Is the flag leaf important in perennial ryegrass seed production?

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Abstract

In cereals, the importance of the flag leaf for contributing carbohydrate during seed fill is well documented. However, in ryegrass this relationship is not so clear. This paper reports on an investigation of the effect on seed yield components of reduced photosynthetic capacity to the flag leaf, stem and head of perennial ryegrass (*Lolium perenne*). The hypothesis was that reduced photosynthetic capacity of the flag leaf does not affect seed yield components in perennial ryegrass. Following flowering, photosynthetic capacity was reduced by defoliation or shading the flag leaf, stem, or head of individual tillers. Seed yield components were measured at harvest. Reduced photosynthetically active radiation (PAR) to the flag leaf and stem did not affect thousand seed weight or seed yield when compared with control plants whereas reducing PAR to the head had a significant effect. The seed head itself may be more important than the flag leaf during seed fill, but when the seed head is shaded substantial re-mobilisation of stored carbohydrates can occur.

Keywords: flag leaf, green leaf area, *Lolium perenne*, photosynthetically active radiation, seed fill, seed yield, thousand seed weight, water-soluble carbohydrates

Introduction

In cereals and temperate forage grasses such as perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) water-soluble carbohydrates (WSC) are the major non-structural storage carbohydrate fraction in vegetative tissues (Chatterton *et al*. 1989; Pollock & Cairns 1991; Cairns *et al*. 2002). These carbohydrates, stored in leaves and stems, are mainly in the form of sucrose and sucrose-derived polymers of fructose (fructans) (Borrell *et al*. 1989; Schnyder *et al*. 1993). Whereas simple linear (2→1)-β-linked fructans (inulins) are common in dicotyledons, temperate forage grasses store a more complex mixed-type of fructan where fructose residues are predominantly linked (2→6)-β (Van Laere & Van den Ende 2002). While much is known about the distribution of WSC and their function during vegetative growth of grasses under conditions that limit photosynthesis, such as defoliation (Cairns & Pollock 1988; Morvan-Bertrand *et al*. 1999, 2001; Turner *et al*. 2001), the mobilisation of WSC from vegetative organs to the seed during reproductive development is less well understood. Breeding for increased seed production in the future will require an understanding of the factors that determine the priority for allocation of assimilates during reproductive development and the contribution of carbohydrate from each organ.

The importance of the flag leaf and stem in supplying carbohydrate during grain filling in cereals is well established and it is generally accepted that stems provide temporary storage of assimilates before and after anthesis, and contribute a significant amount of assimilates to the developing grain (Borell *et al*. 1989). In winter barley (*Hordeum vulgare* L.) high molecular-weight WSC comprise approximately 30% (dry weight) of the stem at the time of maximum stem mass (Bonnett & Incoll 1993). In wheat, [^{14}C]carbon labelling studies showed that pre-anthesis carbon was remobilised from the flag leaf into the stem and subsequently remobilised again into the grain during filling (Yang *et al*. 2004).

As in cereals, stems of forage grasses such as ryegrass and tall fescue (Griffith 2000) accumulate high levels of WSC during reproductive development. In perennial ryegrass at final harvest the stem was found to contain 25% (dry weight) WSC (Warringa & Marinissen 1997). Furthermore, Griffith (1992) showed for Italian ryegrass (*Lolium multiflorum* Lam.) that stem assimilates are remobilised to support both seed development and vegetative growth when plant source-sink relations were artificially modified. Also, chemical and mechanical prevention of lodging of perennial ryegrass increased the mobilisation of carbon from the flag leaf to both the reproductive head and the vegetative tillers (Hampton *et al.* 1987).

Therefore, factors that reduce the amount of photosynthetic material present on the plant (lodging, pathogen attack, defoliation) are all likely to influence the amount of carbohydrate that reaches the seed. The competition for photosynthate between plant organs of economic importance and the remaining vegetative structures, that may also support seed fill, is therefore especially important. The aim of this study was to investigate the importance of the flag leaf, stem, and reproductive head in contributing to seed quality by reducing photosynthetic active capacity from flowering through to harvest.

