Seed Abortion and Yield in Perennial Ryegrass Following Selective Pre-anthesis Defoliation of Reproductive and Vegetative Tillers

V.M. Mares Martins[1] and E.E. Gamble[1,2]

ABSTRACT

The effects of selective pre-anthesis defoliation and two management levels on floret dynamics and seed yield of perennial ryegrass (*Lolium perenne* L.), were investigated near Elora, Ontario, Canada. Low management (LM) was defined as 60 kg N ha^{-1} applied in the spring. High management (HM) was a composite of 120 kg N ha^{-1}, split between autumn (30 kg ha^{-1}) and spring (90 kg ha^{-1}) applications, 2 kg a.i. ha^{-1} of the plant growth regulator paclobutrazol and 0.25 kg a.i. ha^{-1} of the fungicide propiconazole. Paclobutrazol was applied at spikelet initiation and propiconazole at the beginning of ear emergence. Defoliation (factorial combinations of flag and basal leaf removal from reproductive tillers and removal of subtending vegetative tillers) did not affect seed set which was about 75-80% two days after anthesis. Seed set was higher under HM following some defoliation treatments. Effects on the number of florets per spikelet were inconsistent, varying from reductions following some defoliation treatments under LM to no effects. Seed shedding began 6-8 days after anthesis, but no clear pattern associated with management or defoliation treatment was found. Significant seed abortion (about 30% of seed set at day 2) was recorded at day 10 following anthesis, under all treatments and control. However, abortion increased following some defoliation treatments under both management levels. Seed yield was not affected by management or defoliation treatments. The consistent occurrence of early seed abortion under different source-sink relationships would suggest that it is determined by factors other than nutrition.

Additional index words: **abortion, defoliation, floret shedding, paclobutrazol, seed set, sink competition.**

INTRODUCTION

Photoassimilate in crop plants is unevenly partitioned among metabolic sinks. The physiological mechanism of control of assimilate distribution is unclear. A currently accepted view of regulation of partitioning among alternative routes is based on the concept of competition among sinks of different sizes and strengths, located at different distances from metabolic sources (Cook and Evans, 1978). It is also accepted that increases in crop yield are dependent on the balance of assimilate partitioning between harvestable and non-harvestable crop fractions (Gifford, Thorne, Hitz and Giaquinta, 1984). Studies concerning perennial ryegrass seed crops have shown that carbon compounds produced by photosynthesis in leaves, stems and floral structures move along competing alternative routes to various metabolic sinks. Hampton (1983) found a net movement of assimilate from the flag and penultimate leaves down the stem to the subtending vegetative tillers. Clemence and Hebblethwaite (1984) found that the distribution of current assimilate is dependent upon the stage of development of the reproductive crop. They suggested that the flush of vegetative tiller growth which occurs by peak anthesis (Hebblethwaite, Hampton and McLaren, 1982) can compete for and reduce the flux of assimilate to the seed head. Colvill and Marshall (1984) observed that following anthesis, the lower internodes of the stem, rather than the head, are the major sink for assimilate from the leaves.

Based on the conceptual framework of sink competition and resource limitation, paired to the evidence of differential assimilate allocation, several authors have inferred that seed setting in perennial ryegrass is substrate limited (Hampton and Hebblethwaite, 1985a; Marshall, 1985). However, the notion that assimilate supply governs seed production through seed abortion has recently been challenged by Marshall and Ludlam (1989). They hypothesised that if the degree of seed abortion is related to assimilate supply to the head, improving photosynthetic performance via the control of lodging or by fungicide application might be expected to effect a reduction in seed abortion. They found that seed abortion was not affected by experimental treatment and concluded that the high frequency of abortion in ryegrass may be an inherent characteristic related to the outbreeding system rather than an effect related to assimilate supply. Source-sink relationships can also be altered by defoliation, as shown by Chapin and Wardlaw (1988) who found that removal of alternative sources of assimilate modified photosynthetic rates of the remaining tissues and changed assimilate partitioning in barley plants.

The present study tested the hypothesis that specific defoliation of reproductive tillers and removal of subtending vegetative tillers at anthesis will affect seed abortion and seed yield, by modifying source-sink relationships during early seed development. Also, the study tested the hypothesis that level of management and chemical control of lodging alter the response to defoliation by modifying source-sink interactions.

---

[1] Crop Science Department, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

[2] Corresponding author. Accepted for publication 6 May 1993.

