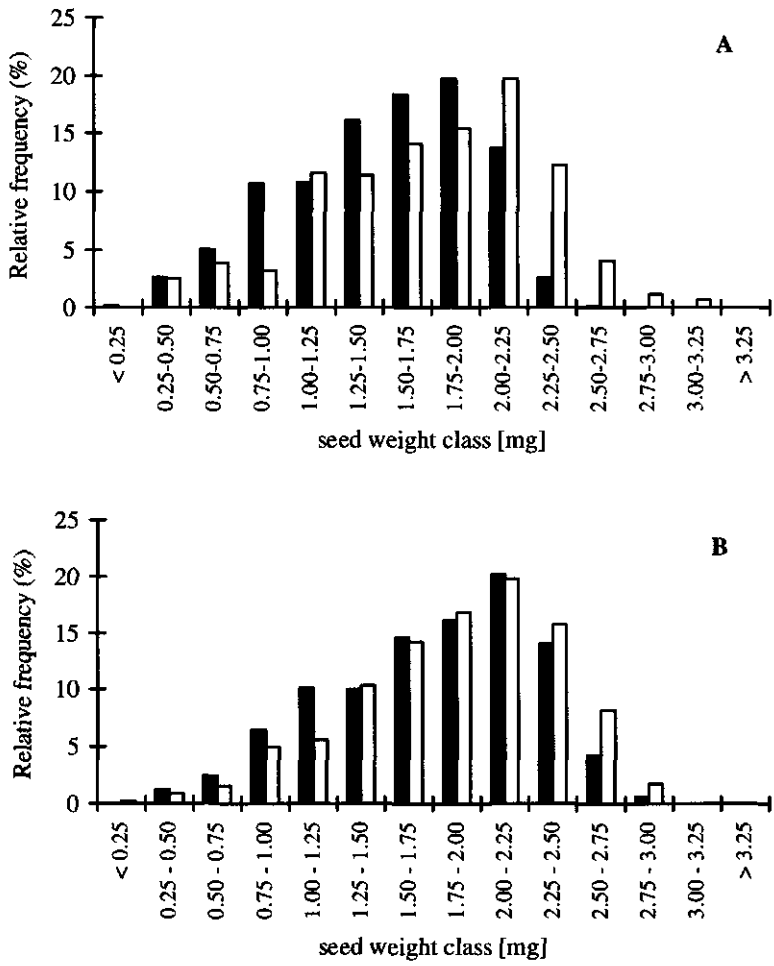

Figure 1. The effect of removal of the ovules from 50 % of the spikelets at anthesis on the relative frequency distribution of the remaining seeds within the main ear of spaced plants of *Lolium perenne* (exp. 1). Removal was carried out at nitrogen levels of 160 mg per plant (A) and 360 mg per plant (B). (■) Control; (□) 50 % ovule removal.

Table 4. Characteristics of the frequency distribution of the seed dry weight in the main ear of spaced plants of *Lolium perenne*, as influenced by the addition of nitrogen and the removal of the ovules from 50% of the spikelets at anthesis (exp. 1). Asterisks indicate a statistically significant effect of ovule removal ($P<0.05$).

Nitrogen level (mg/plant)	Seed weight distribution parameter	Ovule removal		
		None	50%	Relative change (%)
160	Lower quartile (mg)	1.14	1.35	18 *
	Upper quartile (mg)	1.87	2.19	17 *
	Standard deviation	0.48	0.55	15 *
	Mean (mg)	1.47	1.71	16 *
360	Lower quartile (mg)	1.37	1.57	15 *
	Upper quartile (mg)	2.17	2.28	5
	Standard deviation	0.51	0.52	2
	Mean (mg)	1.72	1.94	12 *

Table 5. Characteristics of the frequency distribution of the seed dry weight in the main ear of spaced plants of *Lolium perenne*, as influenced by the removal of 1/3 or 2/3 of the spikelets at anthesis (exp. 2). Different letters indicate a statistically significant effect of ovule removal ($P<0.05$).

Clone	Seed weight distribution parameter	Spikelet removal		
		None	1/3	2/3
B1	Lower quartile (mg)	0.92 a	1.14 ab	1.23 b
	Upper quartile (mg)	1.80	1.93	2.00
	Standard deviation	0.50	0.50	0.46
	Mean (mg)	1.37 a	1.49 b	1.57 b
Mg2	Lower quartile (mg)	1.98	2.10	2.00
	Upper quartile (mg)	2.74	2.83	2.81
	Standard deviation	0.56	0.57	0.57
	Mean (mg)	2.30 a	2.44 b	2.37 ab

The gradient of seed dry weight within a spikelet as affected by shading

In experiment 3 the average moisture concentration of the seeds at harvest was 45 % at 100 % light and 49 % at 25 % light, so ripening was not affected by the shading treatment. In both clones the average seed dry weight did not differ between the four selected spikelets in the untreated ears, which shows that the selected spikelets were uniform.

Linear regression analysis showed that the decrease of seed dry weight towards the tip of the spikelet was linear in clone B1 (R^2 adj. = 97 %) but not in clone Mg2 (Figure 2). In clone Mg2 the following model was used (R^2 adj. = 99 %):

$$y = ymax - s * (p - 1)^2 \quad (mg)$$

where y is the seed dry weight, ymax is the dry weight of the proximal seed, s is a slope parameter and p is the position within the spikelet.

Shading after onset of anthesis did not affect seed dry weight strongly (Figure 2). Reducing the light intensity by 75 % lowered the dry weight of the proximal seed by 10 % in clone B1 and by 9 % in clone Mg2. The dry weight of the proximal seed was higher in clone Mg2 than in clone B1. The slope parameters were not affected by shading. In absolute terms all seeds were affected similarly by shading in clone B1 but in clone Mg2 the effect decreased towards the spikelet tip. In relative terms however, this means that the more distal seeds in clone B1 were more affected by shading than the more proximal seeds (linear model). According to the non-linear model the relative effect of shading was similar for all seed positions within the spikelet in clone Mg2.

Shading reduced seed set in the four central spikelets in the untreated ears from 68 % to 57 % in clone B1, but in clone Mg2 seed set remained at 64 %.

Sink-sink relations within a spikelet

In experiment 3 the final model from the REML analysis indicated an interaction between light intensity, treatment (ovule removal) and position within the spikelet in the effect on seed dry weight. These effects were not strong; seed dry weights increased by 12 % on average (Table 6).

At high light the removal of ovules and thus the reduction of the number of seeds per spikelet did not influence the dry weight of the remaining seeds, an exception being a decrease at position 6 in clone B1. At low light however, mainly the younger, central seeds increased in dry weight if they grew alone.

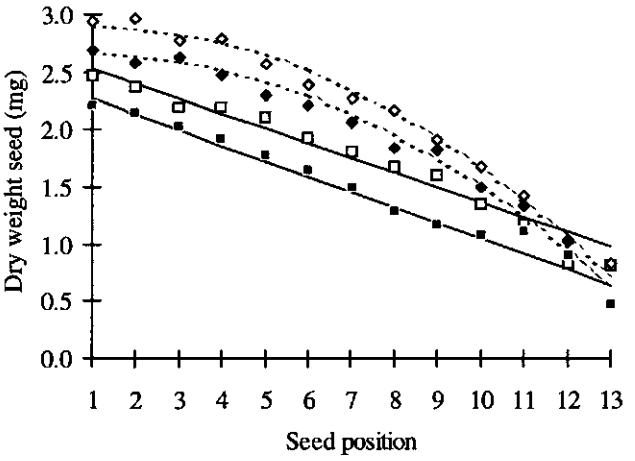

Figure 2. The effect of shading by 75 % on the gradient in seed dry weight within the central spikelets in the ear of two genotypes of *Lolium perenne* grown as spaced plants. Position 1 is the proximal, oldest seed and position 13 the distal, youngest seed. (□, ■) clone B1; (--◇--, ---◆--) clone Mg2; open symbols, 100 % light; closed symbols, 25 % light.

Nitrogen concentration

Increasing the nitrogen supply in experiment 1 increased the nitrogen concentration in all three seed groups (Table 7). Spikelet removal at the higher nitrogen level did not increase the nitrogen concentration in the seeds. At the lower nitrogen level the nitrogen concentration in the lighter seeds increased more strongly after spikelet removal than in the other two seed weight groups. With the exception of the untreated ears at low nitrogen, the differences between seed groups were not statistically significant.

In experiment 2 the nitrogen concentration in the seeds was not affected by spikelet removal. It remained 2.2 % for clone B1 and 2.6 % for clone Mg2.

Ovule removal within a spikelet increased the nitrogen concentration in the remaining seed irrespective of position or light intensity in experiment 3, although the effects were not strong. At 100 % light the nitrogen concentration increased on average from 2.3 % to 2.4% and at 25 % light it increased from 2.5 % to 2.6 %.

Table 6. The effect of ovule removal, light intensity and genotype on the dry weight (mg) of remaining seeds in different positions within the spikelet of *Lolium perenne* (exp. 3). Two seeds in either the proximal positions 1 and 2 or in the central positions 6 and 7 were maintained in the spikelet, while from the other florets the ovules were removed at anthesis. Asterisks indicate a statistically significant effect (P<0.05).