Methods

Perennial ryegrass (cv. Grasslands Commando) was sown in a Wakanui silt loam soil in April 2007 at 7.5 kg/ha with 15 cm row spacing at the AgResearch Lincoln farm, Canterbury, New Zealand (43°40′S, 172°30′E). Plots (9 m x 4 m) were replicated four times. A mineral soil N (sum of ammonium-N and nitrate-N calculated on a dry weight basis) for 0 to 30 cm samples was taken on the 27 September 2007, frozen, and analysed at a commercial laboratory based on a 2M KCl extraction with nitrate-N determined by cadmium reduction and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) colorimetry; and ammonia-N by Berthelot colorimetry. No autumn nitrogen (N) was applied. Spring N was applied as urea with two equal applications, on the 2 and 18 October 2007, giving a total of 150 kg/ha applied N. The plots were irrigated as required to ensure plants were not under moisture stress.

Moddus plant growth regulator (trinexapac-ethyl 250 g/l) at 1.5 l/ha and Opus fungicide (epoxiconazole 125 g/l) at 0.2 l/ha were mixed and applied as one application on 24 October 2007. A tank mix of Amistar (azoxystrobin 250 g/l) at 0.5 l/ha and Proline (prothioconazole 480 g/l) at 400 ml/ha was applied on the 29 November and 20 December 2007.

Post-anthesis, 30 individual tillers per replicate had the blade of the flag leaf removed at the collar (Treatment 1). A control treatment containing 30 tillers with flag leaf blade attached was also tagged. Individual stems (20 per replicate) were enclosed in foil below the head (Treatment 2) as well as the seed head of additional tillers (20 per replicate) (Treatment 3). Five tillers per replicate were also prevented from lodging using ties and wooden stakes (Treatment 4). Tillers were left to develop through to harvest.

At 50% lodging, plant health was assessed using the normalised difference vegetative index (NDVI) and was recorded using a Greenseeker Hand Held^{TM} Optical Sensor Unit attached to a HP iPAQ Personal Digital Assistant (NTech Industries, Inc. Ukiah, California). Lodging was recorded fortnightly from the 1 November 2007 through to harvest. All treatments were hand harvested on the 14 January 2008 at approximately 38% seed moisture content. Tillers were air dried, threshed and cleaned to a 1st Generation Seed Certification standard. Seed yield of the treatment plots was assessed from 1 m^2 quadrats, and separately for marked tillers (g/tiller), number of seeds per tiller and thousand seed weight (TSW) based on 200 seeds per replicate were determined. GenStat (version 10) was used for statistical analysis using a general ANOVA model. Samples were designated as treatments and replicates designated as blocks.

Results

The seed yield of the plots selected for this work averaged 3600 kg/ha. The number of seeds per spikelet was similar (3.0-3.2 seeds per spikelet) for all treatments, including the control treatment, when the flag leaf, stem and head were deprived of PAR during seed fill (Figure 1a). Removing the flag leaf and wrapping the stem gave the highest number of seeds per spikelet. Unsupported tillers reached 50% lodging on the 28th of November and the NDVI reading (0.885) indicated plants were not under stress. Although number of seeds per spikelet in the unlodged treatment was an average of 8% lower than the other treatments, this was not significant and may be an artefact of the small sample size of the unlodged treatment. Blocking all available PAR to the stem and flag leaf had no detrimental effect on TSW when compared with the control treatment (Figure 1b). No statistical difference was observed between lodged and unlodged tillers. In contrast, reducing PAR to the head during seed fill had a significant effect (P<0.01) on TSW.

This TSW result was reflected in seed yield per tiller, which was also decreased when reproductive heads were devoid of PAR during seed fill (Figure 2). A one-tailed t-test confirmed that the treatment with heads shaded was depressed compared with all others (P<0.01), and equates to an average difference of 470 kg/ha of saleable seed. No difference in seed yield was observed when the flag leaf was removed compared with the control treatment.