MATERIALS AND METHODS

Two field experiments were conducted at the Elora Research Station, University of Guelph, on a silt loam soil (Typic Hapludalf-grey brown podsolic). Perennial ryegrass cv. Fiesta II, an early maturing turf type, was sown by drill at a rate of 6 kg ha^{-1} in 7 row plots, 7 m long with 18 cm between rows (Exp 1) and in 4 row plots, 7 m long with 36 cm between rows (Exp 2). Exp 1 was sown in August 1987 and Exp 2 in August 1988. Treatment combinations of level of management and patterns of defoliation were imposed to promote qualitative and quantitative changes in source-sink relationships. Two levels of management were imposed. Low management was defined as 60 kg N ha^{-1} applied in the spring. High management was a composite of 120 kg N ha^{-1}, split between autumn (fall) (30 kg ha^{-1}) and spring (90 kg ha^{-1}) applications, 2 kg a.i. ha^{-1} of the plant growth regulator paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol] and 0.25 kg a.i. ha^{-1} of the fungicide propiconazole [1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl)-1H,1,2,4-triazole]. Paclobturazol was applied at spikelet initiation and propiconazole at the beginning of ear emergence. Spikelet initiation was monitored microscopically by dissection of 24 tillers taken randomly and frequently from the experimental area. Defoliation treatments were factorial combinations of removal of the flag leaf (FLR), removal of the basal leaves from reproductive tillers (BLR) and removal of subtending vegetative tillers (DVT). Defoliation was carried out one week before anthesis in both experiments. Experimental units were replicated four times in a split-plot design with management levels as the main blocks and patterns of defoliation as subplots. An undefoliated control was included within each main plot. Subplots were 1 m long sections of the sown rows.

In Exp 1, twenty five phenologically comparable tillers per subplot were tagged at anthesis for subsequent sampling. Five tagged tillers were sampled every second day, starting at day 2 after tagging. Tillers were identified and preserved in FAA (5:5:45:45 10% formalin, glacial acetic acid, 95% ethanol, distilled water) for subsequent counting of developing seeds. From the preserved tillers, data were collected on the number of empty (non fertilised and aborted) and full (developing seed) florets, taking as subsamples one spikelet from each of the top, middle and bottom sections of the spike. This was carried out for each of the five sampling dates during early seed development. Florets were counted as either empty or full, using a stereoscopic microscope providing 64X magnification. A floret was regarded as full when the endosperm looked plump and milky. A floret was counted as empty when the endosperm was absent, watery or collapsed. Small, round and milky ovaries showing evidence of pollen grains in the vicinity were counted as full. Collapsed, watery ovaries were counted as empty.

Subplots were harvested by hand on July 18, 1988 for Exp 1 and July 31, 1989 for Exp 2. Harvested spikes were dried at low temperature (35°C) for four days and the seed was threshed and cleaned before weighing. Seed yield, thousand seed weight (TSW) and percent germination were determined. Data analyses were performed by the methods of analysis of variance and least square regression.

RESULTS

Seed weight

Thousand seed weight was reduced by HM when the flag and basal leaves were removed from the flowering stem (Table 1). In Exp 2, HM significantly reduced TSW regardless of defoliation treatment.

Table 1. Thousand seed weight (TSW) response to low (LM) and high (HM) management level and defoliation treatment (DVT, subtending vegetative tillers; BLR, basal leaves from reproductive tillers; FLR, flag leaf removal).

	Experiment 1		Experiment 2	
	LM	HM	LM	HM
	TSW g			
Control	1.83	1.81	1.98	1.75
DVT	1.83	1.81	1.99	1.92
BLR+FLR	1.89	1.63	1.99	1.64
DVT+BLR+FLR	1.84	1.81	2.12	1.83
DVT+FLR	1.85	1.78	1.82	1.77
DVT+BLR	1.95	1.71	2.07	1.89
FLR	1.85	1.65	1.89	1.71
BLR	1.96	1.68	2.02	1.75
Mean	1.87	1.74	1.98	1.78

LSD ($P < 0.05$) to compare management x defoliation means Exp 1 = 0.14

LSD ($P < 0.05$) to compare management means Exp 2 = 0.13

Total number of florets per spikelet

Significant interactions between level of management and defoliation treatment occurred. At day 2 after anthesis, the mean number of florets per spikelet under LM (9.4) and HM (9.0) did not differ in intact plants. However, for the DVT, DVT+BLR+FLR, and BLR defoliation treatments, the number of florets per spikelet was significantly lower under LM than under HM. Compared to the control, removal of the vegetative tillers and basal leaves significantly reduced the number of florets under LM. Under HM, the defoliation treatments were inconsistent, as the DVT+FLR treatment significantly reduced and the DVT+BLR+FLR treatment increased the number of florets (Table 2). Differences in the number of florets per spikelet at day 2 after anthesis were not related to any particular section of the spike (data not shown).