Light intensity	Clone	Position within the spikelet	Seed dry weight (mg)		
			Control	Ovule removal	Relative change (%)
100%	B1	1	2.45	2.52	2.9
		2	2.37	2.43	2.5
		6	1.97	1.79 *	-9.1
		7	1.81	1.84	1.7
	Mg2	1	2.94	2.98	1.4
		2	2.96	2.98	0.7
		6	2.42	2.30	-5.0
		7	2.31	2.17	-6.0
25%	B1	1	2.18	2.35 *	7.8
		2	2.16	2.15	0.5
		6	1.61	1.80 *	11.8
		7	1.46	1.62 *	11.0
	Mg2	1	2.70	2.65	-1.9
		2	2.60	2.53	-2.7
		6	2.16	2.37 *	9.7
		7	2.00	2.29 *	14.5

Starch

Spikelet removal in experiment 1 reduced the starch concentration in the remaining seed at low nitrogen (by 7.7 %), but did not do so at the higher nitrogen level (Table 8). Due to the increase in dry weight the amount of starch in the seeds was not reduced at low nitrogen after spikelet removal. The addition of nitrogen did not increase the starch concentration but it did increase starch amount in the seeds as seed dry weight was higher (Table 2).

Table 7. The effect of the addition of nitrogen and the removal of the ovules from 50% of the spikelets at anthesis on the final nitrogen concentration (mg/g) in three groups of seeds within the main ear of spaced plants of *Lolium perenne* (exp. 1). Asterisks indicate a statistically significant effect of ovule removal (P<0.05).

		Nitrogen concentration in seeds (mg/g)		
		Ovule removal		
Nitrogen supply (mg/plant)	Seed weight group	None	50%	Relative change (%)
160	Light	20.4	25.9	27 *
	Medium	21.5	24.6	14 *
	Heavy	22.2	25.1	13 *
360	Light	26.7	28.3	6
	Medium	27.3	28.4	4
	Heavy	27.4	28.5	4

Table 8. The concentration (mg/g) and amount (μg) of starch in the seeds within the main ear of spaced plants of *Lolium perenne* as influenced by the addition of nitrogen and the removal of the ovules from 50 % of the spikelets at anthesis (exp. 1). Asterisks indicate a statistically significant effect (P<0.05).

		Ovule removal	
	Nitrogen level (mg/plant)	None	50%
Starch concentration	160	350	323 *
	360	335	346
Starch amount	160	516	552 *
	360	579	667 *

DISCUSSION

The data show that the assimilate partitioning and the relations between seeds in the ear of *L. perenne* are already largely established at anthesis. Factors controlling ear development determine the ovule dry weight at anthesis and thus to a large extent the final seed dry weight (Chapter 5). After anthesis only a limited increase in seed dry weight remains possible, e.g. by manipulation of sink-sink relations.

The increase in average seed dry weight in response to seed removal is far from proportional to the decrease in the number of seeds present in the ear. Spikelet removal increased the average seed dry weight of the remaining seeds at most by 16% in clone B1 (Table 4) and by 6 % in clone Mg2 (Table 3). Within the central spikelets a reduction of the seed number by a factor 2.9 (from 7.8 to 2.7) increased seed dry weight by 12% on average, but only under low light and in some positions (Table 6). These results support the findings that the availability of assimilates and the relative growth rate do not differ between individual seeds in the intact ear (Chapter 5). Final seed dry weight is largely determined by factors within the seed itself. The ovule dry weight at anthesis is the main factor, accounting for 60 % of the differences in seed dry weight within the ear (Chapter 5). Furthermore, ear photosynthesis contributes largely to the carbon supply to the seeds and 40 - 50 % of ear photosynthesis is accounted for by the palea and lemma in *L. perenne* (Ong et al., 1978).

The reasons for the increase in the dry weight of the seeds remaining after a reduction of seed number may differ for seeds in different spikelets and seeds in the same spikelet. In view of the results a distinction has to be made between treating entire spikelets (exps. 1 & 2) and the removal of some ovules within two central spikelets (exp. 3). The former treatment did affect the dry weight of the remaining seeds under optimal conditions (ample light and nutrients) whereas the latter treatment did not (Tables 3, 6). Seed set in the remaining part of the ear was not affected by spikelet removal (Tables 2, 3).

Seeds in different spikelets

The amount of carbon assimilates in the flowering tiller does not limit seed growth (Chapter 2). The minor effect of 75 % shading on seed dry weight in experiment 3 confirms this (Figure 2). Furthermore, the results from Chapter 5 have shown that the availability of sucrose and nitrogen, the relative growth rate and the rate of starch accumulation of each individual seed do not differ within the ear of L. perenne. This indicates that both the assimilate availability and the capacity of the vascular connections are not limiting within the ear.

From the increase in the dry weight of the seeds remaining after spikelet removal it can be inferred that the growth rate of these seeds has increased, because the duration of growth was the same. Under optimal conditions in the intact ear the seeds did not grow at their maximum rate. Because the treatments started at anthesis any effects on the ovule dry weight will have been minor and thus the relative growth rate will have increased. The increases in seed dry weight were caused by changes in the dry weight of the caryopsis itself, as the dry weight of palea + lemma did not change after spikelet removal.

We hypothesise that spikelet removal triggers an increase in the growth rate of the remaining seeds. This implies that there is an effect on the seeds themselves, leading to a greater sink strength of the seeds, resulting from an effect on sink size (cell number) and/or sink activity (i.e. an effect on sink metabolism; Ho, 1988). Drastic measures such as spikelet removal are needed to actually increase the assimilate flux into the seeds.

The mechanism behind this greater sink strength is unclear. In wheat Jenner (1980) found an increase of 91% in the amount of sucrose and of 252% in the amount of soluble amino-N in the rachis halfway through ripening after reducing the number of spikelets to four. In the present experiments we did not measure the sucrose and nitrogen concentrations of the rachis just after the treatments started. An abundance of carbohydrates in plants tends to favour expression of genes for utilisation of these carbohydrates (Koch, 1996). Clearly, sugars can exert a coarse (long term) control over sink metabolism, thus influencing carbon fluxes within the plant (Farrar, 1992).

A second possible mechanism might be an increased availability of nitrogen leading to a greater sink strength. Radley (1978) found that the removal of the proximal two seeds *within* each spikelet in wheat resulted in increased seed dry weight, rate of starch synthesis, nitrogen concentration and cell number in the endosperm of the third seed. It is unclear whether nitrogen causes the increase in cell number. Donovan (1983) found no effect of an increase in N supply on endosperm cell number in wheat ears on liquid culture.

Comparing the effect of spikelet removal on seed dry weight at different nitrogen supplies at anthesis suggests that nitrogen may not be the factor triggering a higher growth rate in the remaining seeds. The effect of spikelet removal on seed dry weight was independent of the nitrogen supply (Table 4). Only the seeds with the lowest nitrogen concentration did not respond to spikelet removal.

At low nitrogen supply there was a gradient in the nitrogen concentration within the spikelet from the heavier, proximal seeds to the lighter, distal seeds in the control ears of clone B1 (Table 7). After spikelet removal it was mainly the heavier, proximal seeds that responded with an increase in dry weight and not the lighter, distal seeds; this is clear from the frequency distributions of seed dry weight (Figure 2; Table 4). At the higher nitrogen supply there was no gradient in the nitrogen concentration within the spikelet and all seeds increased in dry weight to a similar extent (Figure 2; Table 4).

Finally, a direct hormonal signal after spikelet removal triggering an increased growth rate in the remaining seeds, cannot be excluded.

Averaged across all seeds in a spikelet, the removal of spikelets did interact with nitrogen supply with regard to starch synthesis (Table 8). Spikelet removal reduced the starch concentration at low nitrogen, but not at high nitrogen. At both nitrogen levels the seed dry weight (averaged across the spikelet) increased to a similar extent (Table 4). This differential response of starch synthesis and total dry matter of the seeds might be related to the timing of starch synthesis and cell division in the seeds. Endosperm cell division occurs first and then starch is deposited in these cells (Elgersma & Sniezko, 1988). At low nitrogen the shortage will have increased during seed growth and might thus have affected starch synthesis more than cell division and dry weight.

The effect of spikelet removal on seed dry weight and on final nitrogen concentration were not correlated (Table 7), confirming the conclusions of Jenner (1980) and Swank et al. (1982) that nitrogen and carbon import by the seeds are regulated independently.

Spikelet removal does not always lead to a higher dry weight of the remaining seeds in *L. perenne* (Table 5; Marshall & Ludlam, 1989) or in wheat (Slafer & Savin, 1994); it depends on genotype. Perhaps genotypical differences can be explained by the extent to which seeds grow at their maximum rate in the intact ear. Clone B1 reacted more strongly to spikelet removal than clone Mg2 did (Table 5). The average seed dry weight of clone B1 was lower than of clone Mg2 (Figure 2). This means that the seed growth rate was also lower in clone B1 than in clone Mg2 as the duration of growth was the same.

Seeds within the same spikelet

The removal of some ovules within two central spikelets is quantitatively a minor manipulation at ear level compared with the removal of at least 1/3 of the spikelets. Only minor changes, for instance in the concentration of assimilates in the rachis, will have resulted. This could explain the absence of an effect on the growth rate of the remaining seeds within the spikelet at high light intensity (Table 6). Under low light intensity the effects within a spikelet seem related to the seed position towards the rachis and competition for stem carbohydrate reserves.

Within a spikelet the proximal seeds are closer to the rachis, which means that they are closer to the carbohydrate reserves that are located mainly in the stem (Chapter 4). Under low light conditions the seeds of *L. perenne* depend more on stem carbohydrate reserves for their growth (Chapter 2). The proximal seeds are the first to draw on these reserves because of their position, and by doing so reduce the availability of reserves to the central

seeds. This can explain the positive effect of removal of all other seeds on the dry weight of the central seeds at low light intensity (Table 6).