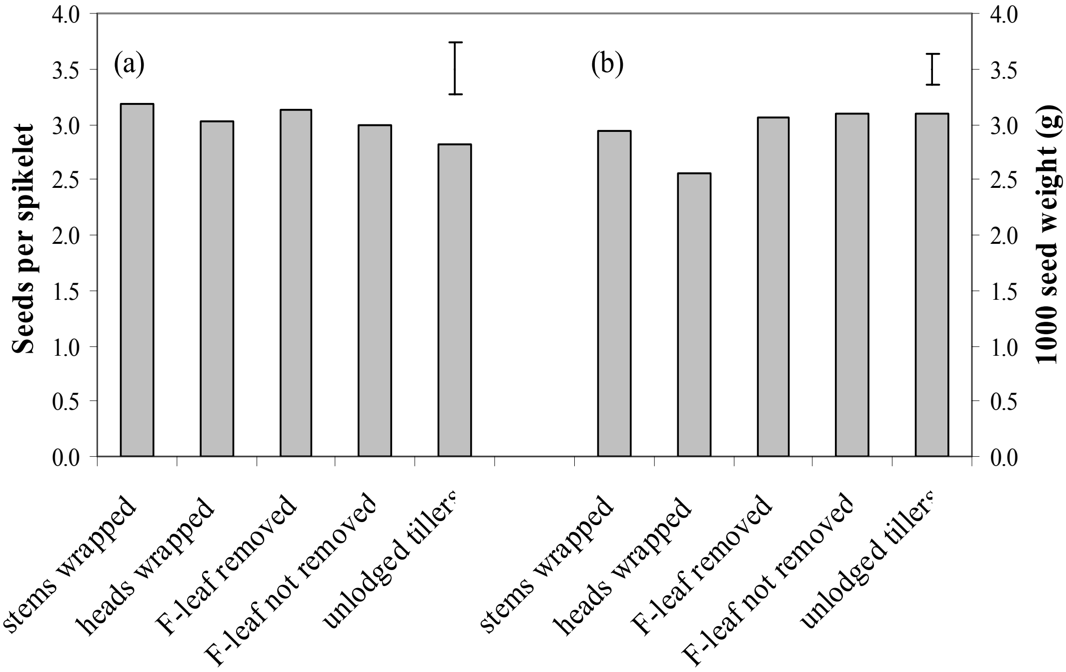

Figure 1 Number of seeds per spikelet (a) and 1000 seed weight (b) for all treatments. Data are a mean of 4 replicates. F-leaf = flag leaf. Bars are LSD (5%)

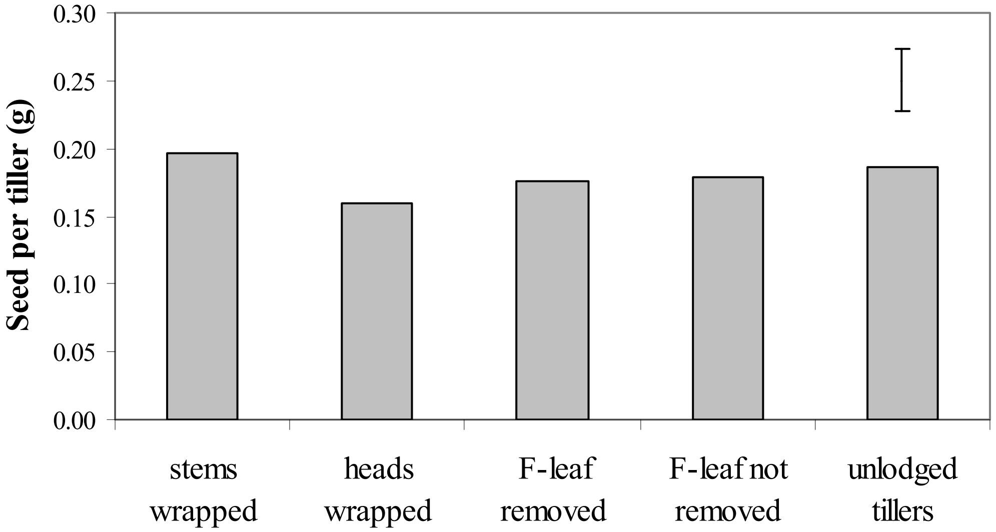

Figure 2 Seed yield per tiller for all treatments. Data are a mean of 4 replicates. F-leaf = flag leaf. Bars are LSD (5%)