Table 2. Mean number of florets per spikelet, 2 and 10 days after anthesis, in response to management level and defoliation treatment for Exp 1.

	Day 2		Day 10	
	LM	HM	LM	HM
		number		
Control	9.4	9.0	8.7	8.5
DVT	7.9	9.5	8.2	7.9
BLR+FLR	8.8	9.2	7.7	9.1
DVT+BLR+FLR	8.5	10.2	8.3	8.7
DVT+FLR	8.8	8.2	8.0	8.6
DVT+BLR	8.1	8.7	7.7	8.8
FLR	8.9	8.9	7.8	9.2
BLR	8.6	9.8	9.4	8.6
Mean	8.6	9.2	8.2	8.7

LSD ($P < 0.05$) management x defoliation means = 0.6.

At the initial count (day 2), the number of florets per spikelet in the middle section of the spike (10.2), averaged over both management levels, was significantly higher ($P < 0.01$) than floret numbers in both the apical (7.6) and the basal (8.9) sections, whereas the mean number of florets at the basal section was higher ($P < 0.01$) than at the apical section. The mean number of florets per spikelet decreased gradually over the 10 day period following anthesis, and the difference between the mean initial count (8.9) and the final count (8.5) was significant ($P < 0.01$). The reduction in the number of florets per spikelet was not uniform during the period for management level, defoliation treatment and position on the spike and no meaningful pattern is suggested by the data (which are not presented). However, the reduction started earlier at the bottom and middle section of the spike and was noticeable ($P < 0.05$) at the third sampling date, whereas for the top section a significant reduction occurred at the fourth date. These differences can be attributed to the asynchronous development of sections of the spike.

Seed set and seed abortion

At day 2 after anthesis, the mean percentage seed set in intact plants was similar for both management regimes (Table 3). However, a significant management level x defoliation interaction occurred and the difference between LM and HM was significant ($P < 0.05$) for the DVT, BLR+FLR, and DVT+BLR+FLR defoliation treatments. Compared to the control, defoliation did not affect seed set at day 2, except for the DVT+BLR treatment under LM (Table 3). Seed set at day 2 was significantly lower at the apical section of the spike (74%) than at the other two sections (78%). The percentage of empty florets (number of empty florets, relative to total number of florets at any given date) increased over time, indicating a reduction in seed initially set. Ten days after anthesis, seed set for all management levels and treatments was significantly lower than seed set at day 2 (Table 3).

The reduction in seed set at any given date, relative to seed set at day 2, provides an estimate of the magnitude of abortion. In the absence of defoliation, statistically similar percentages of fertilised florets were aborted under both HM (32%) and LM (25%) during the 10 day period. Compared to the controls, the percentage of aborted seed at day 10 was increased by the BLR+FLR defoliation treatment under LM and the BLR defoliation treatment under HM (Table 4). Abortion occurred gradually over the 10 day period. However, in most cases the abortion percentages at day 6 were not significantly different from the values observed at day 10 (data not presented).

Table 3. Mean seed set at days 2 and 10 after anthesis in response to management level and defoliation treatment for Exp 1.

	Day 2		Day 10	
	LM	HM	LM	HM
		%		
Control	82.5	74.5	66.3	53.5
DVT	84.1	72.1	59.5	58.1
BLR+FLR	87.1	71.1	52.3	66.3
DVT+BLR+FLR	81.4	72.2	64.0	57.3
DVT+FLR	77.5	79.7	60.4	48.0
DVT+BLR	62.9	66.5	64.0	52.6
FLR	80.1	77.8	61.6	60.2
BLR	79.7	79.7	62.2	50.5
Mean	79.5	74.2	61.3	55.8

LSD ($P < 0.05$) to compare management x defoliation means = 8.2.

Table 4. Mean seed abortion at day 10 after anthesis in response to management level and defoliation treatment for Exp 1.

Defoliation	LM	HM
	%	
Control	25.2	31.5
DVT	27.3	31.8
BLR+FLR	47.0	26.9
DVT+BLR+FLR	23.1	30.4
DVT+FLR	28.1	35.2
DVT+BLR	24.1	19.2
FLR	33.0	20.7
BLR	13.6	44.2
Mean	27.7	30.0

LSD ($P < 0.05$) to compare management x defoliation means = 11.8.