At high light intensity the contribution of stem carbohydrate reserves to seed growth will be smaller and that of current photosynthesis will be greater. Then the presence of other seeds in the spikelet does not influence the dry weight of either proximal or central seeds (Table 6). This is in accordance with the large contribution of the palea and lemma to ear photosynthesis (Ong et al., 1978).

Our results indicate that the capacity of the vascular connections within the spikelet do not limit the assimilate supply to the more distal seeds. The relative increases in the nitrogen concentration (Table 7) and amount (data not shown) were much greater in the lighter, more distal seeds than in the heavier, more proximal seeds. However, the results of experiment 3 show that under conditions of sharply reduced photosynthesis when the seeds within the spikelet depend greatly on stem reserves, the structure or architecture of the vascular connections is limiting to the central seeds. The proximal seeds then are closer to the source.

Because the proximal seeds seem to monopolise reserves from the central seeds at low light it would be expected that shading would affect the central and more distal seeds more than the proximal seeds. This is true for clone B1 but not for clone Mg2 (Figure 2). In clone Mg2 shading did not affect the central seeds more than the proximal ones, but the central seeds did benefit from the removal of the proximal seeds from the spikelet at low light (Table 6). Possibly the presence of proximal seeds in a spikelet also has a direct growth-reducing effect on the younger, central seeds, apart from the competition for carbohydrate reserves at low light. Bangerth (1989) hypothesised that the sequence of sink/seed development determines the dominance effect. The older seeds might then directly inhibit growth of the younger seeds by inhibiting the export of auxin from the younger seeds. However, at high light the central seeds did not benefit from the removal of the proximal seeds in the spikelet which does not support the presence of such a dominance effect in *L. perenne*.

Within a spikelet there also seems to be no relation between the final nitrogen concentration in the seeds and the effect of ovule removal. At both light intensities and in all seed positions the nitrogen concentration increased when ovule number in the spikelet was reduced at anthesis, but seed dry weight did not always increase (Table 6).

Overview

The present work shows that the assimilate partitioning and the sink-sink relations between seeds in the ear of *L. perenne* are already largely determined at anthesis. Factors that

control early ear development determine the ovule dry weight at anthesis and thereby to a large extent determine the final seed dry weight. The partitioning of assimilates after anthesis seems to be determined by the sequence of differentiation of the florets during ear development, resulting in a gradient in seed dry weight along the ear. After anthesis only a limited increase in seed dry weight remains possible. The presence of the proximal seeds in a spikelet does not inhibit growth of the more distal seeds and therefore does not affect seed yield of perennial ryegrass. Increasing seed yield by manipulations after anthesis is not feasible.

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REFERENCES

Anslow, R.C., 1964. Seed formation in perennial ryegrass. II Maturation of seed. *Journal of the British Grassland Society* 19: 349-357.

AOAC, 1984. Williams S., (ed). Official methods of analyses of the association of official analytical chemists, The William Byrd Press Inc, Richmond, Virginia, USA.

Aspinall, D., 1963. The control of tillering in the barley plant. II. The control of tiller-bud growth during ear development. *Australian Journal of Biological Sciences* 16: 287-304.

Bangerth, F., 1989. Dominance among fruits/sinks and the search for a correlative signal. *Physiologia Plantarum* 76: 608-614.

Bremner, P.M & H.M. Rawson, 1978. The weights of individual grains of the wheat ear in relation to their growth potential, the supply of assimilate and interaction between grains. *Australian Journal of Plant Physiology* 5: 61-72.

Donovan, G.R., 1983. Effect of nitrogen concentration on endosperm DNA content and cell number in wheat ears grown in liquid culture. *Australian Journal of Plant Physiology* 10: 569-572.

Elgersma, A., 1990a. Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). *Plant Breeding* 105: 117-125.

Elgersma, A., 1990b. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Elgersma, A., & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S:59-68.

Evans, L.T., J. Bingham & M.A. Roskams, 1972. The pattern of grain set within the ears of wheat. *Australian Journal of Biological Science* 25:1-8.

Farrar, J.F., 1992. The whole plant: carbon partitioning during development. In: Pollock C.J., J.F Farrar & A.J.Gordon (eds.) *Carbon partitioning within and between organisms*. Oxford: Bios Scientific Publishers, pp.163-179.

Hampton, J.G. & P.D. Hebblethwaite, 1983. Yield components of the perennial ryegrass (*Lolium perenne* L.) seed crop. *Journal of Applied Seed Production* 1: 23-25,

Ho, L.C., 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 355-378.

Horeman, G.H., 1989. Effects of fungicides on perennial ryegrass seed production. In: *Proceedings of the XVIth International Grassland Congress*, 1989. Nice, France. pp. 667-668.

Jenner, C.J., 1980. Effects of shading or removing spikelets in wheat: testing assumptions. *Australian Journal of Plant Physiology* 7: 113-121.

Klinck, H.R. & S.L. Slim, 1976. The influence of source of photosynthate and sink size on grain yield in oats (*Avena sativa* L.). *Annals of Botany* 40: 785-793.

Koch, K.E., 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*. 47: 509-540.

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in Lolium perenne L. *Annals of Botany* 63:19-27.

McCullagh, P. & J.A. Nelder, 1989. Generalized linear models. 2nd edition Cambridge: Cambridge University Press.

Meijer, W.J.M., 1985. The effect of uneven ripening on floret site utilization in perennial ryegrass seed crops. *Journal of Applied Seed Production* 3: 55-57.

Miralles, D.J. & G.A. Slafer, 1995. Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. *Field Crops Research* 43: 55-66.

Ong, C.K., K.E. Colvill & C. Marshall, 1978a. Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L.. *Annals of Botany* 42: 855-862.

Payne, R.W., P.W. Lane, A.E. Ainsley, K.E. Bicknell, P.G.N. Digby, S.A. Harding, P.K. Leech, H.R. Simpson, A.D. Todd, P.J. Verrier & R.P. White, 1987. *Reference Manual.Genstat 5 Committee Rothamsted Experimental Station.* Oxford: Clarendon Press.

Peterson, D,M,, 1983. Effects of spikelet removal and post-heading thinning on distribution of dry matter and N in oats. *Field Crops Research* 7: 41-50.

Radley, M., 1978. Factors affecting grain enlargement in wheat. *Journal of Experimental Botany* 29: 919-934.

Rawson, H.M. & L.T. Evans, 1970. The pattern of grain growth within the ear of wheat. *Australian Journal of Biological Sciences* 23: 753-764.

Simmons, S.R. & D.N. Moss, 1978. Nitrogen and dry matter accumulation by kernels formed at specific florets in spikelets of spring wheat. *Crop Science* 18: 139-143.

Slafer, G.A. & R. Savin, 1994. Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Research* 37: 39-49.

Sofield, I., L.T. Evans, M.G. Cook & I.F. Wardlaw, 1977. Factors influencing the rate and duration of grain filling in wheat. *Australian Journal of Plant Physiology* 4: 785-797.

Steiner, A.A., 1984. *The universal nutrient solution.* ISOSC Proceedings of the 6th International Congress on Soilless Culture. Wageningen, the Netherlands, pp. 633-649.

Struik, P.C. & T. Makonnen, 1992. Effects of timing, intensity and duration of pollination on kernel set and yield in maize (*Zea mays* L.) under temperate conditions. *Netherlands Journal of Agricultural Science* 40: 409-429.

Swank, J.C., F.E. Below, R.J. Lambert & R.H. Hageman, 1982. Interaction of carbon and nitrogen metabolism in the productivity of maize. *Plant Physiology* 70: 1185-1190.

Walpole, P.R. & D.G. Morgan, 1973. The effects of floret sterilization on grain number and grain weight in wheat ears. *Annals of Botany* 37: 1041-1048.

CHAPTER 7

GENERAL DISCUSSION

GENERAL DISCUSSION

The work presented in this thesis aimed to elucidate which factors determine seed filling and seed dry weight in perennial ryegrass (*Lolium perenne* L.). The variation in individual seed dry weight within a standing seed crop of perennial ryegrass is considerable. The gap between the number of seeds present just before harvest and cleaning and afterwards is large. Some seeds are lost through shedding but on average 70 % of them are too light and are lost during harvest and cleaning (Meijer, 1985; Horeman, 1989; Elgersma, 1990). This suggests that seed filling and not seed set determines to a large extent the number of harvestable seeds and thus seed yield.

In this thesis several hypotheses were tested. Firstly, seed growth might be limited by the total amount of carbon assimilates available in the flowering tiller. Furthermore the partitioning of assimilates to a seed might be limited by the presence of competing organs, such as the stem or new vegetative tillers and, finally, processes within the ear itself might determine seed filling. Processes within the ear were investigated to clarify which factors contribute to the considerable variation of seed dry weight found within the ear of perennial ryegrass. The rate and duration of growth in several positions within the ear were measured, as well as the availability of assimilates and the rate of starch accumulation. Individual seeds might also compete for assimilates, thereby reducing seed set or dry weight of neighbouring seeds. These possible causes of low and variable seed yield in perennial ryegrass seed crops are further discussed below.