Discussion

The two major yield components of perennial ryegrass (*Lolium perenne*) seed crops are the number of seeds per unit area and the average seed weight. In the present study, seed yield and number of seeds per spikelet were high compared with other seed yield and seeds per spikelet data reported for forage ryegrass (Rolston 1995; Rolston & Chynoweth 2006). Research has shown that increasing seed yields are often correlated with an increased number of seeds per spikelet (Chynoweth *et al.* 2010). In this study, TSW was unaffected when the flag leaf and stem were deprived of PAR during seed fill. In contrast, reducing all available PAR to the seed head had a significant effect and decreased average TSW by 16%. Similarly, in a glasshouse experiment, Warringa *et al.* (1998) reported a 10% difference in seed weight when whole tillers of perennial ryegrass were shaded by 75%. These results suggest that shading induced by lodging may have a negative effect on perennial ryegrass seed yields through reduced light interception of the seed head itself.

Lodging is common in perennial ryegrass especially when grown with optimum rates of nitrogen fertiliser (Rolston *et al.* 2007) and is often associated with decreased seed yields (Chynoweth *et al.* 2010). In the present study no difference was observed in seed yield components between lodged and unlodged tillers. However, this may be due to the small sample size and requires further investigation. Lodging has been associated with an increase in the production of vegetative tillers that could act as a sink for WSC and could compete with the seed head for assimilates (Clemence & Hebblethwaite 1984; Hampton *et al.* 1987). Although carbohydrates are remobilised from reproductive tillers during seed filling, the reported contribution to subtending vegetative tissues varies. The results of Warringa and Marrinissen (1997) indicate that after anthesis there is little net exchange of [^{13}C]carbon between main tillers and subtending tillers. Similarly, Matthew (2002) labelled flowering tillers of perennial ryegrass with [^{14}C]carbon followed by different defoliation treatments. Stems and seed heads contained the largest amount of radiocarbon whereas translocation into daughter tillers accounted for only 3.5% of [^{14}C]carbon recovered. However, this small fraction in daughter tillers maybe limited by the relative size of the daughter tillers. In contrast, Clemence and Hebblethwaite (1984) found that while the main export of assimilated carbon moved from the subtending leaf of the flag leaf to the flag leaf and then to the stem, allocation of [^{14}C]carbon to younger tillers increased to 24% during seed development.

Positive correlations have been found between grain yield and flag leaf area for wheat (*Triticum aestivum* L.) (Subhani & Chowdhry 2000; Dere & Yildirim 2006). In the present study, there was no observable difference in seed yield when photosynthetic capacity of the flag leaf and stem were reduced during seed fill. Similarly, Warringa and Kreuzer (1996) found that defoliation or tiller removal from perennial ryegrass after anthesis reduced WSC concentration but did not affect seed yield. Clemence and Hebblethwaite (1984) found that the flag leaf of perennial ryegrass was relatively much more important in perennial ryegrass than in poa (*Poa annua* L.). This result was attributed to there being a much larger flag leaf in ryegrass. Bonnett and Incoll (1993) found that the timing of mobilisation of WSC in barley could be altered and the effect of shading on the reduction of WSC and seed yield was dependent upon the timing of the treatment. Shading whole barley plants to 45% of incident radiation from flowering had a significant negative effect on the mass of the ear. However, the flag leaves of shaded plants lost as much mass and at the same time as unshaded plants. In contrast, shading from 14 days after flowering had no effect on ear mass and, while flag leaves lost as much mass as unshaded plants, this began three days earlier.

The results of the present study in a high yielding perennial ryegrass crop indicate that head photosynthesis contributes explicitly to seed fill. Furthermore, the reproductive head itself maybe more important than the flag leaf in contributing to seed weight and determining tiller seed yield.

Acknowledgements

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