Seed yield

Seed yield was not affected by level of management or defoliation treatment in either experiment. The mean seed yield produced in Exp 2 (48.5 g m^{-1}) was 61% higher than seed yield in Exp 1 (30.2 g m^{-1}) and the difference was highly significant. The difference can be attributed to the different row spacing used in the two experiments and also to seasonal differences. For Exp 1 the growing season was rather dry whereas rainfall was not limiting for Exp 2.

DISCUSSION

Seed yield in perennial ryegrass appears not to be limited by initial seed set (successfully pollinated and fertilised florets) which was about 80%. The observed mean seed set 10 days after anthesis (59%) was consistent with the 60-70% seed set eight days after anthesis determined by Hampton and Hebblethwaite (1985a). The virtual absence of any effects of defoliation on initial seed set suggests that the nutrient requirements for pollination and fertilisation are small relative to the total nutrient budget of the plant and that these requirements are possibly satisfied by the photosynthetic activity of the floral organs. Bazzaz and Carlson (1979) have shown that individual flowers of *Ambrosia trifida* L. retained their ability to generate seed after being removed from the parent plant at the time of pollination and that the weight of seed thus produced was comparable to that observed for control plants.

Our data strongly suggest that substantial seed abortion happens earlier than previously suggested (Marshall and Ludlam, 1989) and that a high proportion of the abortion is completed by the end of the first week following pollination. Although the effect of the most severe defoliation treatment (BLR and FLR under low management) suggests that assimilate supply was involved as a factor contributing to differences in seed abortion, the overall occurrence of similar magnitudes of seed abortion under most treatments and controls might be taken as indication that early seed abortion was independent of nutrient supply. An alternative interpretation to explain the occurrence of heavy early abortion in outbreeding perennial species has been recently discussed by Charlesworth (1989). She pointed out that outcrossers are liable to high genetic loads that result from a high rate of mutations to harmful recessive alleles, and suggested that these mutations are eliminated in the earliest stage of development. A mechanism of diplontic selection for preserving the integrity of the genome at the apical meristems, by elimination of mutant cells as they appear, has been proposed by Hardwick (1986). This process guarantees both the stability of the pool of undifferentiated meristematic cells and the elimination of mutants before differentiation and organogenesis. We speculate that a similar mechanism can operate at the zygotic stage, before embryogenesis, even if mutations are not strictly lethal.

The observed progressive reduction in the number of florets per spikelet indicates that some shedding of immature seeds started soon (six to eight days) after anthesis. An early proneness to shedding is consistent with the fact that a primary abscission layer at the base of the floret is already recognisable very early during floral differentiation in grass species, as has been demonstrated in guineagrass (*Panicum maximum* Jacq.) by Weiser, Smith and Varnell (1979) and cocksfoot (*Dactylis glomerata* L.) by Piccirilli and Falcinelli (1989). Earlier differences in floret number among defoliated plants might be attributed to a sudden and temporary stress to the plants, more acute under LM, inducing a premature abscission of some florets. Short term assimilate, water or nitrogen shortages may be involved. In perennial ryegrass, the pattern of assimilate distribution is rapidly reorganised following defoliation in order to support affected tillers, which become assisted by other tillers by way of a re-established interdependence (Gifford and Marshall, 1973).

Lodging in Exp 1 and Exp 2 was consistent with the responses to paclobutrazol reported by Hampton and Hebblethwaite (1985b) and Hebblethwaite, Batts, Barrett and Wiltshire (1986). They indicated that lodging in perennial ryegrass was completely prevented by either 1 or 2 kg ai ha^{-1} applied at spikelet initiation.

The observed decrease in TSW produced by HM was consistent with previous observations following paclobutrazol applications (Hampton and Hebblethwaite, 1984) and could be due to an increased retention of lighter seed which otherwise would be shed. The suggested increased seed retention is consistent with the observed trend of higher seed yields caused by HM in both experiments. Evidence of increased floret and seed retention, brought about by paclobutrazol, has been produced (Mares Martins and Gamble, 1993a,b).