Amount of carbon assimilates

Literature suggests that the amount of carbon assimilates available does not control the seed yield of perennial ryegrass seed crops (Spiertz & Ellen, 1972; Ong et al., 1978; Colvill & Marshall, 1984; Marshall & Ludlam, 1989). The present work has provided evidence that the amount of carbon assimilates does not limit seed filling. It has been shown that the reproductive tiller can support seed yield per ear over a broad range of light intensities during seed filling (Chapter 2). With the exception of the lowest light intensity used (24 %; 1.1 MJ/m^2), seed yield per ear was not affected by light intensity and was supported by stem reserves. Reducing the light intensity from 115 % to 24 % reduced seed yield of the main ear by only 14 % and average seed dry weight by only 4 %. The amount of water-soluble carbohydrates (WSC) in the main stem + rachis, however, was reduced by 37 %.

Taking growth respiration into account (Penning de Vries et al., 1983), it can be caculated that the amount of WSC present in the stem + rachis at final harvest could have supported an extra 37 % of seed yield per ear at 24 % light and an extra 49 % of seed yield

per ear at 115 % light. In another experiment and another genotype grown at full light this extra amount was 29 % (Chapter 3).

Labelling with ^{13}C showed that carbon assimilates fixed before anthesis contributed 16 % to final seed carbon, when correcting for the palea and lemma that are present already at anthesis. The amount of WSC in the stem increased from ear emergence onwards until one week before final harvest, before declining slightly. Part of the label fixed at ear emergence was redistributed from the stem to the seeds (Chapter 3). This confirms that the stem is a temporary storage organ that can support seed filling (Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984; Griffith, 1992). Stem WSC reserves are still abundant at maturity and the seeds do not seem to be able to use the available resources fully. Obviously, a sufficient supply of assimilates is a prerequisite for a high seed yield but apparently the sink organs themselves determine the partitioning of assimilates (Gifford & Evans, 1981; Marcelis, 1996 and references therein), and thus seed yield.

Stem versus ear?

Ryle (1970) suggested that competition between the elongating stem and the developing ear could be a factor depressing seed yield. Furthermore, in a field experiment Clemence & Hebblethwaite (1984) found that during early seed growth the stem was a net sink organ and the ear a net source organ, a situation which reversed during seed filling. This suggests that the elongating stem is a constraint during early seed development but not during seed filling (Clemence, 1982).

The stem is a strong sink organ during its elongation (Ryle, 1970) and elongation proceeds until after the onset of anthesis. In Chapter 3 it was reported that during anthesis ^{13}C-carbon from the leaves was imported by the stem and not by the developing seeds, confirming results obtained by Clemence & Hebblethwaite (1984). It is not clear whether or not this causes a reduction in seed number or yield per ear. However, abortion under non-stress conditions is thought to have genetic and cytological causes associated with outbreeding and not to be caused by competition for assimilates (Elgersma & Sniezko,1988; Marshall & Ludlam, 1989). Stem elongation was not manipulated in this study as the results showed that the stem is a source organ during seed filling (Chapter 3).

Do new vegetative tillers depress seed yield?

The greenhouse experiments with spaced plants in which tiller number at onset of anthesis was manipulated demonstrated that competition between new vegetative tillers and the

seeds for both carbohydrates and nitrogen is not a major cause of low seed yields (Chapter 4). Although young developing tillers draw on the same stem WSC reserves as the seeds do, this does not limit seed yield per ear, as WSC reserves are abundantly available. Stem WSC reserves even increased concomitantly with the increase in the number of younger tillers (Chapter 3). Nitrogen was largely redistributed from vegetative organs to the seeds. At final harvest 40 - 63 % of the nitrogen in the flowering tillers was located in the seeds. This percentage increased with seed yield per ear (different clones) and decreased with an increasing nitrogen supply (Chapters 3, 4). Although strong regrowth of tillers did reduce the amount of nitrogen in the seeds in one clone, yield per ear was not affected (Chapter 4).

Competition for assimilates between seeds and new vegetative tillers has been suggested as a major cause of the low and variable seed yield in perennial ryegrass seed crops (Hampton et al., 1983; Clemence & Hebblethwaite, 1984). Around the time of anthesis the tillering rate may increase, because of the plant's perennial habit (Hebblethwaite et al., 1980; Parsons and Robson, 1981; Elgersma, 1990).

In field experiments, however, there is usually no significant relation between the seed yield and the number of vegetative tillers at final harvest (Figure 1). Only the data from Hebblethwaite and Burbidge (1976) show a weak negative correlation (R^2 adj. = 28 %) between seed yield and the number of vegetative tillers. Some treatments in their study resulted in an abnormally low seed yield, associated with a low number of ears. The low density of flowering tillers could be the cause of the development of new vegetative tillers. Results presented by Spiertz and Ellen (1972) showed that both the number of ears and the number of vegetative tillers increased with an increasing light intensity, leading to a positive correlation between seed yield and the number of vegetative tillers.

In the field a reduced seed yield and an increased growth of new vegetative tillers could very well be the consequence of a common cause, such as lodging, and not causally related.

Processes within the ear

Within the ear there is a large variation in final seed dry weight (Anslow, 1964; Chapter 5). Mainly *within* a spikelet the seed dry weight decreases sharply from the proximal to the distal position. The present work showed a decrease from 1.86 mg to 0.71 mg per seed (Chapter 5). As a result of this variation in seed dry weight within the ear, many seeds will be too light and will not be recovered after harvest and cleaning.

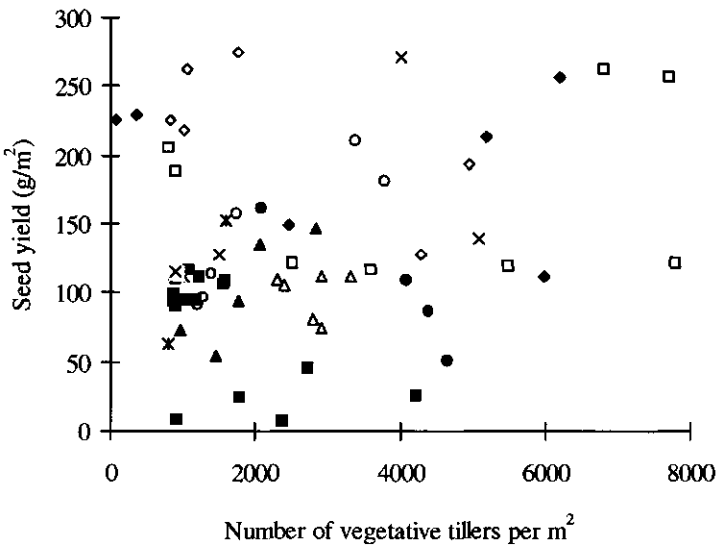

Figure 1. The seed yield of a seed crop of perennial ryegrass related to the number of vegetative tillers at final harvest. Data extracted from: (▲) Hampton, 1986; (□) Hampton & Hebblethwaite, 1984a; (●) Hampton & Hebblethwaite, 1984b; (◆) Hampton & Hebblethwaite, 1985; (■) Hebblethwaite & Burbidge, 1976; (○) Hebblethwaite & Ivins, 1977; (◇) Hebblethwaite et al., 1985; (✳) Spiertz & Ellen, 1972; (+) Wiltshire et al., 1989; (△) Wright & Hebblethwaite, 1979.

Monitoring the seed growth and ripening in 12 different positions in the ear (Chapter 5) revealed that the lighter, more distal seeds in a spikelet grew slower and for a shorter period. About 60 % of the differences in seed dry weight between positions in the ear could be attributed to differences in growth rate and about 30 % to differences in duration of growth.

The lower growth rate of the distal seeds was attributable to a lower ovule dry weight at anthesis and not to a lower relative growth rate. Neither the availability of sucrose and nitrogen nor the ability to convert sucrose into starch could explain differences in dry weight between the seeds in the different positions (Chapter 5). Akpan & Bean (1977) also found a positive correlation between the size of the ovule and seed dry weight in *L. perenne* and *L. multiflorum*. Both decreased with increasing temperature after ear emergence.

The shorter duration of growth was in turn due to the distal florets flowering later, but ripening was concomitant with the proximal seeds (Chapter 5).

Mutual interaction between seeds of different spikelets in the ear of perennial ryegrass is not strong (Chapter 6). In one of the two clones investigated, spikelet removal at anthesis (1/2 or 2/3 of the spikelets) increased average seed dry weight in the remaining spikelets by 16 %. Spikelet removal increased the growth rate of the remaining seeds. The treatment at anthesis is unlikely to have affected the ovule dry weight, and therefore the relative growth rate of the seeds must have increased. The exact cause of this increase is not clear. Competition for assimilates between spikelets seems unlikely in view of the abundance of reserves in the stem (Chapters 2, 3).

Within a spikelet, seeds do compete, but only when the availability of carbon assimilates is reduced by shading by 75 % (Chapter 6). Under such conditions the central seeds grew about 12 % heavier after all other seeds in the spikelet had been removed at anthesis. The proximal seeds did not benefit from the removal of the other seeds within the spikelet. These proximal seeds have better access to the WSC reserves because they are closer to the rachis. This means they can better offset a reduced photosynthetic rate by using reserves, thereby withholding part of these reserves from the central seeds. The capacity of the vascular connections within the spikelet does not seem to be limiting. However, under conditions of sharply reduced photosynthesis when the seeds within the spikelet depend greatly on stem reserves (Chapter 2), the structure or architecture of the vascular connections is limiting to the central seeds. The proximal seeds are then closer to the source than the central seeds (Chapter 6).

Bangerth (1989) hypothesised that the often found dominance effect from an older to a younger fruit (he suggested the term 'primigenic dominance') is determined by the sequence in which these fruits develop. The older seeds would then directly inhibit growth of the younger seeds through inhibition of the export of auxin from the younger seeds, irrespective of the assimilate availability in the plant. However, in the experiments it was found that the central seeds did not benefit from the removal of the proximal seeds in the spikelet at high light, suggesting that no such dominance effect exists in perennial ryegrass.

The weak interaction between seeds in the ear of perennial ryegrass is in accordance with the findings that the availability of assimilates and the relative growth rate do not differ between individual seeds in the intact ear (Chapter 5). The amount of growth is largely determined by factors within the seed itself. A measure for that is the ovule dry weight at anthesis which is the main factor accounting for differences in seed dry weight within the ear. Furthermore, ear photosynthesis contributes largely to the carbon supply to the seeds, and 40 - 50 % of ear photosynthesis is accounted for by the palea and lemma in perennial ryegrass (Ong et al., 1978).

Although the ovule dry weight at anthesis accounts for 60 % of the variation in seed dry weight within the ear, it is unlikely that differences in seed dry weight between different years or genotypes will be solely attributable to ovule dry weight. Obviously, adverse

conditions after anthesis will reduce the growth rate and dry weight of the seeds without affecting the ovule dry weight. The relative growth rate and the duration of growth may very well differ between genotypes or years with, for instance, a different temperature during seed growth. In perennial ryegrass the duration of growth seems more sensitive to temperature than the rate of growth (Shah et al., 1991).

The results mentioned above show that the seed yield of perennial ryegrass is mainly determined by processes within the seed itself. The assimilate partitioning until maturity and the relation between seeds in the ear of perennial ryegrass are largely determined at anthesis. Factors controlling ear development determine the ovule dry weight at anthesis and to a large extent the final seed dry weight within the ear (Chapter 5). The partitioning of assimilates after anthesis seems to be determined by the sequence of differentiation of the florets during ear development, resulting in a gradient in ovule dry weight and thus seed dry weight along the ear.

Implications for yield formation

Although this study was conducted in the greenhouse with spaced plants some implications regarding crop growth may be derived from it.

When a particular seed crop of perennial ryegrass is harvested, the number of seeds that will be obtained after harvest and cleaning determines seed yield. Within a crop there is a large variation in age of the flowering tillers. This means that flowering and maturation in the crop occur over a period. Some losses occur before harvest through the shattering of lighter, ripe seeds. The distal seeds in the upper and central spikelets are shattered first (Elgersma et al., 1988). These losses vary between zero and 15 % of the total seed yield (Andersen & Andersen, 1980; Chapter 1 figure 1).

Losses during harvest and cleaning are determined by a weight limit set by criteria to do with the purity and germination of the seeds. Seeds that are lighter than this weight limit will be lost. During cleaning seeds will have to be separated from dust and other plant parts such as leaf and rachis fragments, empty florets and seeds from other species. Seed cleaning is based on differences in specific gravity, size and shape. With regard to these properties many lighter, but viable seeds overlap with empty florets of perennial ryegrass and with seeds from other unwanted species (H. Nijenstein, pers. comm.) and will thus be lost. Anslow (1964) has shown that lighter seeds, i.e. more distal seeds within a spikelet and seeds from younger, later emerged ears, have a lower germination capacity. Within an ear, the central spikelets have a slightly higher germination capacity than the lower and upper ones. In general the germination capacity is high enough in cleaned perennial

ryegrass samples (R. v.d. Born, pers. comm.). It is the criteria for purity that mainly determine the intensity of the cleaning process and hence the seed losses.

The weight limit set by harvest and cleaning means that a small increase in the dry matter partitioning towards the seeds can result in a strong increase in seed yield if the dry weight of many seeds will be increased beyond this weight limit. The next example will illustrate this.

Figure 2 shows the gradient in seed dry weight in a spikelet with well filled seeds and in a spikelet with seeds that are on average 10 % lighter. These data come from a shading experiment (Chapter 6) but one could imagine them being from two different years or genotypes. The lighter spikelet will yield only 2 seeds after harvest and cleaning, but the heavier spikelet can yield 4 seeds. In this example this would mean a 110 % increase of the seed yield per ear after a 10 % increase in average seed dry weight. Calculations after harvest and cleaning show that on average between one and three seeds per spikelet are recovered after harvest and cleaning (Hebblethwaite and Burbidge, 1976; Hebblethwaite and Ivins, 1977; Wiltshire et al., 1989) and that only very little of the potential seed yield is realised.

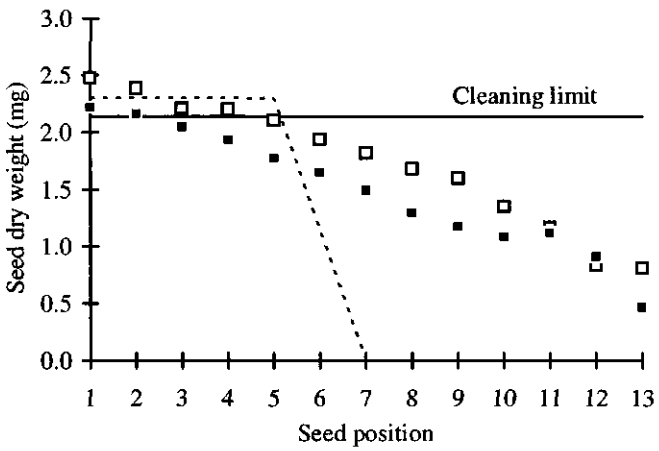

Figure 2. The gradient in seed dry weight within a spikelet of *Lolium perenne* of well filled seeds (□) or seeds that are on average 10 % lighter (■). (----) a more ideal gradient of seed dry weight.

The variable effects of growth regulators or fungicides on seed yield could also be attributable to a slight increase in the seed dry weight of many seeds that otherwise would have been too light. It has been found that the number of seeds per spikelet calculated after harvest and cleaning increased after these chemicals had been applied (Hampton &

Hebblethwaite, 1984c; Hebblethwaite, 1987; Horeman, 1989). The increases in seed yield after the use of fungicides were not related to the prevention of any infection or disease, as these did not occur. Both growth regulators and fungicides have led to a delayed leaf senescence (Hampton & Hebblethwaite, 1984c; Hebblethwaite, 1987; Davies at al., 1988) which suggests that the seeds had a longer growth duration. Horeman (1989), however, found that ripening of the seeds in perennial ryegrass was not affected by fungicide treatments. In spring barley an increased seed dry weight after fungicide treatment resulted from a higher growth rate of the seeds. Dehydration of the seeds was also delayed but only after the maximum seed dry weight had already been reached (Cochrane, 1996).

Growth regulators delay or prevent lodging by decreasing stem length in perennial ryegrass (Hebblethwaite, 1987; Martins, 1990). This could point towards a competition between the elongating stem and the developing ear, as suggested by Ryle (1970). A possible effect on ovule dry weight remains hypothetical, but future research should consider this.

The results presented in Chapter 5 have shown that final seed dry weight is to a large extent determined by factors controlling ear development and thus the ovule dry weight at anthesis. This means that ear and crop development are crucial processes in setting the yield potential of a seed crop. This is illustrated by the fact that older tillers that have developed in autumn and winter, have more spikelets per ear and contribute most to the seed yield the next summer (Griffiths et al., 1973; Hill & Watkin, 1975). Future research should therefore focus on factors affecting ear development, the variation in ear size and the variation in ovule dry weight within and between ears.

Implications for plant breeding

To increase the seed yield per ear of perennial ryegrass a breeder should select for genotypes with more spikelets per ear, fewer florets per spikelet with a different gradient in seed dry weight within the spikelet, and for a longer duration of growth. This is discussed further below.

Given the sharp gradient in seed dry weight within the spikelet (Figure 2) it seems reasonable to expect that genotypes with more spikelets per ear will have a higher seed yield per ear. More spikelets per ear means more proximal, heavier seeds. An obvious prerequisite for successful selection on spikelet number per ear is that the average seed dry weight and seed set are not lower.

The loss of seeds that are too light could be minimised by reducing the gradient in seed dry weight within a spikelet. To achieve this the gradient in ovule dry weight within a spikelet should be less or the distal seeds should ripen later than the proximal seeds,

increasing their duration of growth. Obviously, the absolute level of seed dry weight within a spikelet will affect total seed yield, but it is less critical to seed losses. It is the *variation* in seed dry weight at harvest that largely determines the losses during harvest and cleaning. However, it will be biologically impossible for all seeds in a spikelet to reach a similar final dry weight. The sequence of differentiation of florets that determines the gradient in seed dry weight within a spikelet is to a large extent a fixed plant trait. Therefore distal seeds in a spikelet will probably always be too light to be recovered after harvest and cleaning. The ideal gradient of seed dry weight within a spikelet therefore is horizontal for the first proximal and central positions followed by a sharp decline (Figure 2). The number of florets per spikelet should thereby be reduced; similar to wheat where a spikelet contains about four developed seeds and a few small, apical florets (Rawson & Evans, 1970; Miralles & Slafer, 1995). How many seeds per spikelet with a similar dry weight a genotype will be able to sustain will mainly depend on the duration of growth of the younger seeds relative to that of the older seeds. If there are fewer florets per spikelet the variation in seed dry weight will be less and thus the cleaning process will be more efficient and fewer seed will be lost.

Selecting genotypes with a longer duration of growth would also boost seed yield. More seeds would then be able to attain an adequate dry weight and be recovered after harvest and cleaning.