Because seed yield was higher in a moist year but consistently unaffected by either management level or defoliation, the implication is that soil moisture had an overriding effect and that the basal N level (60 kg ha^{-1}) was enough to sustain yield. The recorded lack of effect of level of management on seed yield is consistent with the wide variability of the response of perennial ryegrass to paclobutrazol, as reviewed by Hebblethwaite (1987). Although this variability has been mostly attributed to climatic fluctuations, the complexity of the set of morphological and physiological effects of paclobutrazol leading to either yield increases or decreases, has not yet been elucidated. The lack of effect of defoliation treatments on seed yield suggests that the relative strength of the spike as a metabolic sink is not necessarily increased or decreased by the combined removal of the subtending vegetative tillers and the leaves, other than the flag leaf, from the reproductive tillers. It also suggests that the availability of assimilate for seed production is not impaired by the removal of the flag leaf and all other leaves from the flowering stem, pointing to a high degree of dependency on the spike and stem to provide assimilate for seed development.

ACKNOWLEDGMENTS

Appreciation is given to the International Development Research Centre (IDRC) for the scholarship awarded to the senior author, and to the Ontario Ministry of Agriculture and Food (OMAF) for additional financing of the research.

REFERENCES

1. Bazzaz, F.A. and Carlson, R.W. 1979. Photosynthetic contribution of flowers and seeds to reproductive effort of an annual coloniser. *New Phytologist* 82: 223-232.

2. Chapin, F. and Wardlaw, I.F. 1988. Effect of phosphorus deficiency on source-sink interactions between the flag leaf and developing grain in barley. *Journal of Experimental Botany* 39: 165-177.

3. Charlesworth, D. 1989. Why do plants produce so many more ovules than seeds? *Nature* 338: 21-22.

4. Clemence, T.G.A. and Hebblethwaite, P.D. 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.

5. Colvill, K.E and Marshall, C. 1984. Tiller dynamics

and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology* 104: 543-557.

6. Cook, M.G. and Evans, L.T. 1978. Effect of relative size and distance of competing sinks on the distribution of photosynthetic assimilates in wheat. *Australian Journal of Plant Physiology* 5: 495-509.

7. Gifford, R.M. and Marshall, C. 1973. Photosynthesis and assimilate distribution in *Lolium multiflorum* Lam. following differential tiller defoliation. *Australian Journal of Biological Sciences* 26: 517-526.

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

9. Hampton, J.G. 1983. Chemical manipulation of *Lolium perenne* grown for seed production. PhD Thesis. University of Nottingham.

10. Hampton, J.G. and Hebblethwaite, P.D. 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.

11. Hampton, J.G. and Hebblethwaite, P.D. 1985a. The effect of growth retardant application on floret site utilisation and assimilate distribution in ears of perennial ryegrass cv. S.24. *Annals of Applied Biology* 107: 127-136.

12. Hampton, J.G. and Hebblethwaite, P.D. 1985b. The effect of the growth regulator paclobutrazol (PP333) on the growth, development and yield of *Lolium perenne* grown for seed. *Grass and Forage Science* 40: 93-101.

13. Hardwick, R.C. 1986. Physiological consequences of modular growth in plants. *Philosophical Transactions of the Royal Society, London* B 313: 161-176.

14. Hebblethwaite, P.D., Hampton, J.G. and McLaren, J.S. 1982. The chemical control of growth, development and yield of *Lolium perenne* grown for seed. In: Chemical Manipulation of Crop Growth and Development. (ed. J.S. McLaren), 505-523, Butterworths, London.

15. Hebblethwaite, P.D., Batts, G.R., Barrett, S.K. and Wiltshire, J.J.J. 1986. Growth and seed yield responses to timing of paclobutrazol (PP333) application in *Lolium perenne* L. *Journal of Applied Seed Production* 4: 52-56.

16. 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.

17. Mares Martins, V.M. and Gamble, E.E. 1993a. Floret dynamics in perennial ryegrass in response to chemical manipulation of the seed crop. *Journal of Applied Seed Production* 11: 39-47.

18. Mares Martins, V.M. and Gamble, E.E. 1993b. Responses of two perennial ryegrass cultivars grown for seed to nitrogen, paclobutrazol and propiconazole. *Journal of Applied Seed Production* 11: 64-73.

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

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

21. Piccirilli, M. and Falcinelli, M. 1989. Anatomy of seed dispersal mechanisms in high and low seed shattering cultivars of orchardgrass. *Crop Science* 29: 972-976.

22. Weiser, G.C., Smith, R.L. and Varnell, R.J. 1979. Spikelet abscission in guineagrass as influenced by auxin and gibberellin. *Crop Science* 19: 231-235.