Comparing clones B1 and Mg2 (Chapter 6) shows that the gradient of seed dry weight within a spikelet is variable. The ovule dry weight in the distal florets was lower in clone B1, but not in the proximal part of the spikelet (Figure 3). The 20 % higher average seed dry weight of clone Mg2 (Chapter 4, 6) implies that the relative growth rate of the seeds of clone Mg2 is higher than that of clone B1. Although the ovule dry weight at anthesis explains most of the differences in seed dry weight within the ear (Chapter 5) this does not mean that differences between clones arise solely from ovule dry weight.

The number of spikelets per ear and the number of florets per spikelet are easy to assess, but in selecting for a higher seed yield a breeder would also benefit from a visible, or easily measurable trait that correlates with the gradient of seed dry weight within a spikelet. Unfortunately, no such trait is available at present.

Conclusion

The present work has shown that seed filling and seed yield of perennial ryegrass are not limited by the amount of assimilates available in the flowering tiller. The stem contains sufficient carbohydrates but the seeds do not fully use these reserves. The large amount of

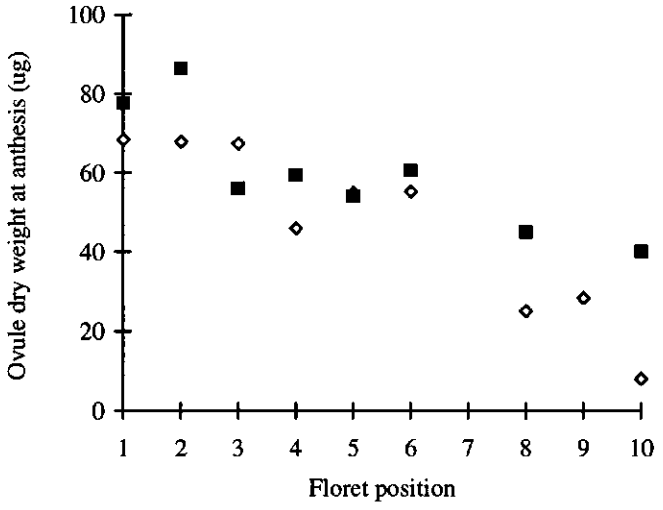

Figure 3. The change in the dry weight of the ovule at anthesis with position in the spikelet. Position 1 is the proximal position and position 10 is the distal position. The number of replicate measurements ranges from one to three. (◇) clone B1; (■) clone Mg2.

stem carbohydrates allows new vegetative tillers to develop around the time of anthesis without any detrimental effect on seed yield per ear, as was shown in spaced plants. Nitrogen is largely redistributed from the vegetative plant parts to the seeds and does not limit seed yield per ear. Within the ear the relative growth rate and the rate of starch accumulation do not differ between the seeds, which means that the main constraint to seed filling lies within the seed itself. The assimilate partitioning and final seed dry weight are already largely determined at anthesis. Factors controlling ear development and thus ovule dry weight at anthesis largely determine seed dry weight and the variation therein. Therefore to increase the seed yield of perennial ryegrass the variation in seed dry weight within an ear and between ears should be minimised, to limit losses of recoverable seeds during harvest and cleaning.

REFERENCES

Akpan, E.E.J. & E.W. Bean, 1977. The effects of temperature upon seed development in three species of forage grasses. *Annals of Botany* 4: 689-695.

Andersen, S. & K. Andersen, 1980. The relationship between seed maturation and seed yield in grasses. In: P.D. Hebblethwaite (ed.) *Seed Production*. London: Butterworths, pp.151-172.

Anslow, R.C., 1964. Seed formation in perennial ryegrass. II Maturation of seed. *Journal of the British Grassland Society* 19: 349-357.

Bangerth, F., 1989. Dominance among fruits/sinks and the search for a correlative signal. *Physiologia Plantarum* 76: 608-614.

Clemence, T.G.A., 1982. Seed production by amenity ryegrass (*Lolium perenne*). PhD thesis, University of Nottingham, School of Agriculture, Nottingham.

Clemence, T.G.A. & P.D. Hebblethwaite, 1984. An appraisal of ear, leaf and stem $^{14}CO_2$-assimilation, ^{14}C-assimilate distribution and growth in a reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Biology* 105: 319-327.

Cochrane, M.P., 1996. Effects of foliar applications of fungicides during plant growth on grain development and grain germinability in spring barley. *Annals of Applied Biology* 128: 21-35.

Colvill, K.E. & C. Marshall, 1984. Tiller dynamics and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology* 104: 543-557.

Davies, T.D., G.L. Stevens & N. Sankhla, 1988. Triazole plant growth regulators. *Horticultural Review* 10:63-105.

Elgersma, A., 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.

Elgersma, A. & R. Sniezko, 1988. Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S: 59-68.

Elgersma, A., J.E. Leeuwangh & H.J. Wilms, 1988. Abscission and seed shattering in perennial ryegrass (*Lolium perenne* L.). *Euphytica* S: 51-57.

Gifford, R.M. & L.T. Evans, 1981. Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology* 32: 485-509.

Griffith, S.M., 1992. Changes in post-anthesis assimilates in stem and spike components of Italian ryegrass (*Lolium multiflorum* Lam). I. Water soluble carbohydrates. *Annals of Botany* 69: 243-248.

Griffiths, D.J., H.M. Roberts & J. Lewis, 1973. The seed yield potential of grasses. *Report Welsh Plant Breeding Station* 1973, pp. 117-123.

Hampton, J.G., 1986. The effect of chlormequat chloride application on seed yield in perennial ryegrass (*Lolium perenne* L.). *Journal of Applied Seed Production* 4: 8-13.

Hampton, J.G. & P.D. Hebblethwaite, 1984a. Experiments with vegetative tiller manipulation in the perennial ryegrass (*Lolium perenne* L.) seed crop by the application of growth regulators. *Journal of Applied Seed Production* 2: 1-7.

Hampton, J.G. & P.D. Hebblethwaite, 1984b. The influence of rainfall on paclobutrazol (PP333) response in the perennial ryegrass (*Lolium perenne* L.) seed crop. *Journal of Applied Seed Production* 2: 8-12.

Hampton, J.G. & P.D. Hebblethwaite, 1984c. The effect of fungicide application on seed yield in perennial ryegrass cv. S.24. *Annals of Applied Biology* 104: 231-239.

Hampton, J.G. & P.D. Hebblethwaite, 1985. A comparison of the effects of the growth regulator paclobutrazol (PP333) and flurprimidol (EL500) on the growth, development and yield of *Lolium perenne* grown for seed. *Journal of Applied Seed Production* 3: 19-23.

Hampton, J.G., T.G.A. Clemence & P.D. Hebblethwaite, 1983. Nitrogen studies in *Lolium perenne* grown for seed. IV. Response of amenity types and influence of a growth regulator. *Grass and Forage Science* 38: 97-105.

Hebblethwaite, P.D., 1987. The chemical control of growth, development and yield in *Lolium perenne* grown for seed. *Journal of Applied Seed Production* 5: 54-59.

Hebblethwaite, P.D. & A. Burbidge, 1976. The effect of maleic hydrazide and chlorocholine chloride on the growth, seed yield components and seed yield of S.23 ryegrass. *Journal of Agricultural Science, Cambridge* 86: 343-353.

Hebblethwaite, P.D. & J.D. Ivins, 1977. Nitrogen studies in *Lolium perenne* grown for seed. 1. Level of application. *Journal of the British Grassland Society* 32: 195-204.

Hebblethwaite, P.D., D. Wright & A. Noble, 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). In: P.D. Hebblethwaite (ed.) *Seed Production*, pp. 71-90. Butterworths, London.

Hebblethwaite, P.D. & J.G. Hampton, G.R. Batts & S. Barrett, 1985. The effect of time of application of the growth retardant flurprimidol (EL500) on seed yields and yield components in *Lolium perenne* L. *Journal of Applied Seed Production* 3: 15-19.

Hill, M.J. & B.R. Watkin, 1975. Seed production studies on perennial ryegrass, timothy and prairie grass. I. Effect of tiller age on tiller survival, ear emergence and seedhead components. *Journal of the British Grassland Society* 30: 63-71.

Horeman, G.H., 1989. Effects of fungicides on perennial ryegrass seed production. In: *Proceedings of the XVIth International Grassland Congress*, 1989. Nice, France. pp. 667- 668.

Marcelis, L.F.M., 1996. Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany* 47: 1281-1291.

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in *Lolium perenne* L. *Annals of Botany* 63: 19-27.

Martins, V.M.M., 1990. The effect of plant growth regulators on floret dynamics and seed production of perennial ryegrass (*Lolium perenne* L.). PhD thesis, University of Guelph. 162 pp.

Meijer, W.J.M., 1985. The effect of uneven ripening on floret site utilization in perennial ryegrass seed crops. *Journal of Applied Seed Production* 3: 55-57.

Miralles, D.J., G.A. Slafer, 1995. Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. *Field Crops Research* 43: 55-66.

Ong, C.K., K.E. Colvill & C. Marshall, 1978. Assimilation of $^{14}CO_2$ by the inflorescence of *Poa annua* L. and *Lolium perenne* L. *Annals of Botany* 42: 855-862.

Parsons, A.J. & M.J. Robson, 1981. Seasonal changes in the physiology of S.24 perennial ryegrass (*Lolium perenne* L.). 3. Partitioning of assimilates between root and shoot during the transition from vegetative to reproductive growth. *Annals of Botany* 48: 733-744.

Penning de Vries, F.W.T., H.H. van Laar & M.C.M. Chardon, 1983. Bioenergetics of fruits, and storage organs. In: *Proceedings Symposium Potential Productivity of Field Crops under Different Environments*, pp. 37-59. International Rice Research Institute, Manila, Philippines.

Rawson, H.M. & L.T. Evans, 1970. The pattern of grain growth within the ear of wheat. *Australian Journal of Biological Sciences* 23: 753-764.

Ryle, G.J.A., 1970. Distribution patterns of assimilated ^{14}C in vegetative and reproductive shoots of Lolium perenne and Lolium temulentum. *Annals of Applied Biology* 66: 155-167.

Shah, S.G., C.J. Pearson & A.C. Kirby, 1991. Variable paths to seed production within the Kangaroo valley cultivar of *Lolium perenne*. *Australian Journal of Agricultural Research* 42: 151-159.

Spiertz, J.H.J & J. Ellen, 1972. The effect of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. 'Cropper') and its effects on seed production. *Netherlands Journal of Agricultural Science* 20: 232-246.

Wiltshire, J.J.J., P.D. Hebblethwaite, R.E. Esslemont & D.A. McGilloway, 1989. The use of growth regulator RSW0411 in seed crops of *Lolium perenne* L. *Grass and Forage Science* 44: 139-150.

Wright, D., P.D. Hebblethwaite, 1979. Lodging studies in *Lolium perenne* grown for seed. 3. Cemical control of lodging. *Journal of Agricultural Science, Cambridge* 93: 669 - 679.

SUMMARY

In the Netherlands, 14 different grass species are grown for seed production, with perennial ryegrass (*Lolium perenne* L.), smooth-stalked meadow grass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.) as the main species. About half the acreage is occupied by perennial ryegrass. The largest part of the grass seed production is used for pastures, lawns or sports-fields. Perennial ryegrass is the dominant species in production grasslands in North-western Europe. It has a high persistence and dry matter production, and a good digestibility, intake and palatability. Also cultivars for turf have been developed. The use of perennial ryegrass forces the breeders to largely focus on vegetative traits. Because grasses are commercially propagated by seed, however, the ability to produce sufficient seed is also important for a cultivar to become economically successful. Therefore the breeder is looking for that rare combination of excellent vegetative traits and a sufficient reproductive capability.

The yield of a perennial ryegrass seed crop varies greatly between 1 and 2 Mg/ha, which is mainly (60 - 98 %) attributable to variation in the number of seeds recovered after harvest and cleaning and to a lesser degree to variation in average seed dry weight of the harvested seed. However, within a standing seed crop of perennial ryegrass the variation in individual seed dry weight is considerable. A large gap exists between the number of seeds present just before harvest and cleaning and the number finally recovered. Some seeds are lost through shattering but on average 70 % of them are too light and are lost during the threshing and cleaning operations. This suggests that seed filling and not seed set to a large extent determines the number of harvestable seeds. The variation in seed yield of a perennial ryegrass seed crop is mainly caused by the variation in the number of seeds that reach an adequate dry weight.

The central aim of this study was to identify the factors that determine seed filling and seed dry weight in perennial ryegrass. Several hypotheses were tested. Firstly, the amount of carbon assimilates in the reproductive tiller after anthesis might determine seed yield. Secondly, competing sink organs (the stem or new vegetative tillers) might impede seed filling and thirdly, processes within the ear itself might determine seed filling. Experiments were carried out in the greenhouse with clones grown as spaced plants.

Chemical analysis of the various plant organs indicated that the amount of carbon assimilates in the reproductive tiller was not limiting to seed filling (Chapter 2). It was shown that the reproductive tiller can support seed filling over a broad range of light intensities after anthesis. Reducing the light intensity from 115 % to 24 % (1.1 MJ/m^2) reduced seed yield of the main ear by only 14 % and average seed dry weight by only 4 %. The amount of water-soluble carbohydrates (WSC) in the main stem + rachis, however,

was reduced by 37 %, suggesting that the stem can support seed filling. Although the WSC amount in the stem + rachis at final harvest was strongly reduced at 24 % light it could have supported an extra 37 % of seed yield, taking growth respiration into account. A ^{13}C-labelling experiment under normal light conditions (Chapter 3) showed that part of the carbon fixed at ear emergence was redistributed from the stem to the seeds. Carbon assimilates fixed before anthesis contributed 16 % to final seed carbon. This confirms that the stem is a temporary storage organ that supports seed filling. Stem WSC reserves are abundant until maturity but the seeds do not fully use these reserves.

During stem elongation the stem is a stronger sink organ than the developing ear and the young seeds (Chapter 3). It was suggested in the literature that the elongating stem would be a constraint to seed yield. However, abortion of young seeds under non-stress conditions is thought to have genetic and cytological causes associated with outbreeding and not to be caused by competition for assimilates.

Greenhouse experiments with spaced plants in which tiller number at onset of anthesis was manipulated showed that competition between new vegetative tillers and the seeds for both carbohydrates and nitrogen is not a major cause of low seed yields (Chapter 4). Although young developing tillers drew on the same stem WSC reserves as the seeds did, this did not limit seed yield per ear as WSC reserves were abundantly available. Stem WSC reserves even increased concomitantly with the number of younger tillers (Chapter 3). Nitrogen was largely redistributed from the vegetative plant parts to the seeds and not limiting seed yield per ear (Chapter 4).

Competition for assimilates between seeds and new vegetative tillers has been suggested as a major cause of the low and variable seed yield in perennial ryegrass seed crops. In field experiments, however, there is usually no significant relation between the seed yield and the number of vegetative tillers at final harvest. A reduced seed yield and an increased growth of new vegetative tillers could very well be the consequence of a common cause, such as lodging, and not causally related. The results so far suggest that seed filling is determined by processes within the ear itself.

Within the ear there is a large variation in seed dry weight (Chapter 5). Mainly within a spikelet seed dry weight decreases sharply, viz. from the proximal, basal to the distal, upper position. The present work showed a decrease from 1.86 mg to 0.71 mg per seed. The lighter, more distal seeds in a spikelet grew slower and for a shorter period. About 60 % of the differences in seed dry weight between positions in the ear could be attributed to differences in growth rate and about 30 % to differences in duration of growth. The lower growth rate of the distal seeds was caused by a lower ovule dry weight at anthesis and not by a lower relative growth rate. The availability of sucrose and nitrogen nor the ability to convert sucrose into starch could explain differences in dry weight between the seeds in the various positions. The shorter duration of growth of the distal seeds in its turn was due to

the distal florets flowering later than, but ripening concomitantly with the proximal seeds (Chapter 5). The main factor determining 60 % of the differences in seed dry weight within the ear was found to be the ovule dry weight at anthesis.

Reducing the number of seeds per ear by removing spikelets or seeds within a spikelet did not strongly affect the dry weight of the remaining seeds (Chapter 6). Furthermore, the effect depended on genotype. In one of the two clones investigated spikelet removal at anthesis (1/2 or 2/3 of the spikelets) increased average seed dry weight by 16 % in the remaining spikelets; it had no effect in the second genotype. The weak interaction between seeds in the ear of perennial ryegrass is in accordance with the findings that the availability of assimilates and the relative growth rate do not differ between individual seeds in the intact ear (Chapter 5). Seed growth is largely determined by factors within the seed itself. The assimilate partitioning until maturity and the relation between seeds within the ear of perennial ryegrass are already largely determined at anthesis. Factors controlling ear development and the sequence of differentiation of the florets determine the ovule dry weight at anthesis and thus to a large extent the variation in final seed dry weight within the ear (Chapter 5).

In the General Discussion (Chapter 6) the implications of the results for yield formation and plant breeding are discussed. It is argued that a small increase in assimilate partitioning towards the seeds can have a more than proportional effect on seed yield. This could explain the sometimes strong increases in seed yield found after the use of fungicides in the absence of any infection or disease. In order to increase the seed yield per ear a breeder should select for genotypes with more spikelets per ear, fewer florets per spikelet with a different gradient in seed dry weight and for a longer duration of growth. In this way the variation in seed dry weight could be minimised to limit losses of recoverable seeds during harvest and cleaning.

SAMENVATTING

Fysiologische beperkingen in de zaadgroei bij Engels raaigras (*Lolium perenne* L.).

In Nederland worden 14 verschillende graszaadgewassen geteeld, waarvan Engels raaigras (*Lolium perenne* L.), veldbeemd (*Poa pratensis* L.) en roodzwenk (*Festuca rubra* L.) de belangrijkste zijn. Ongeveer de helft van het areaal graszaad is Engels raaigras. Het meeste graszaad wordt gebruikt voor inzaai van weiden, gazons of sportvelden. Engels raaigras is de meest voorkomende soort in graslanden in Noord-West Europa. Het heeft een hoge persistentie en droge-stof produktie, een goede verteerbaarheid en smaak en een hoge opname. Er zijn ook speciale rassen voor grasvelden ontwikkeld. De veredelaars richten zich vooral op de vegetatieve eigenschappen. De commerciële vermeerdering van rassen vindt echter plaats via zaad en daarom is een voldoende zaadproduktie van belang om van een ras een economische succes te maken. De veredelaar is op zoek naar de zeldzame combinatie van uitstekende vegetatieve eigenschappen en voldoende reproduktieve eigenschappen.

De opbrengst van een zaadgewas van Engels raaigras variëert tussen de 1.000 en 2.000 kg/ha. Deze variatie is vooral toe te schrijven (60 - 98 %) aan variatie in het aantal gewonnen zaden na oogst en schoning en in mindere mate aan variatie in het individueel zaadgewicht. Binnen een staand zaadgewas Engels raaigras is er echter een aanzienlijke variatie in zaadgewicht. Er bestaat een groot verschil tussen het aantal zaden dat aanwezig is vlak voor de oogst en het aantal dat uiteindelijk gewonnen wordt. Een deel van het zaad gaat verloren door uitval, maar gemiddeld 70 % is te licht en gaat verloren tijdens het dorsen en schonen. Dit wijst erop dat zaadvulling en niet zaadzetting grotendeels het aantal oogstbare zaden bepaalt. De variatie in de zaadopbrengst van Engels raaigras wordt hoofdzakelijk bepaald door de variatie in het aantal zaden dat een voldoende gewicht bereikt.

Het hoofddoel van deze studie was de factoren te identificeren die de zaadvulling en het zaadgewicht van Engels raaigras bepalen. Meerdere factoren zijn onderzocht. Ten eerste, de hoeveelheid koolstofassimilaten in de reproductieve spruit zou de zaadvulling kunnen bepalen. Ten tweede, concurrerende organen (zoals de stengel en nieuwe vegetatieve zijspruiten) belemmeren mogelijk de zaadvulling. Ten derde, wellicht bepalen processen binnen de aar zelf de zaadvulling. De experimenten werden in de kas uitgevoerd met klonen opgekweekt als vrijstaande planten.

Chemische analyse van de verschillende plantenorganen gaf aan dat de hoeveelheid koolstofassimilaten in de reproductieve spruit niet beperkend was voor de zaadvulling (Hoofdstuk 2). Over een breed traject van lichtintensiteiten na bloei bleek de reproductieve

spruit in staat de zaadvulling te ondersteunen. Een reductie van de lichtintensiteit van 115 % naar 24 % (1.1 MJ/m^2) verminderde de zaadopbrengst van de hoofdaar met slechts 14 % en het gemiddeld zaadgewicht met slechts 4 %. De hoeveelheid water-oplosbare koolhydraten (WOK) in de stengel + rachis nam echter met 37 % af, hetgeen suggereert dat de stengel de zaadvulling kan ondersteunen. Hoewel de hoeveelheid WOK in de stengel + rachis bij eindoogst sterk afnam bij 24 % licht, zou deze hoeveelheid nog een 37 % extra zaadopbrengst hebben kunnen ondersteunen. Hierbij is gecorrigeerd voor de groeiademhaling. Een ^{13}C-labelling experiment onder normale lichtcondities (Hoofdstuk 3) liet zien dat een deel van de koolstof die was vastgelegd bij aarverschijning weer herverdeeld werd van de stengel naar de zaden. Koolstofassimilaten die waren vastgelegd voor de bloei droegen 16 % bij aan de uiteindelijke hoeveelheid koolstof in de zaden. Dit bevestigt dat de stengel een tijdelijk opslagorgaan is dat de zaadvulling ondersteunt. WOK reserves zijn overvloedig in de stengel aanwezig maar de zaden gebruiken deze niet volledig.

Tijdens de stengelstrekking is de stengel een sterkere sink dan de zich ontwikkelende aar en de jonge zaden (Hoofdstuk 3). In de literatuur is gesuggereerd dat de zich strekkende stengel een beperking zou zijn voor de zaadopbrengst. Abortie van jonge zaden onder stress-vrije omstandigheden wordt echter toegeschreven aan genetische en cytologische oorzaken die samenhangen met kruisbestuiving en niet met competitie om assimilaten.

Kasexperimenten met vrijstaande planten waarin het aantal zijspruiten bij bloei werd gevariëerd, gaven aan dat competitie tussen nieuwe vegetatieve zijspruiten en de zaden om koolhydraten of stikstof geen belangrijke oorzaak kan zijn voor variatie in zaadopbrengsten (Hoofdstuk 4). Hoewel de zich ontwikkelende zijspruiten uit dezelfde stengelreserves putten als de zaden, verlaagde hun groei niet de zaadopbrengst per aar omdat reserves ruimschoots beschikbaar waren. De stengelreserves namen zelfs gelijktijdig toe met het aantal zijspruiten (Hoofdstuk 3). Stikstof werd grotendeels herverdeeld van de vegetatieve plantendelen naar de zaden en was geen beperking voor de zaadopbrengst per aar (Hoofdstuk 4).

In de literatuur is gesuggereerd dat competitie tussen de zaden en nieuwe vegetatieve zijspruiten een belangrijke oorzaak zou zijn voor de lage en variabele zaadopbrengst van Engels raaigras. Uit veldexperimenten blijkt over het algemeen echter geen verband tussen de zaadopbrengst en het aantal vegetatieve zijspruiten bij de eindoogst. Een lage zaadopbrengst en meer vegetatieve zijspruiten zouden zeer wel een gemeenschappelijke oorzaak kunnen hebben, zoals legering, zonder causaal gerelateerd zijn. Tot dusver wijzen de resultaten erop dat de zaadvulling bepaald wordt door processen binnen de aar zelf.

De variatie in zaadgewicht binnen de aar is groot (Hoofdstuk 5). Vooral binnen een pakje neemt het zaadgewicht sterk af van de proximale, onderste naar de distale, bovenste

positie. In de proeven werd een afname van 1.86 mg naar 0.71 mg per zaad gevonden. De lichtere, meer distale zaden groeiden langzamer en korter. Ongeveer 60 % van de verschillen in zaadgewicht tussen de posities in de aar kon worden toegeschreven aan verschillen in groeisnelheid en ongeveer 30 % aan verschillen in groeiduur. De lagere groeisnelheid van de distale zaden werd veroorzaakt door een lager vruchtbeginselgewicht bij bloei en niet door een lagere relatieve groeisnelheid. De beschikbaarheid van sucrose en stikstof noch het vermogen om sucrose om te zetten in zetmeel kon de verschillen in gewicht tussen zaden in de verschillende posities verklaren. De kortere groeiduur van de distale zaden op haar beurt was het gevolg van het feit dat de distale bloempjes later bloeiden maar gelijktijdig afrijpten met de proximale zaden (Hoofdstuk 5). De belangrijkste factor, die 60 % van de verschillen in zaadgewicht binnen de aar verklaarde, bleek het vruchtbeginselgewicht bij bloei te zijn.

Een reductie van het aantal zaden per aar door het wegnemen van hele pakjes of van zaden binnen een pakje had niet veel effect op de achterblijvende zaden (Hoofdstuk 6). Het effect hing ook af van het genotype. In één van de twee onderzochte klonen verhoogde het wegnemen van pakjes (1/2 of 2/3 van de pakjes) het gemiddeld zaadgewicht in de achterblijvende pakjes met 16 %, maar niet in het tweede genotype. De zwakke interactie tussen de zaden in de aar van Engels raaigras is in overeenstemming met het resultaat dat de beschikbaarheid van assimilaten en de relatieve groeisnelheid niet verschillen tussen individuele zaden in de intacte aar (Hoofdstuk 5). De groei wordt hoofdzakelijk bepaald door factoren in het zaad zelf. De verdeling van assimilaten en de relatie tussen zaden binnen de aar van Engels raaigras liggen al grotendeels vast bij bloei. Factoren die de aarontwikkeling en de volgorde van bloemdifferentiatie controleren, bepalen het vruchtbeginselgewicht bij bloei en dus grotendeels de variatie in het zaadgewicht binnen de aar (Hoofdstuk 5).

In de Algemene Discussie (Hoofdstuk 6) worden de implicaties voor de opbrengstvorming tijdens de teelt en voor de veredeling besproken. Daarin wordt aangetoond dat een kleine verhoging van de assimilatenverdeling naar de zaden een meer dan evenredig effect op de zaadopbrengst kan hebben. Dit zou de soms sterke verhogingen in zaadopbrengst kunnen verklaren, die zijn waargenomen na het gebruik van fungiciden zonder dat er infectie of ziekte optrad. Om de zaadopbrengst van Engels raaigras te verhogen zou een veredelaar genotypen moeten selecteren met meer pakje per aar, minder bloempjes per pakje met een afwijkend verloop in zaadgewicht, en een langere groeiduur. Op deze manier zou de variatie in zaadgewicht beperkt kunnen worden en dus de verliezen van oogstbare zaden tijdens oogst en schoning.

CURRICULUM VITAE

Johan Warringa werd geboren op 19 november 1965 te Emmen. Na het behalen van het VWO diploma aan de Christelijke Scholengemeenschap te Emmen studeerde hij in de periode 1984 - 1988 aan de toenmalige Rijks Hogere Landbouwschool te Groningen. Hij behaalde daar het diploma in de studierichting Nederlandse Landbouw, oriëntatie Akkerbouw. In augustus 1988 begon hij met de studie Landbouwplantenteelt aan de Landbouwuniversiteit te Wageningen. In augustus 1991 behaalde hij het doctoraalexamen met als doctoraalvakken Landbouwplantenteelt en Plantenfysiologie. In april 1992 trad hij in dienst van de toenmalige vakgroep Landbouwplantenteelt en Graslandkunde (thans Agronomie) van waaruit hij gedetacheerd werd bij het toenmalige Centrum voor Agrobiologisch Onderzoek (CABO-DLO), het huidige DLO-Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek (AB-DLO). Tot november 1996 werkte hij hier aan het onderzoek dat in dit proefschrift beschreven is.