

AN ABSTRACT OF THE THESIS OF
Timothy E. Fiez for the degree of Master of Science in
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Title: GROWTH AND DEVELOPMENT OF THREE MEADOWFOAM

(*Limnanthes* spp.) LINES

Redacted for privacy

Abstract approved: _____

Dr. Gary D. Jolliff

Meadowfoam (*Limnanthes* R. Br. spp.), a source of C₂₀ and C₂₂ fatty acids, is a new industrial oilseed crop adapted to the Willamette Valley of Oregon. Increased oil yield per land area may increase the rate of meadowfoam commercialization. Using two half-sib *L. floccosa* x *L. alba* lines, 85-765 and 85-729, and *L. alba* 'Mermaid', this study was conducted to establish relationships between oil yield, yield components, and agronomical, phenological, and morphological traits. This information may enhance the development of high oil yielding meadowfoam cultivars and cultural practices. This study was also conducted to characterize meadowfoam growth and development, to quantify ethanol soluble carbohydrates and starch present in above-ground plant organs, and to relate growth, development, and carbohydrate quantities to seed yield.

This information is not present in the literature and is also needed to increase meadowfoam oil yield. The three lines were grown in solid stand in 1987-88 and 1988-89 at the Oregon State University Schmidt Farm on an Amity silt loam (fine-silty, mixed, mesic Argiaquic Xeric Agialboll). Line 85-765 produced the greatest oil yield in 1987-88. Seed weight of line 85-765 was 18 and 13% greater than lines 85-729 and Mermaid, respectively, and seed oil content was 18% greater than Mermaid. Both lines 85-765 and Mermaid produced greater oil yields than line 85-729 in 1988-89. Line 85-765 produced a 7% greater seed weight and Mermaid produced 55% more seeds per flower than line 85-729. Seed weight of line 85-765 was also 9% greater than Mermaid in 1988-89, but Mermaid produced 31% more seeds per flower than line 85-765. Seed weight differences were apparently due to variation in seed growth rate and not seed growth duration. Differences in seeds per flower were not related to pollinator activity or flower phenology. Organ dry weights and leaf area indices (LAI) were measured at 13 and 11 intervals after emergence in 1987-88 and 1988-89, respectively. Ethanol soluble carbohydrates and starch were quantified when stem weight peaked and at physiological maturity. There were no differences in predicted mean values of total above-ground and individual organ dry weights and LAI between lines in either season. In both seasons, total above-

ground dry matter peaked between mid and last bloom. During flowering when stem weight peaked, stems and flowers contained 283 and 148 g kg⁻¹ ethanol soluble carbohydrates, respectively, averaged over lines and years. LAI peaked prior to flowering and was less than 0.1 at last bloom each season, while 35 and 40% of the seed fill period occurred after last bloom in 1987-88 and 1988-89, respectively, averaged across lines. Seed yield was correlated with the amount of potentially remobilizable carbohydrate accumulated when stem weight peaked ($r=0.54$). Thus, developing seeds obtain assimilates mainly from sources other than current leaf photosynthesis.

GROWTH AND DEVELOPMENT OF THREE MEADOWFOAM

(*Limnanthes* spp.) LINES

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GROWTH AND DEVELOPMENT OF THREE MEADOWFOAM

(*Limnanthes* spp.) LINES

INTRODUCTION

Increasing public opposition to open field burning which is used to sanitize and remove straw from grass seed production fields continues to spur searches for alternatives to this procedure (Stiak, 1989). One alternative is to produce crops which do not necessitate open field burning. However, on approximately 80,000 ha of the Willamette Valley of Oregon, the poorly drained "white land", annual ryegrass (*Lolium multiflorum* Lam.) is the only currently adapted cash crop (Jolliff et al., 1981).

In 1963, the New-Crops Research Project at Oregon State University began screening plant introductions to identify plants with economic potential that were adapted to the Willamette Valley. Meadowfoam, a herbaceous winter annual, was the only species out of 122 tested that was adapted to the poorly drained soils currently producing annual ryegrass (Jolliff et al., 1981). Meadowfoam is native to California, southern Oregon, and Vancouver Island, British Columbia; within this native range, it is usually confined to very moist areas (Mason, 1952). In

1959, USDA research scientists determined that meadowfoam seed oil contained desirable chemical products (Earle et al., 1959). Meadowfoam seed oil is unique in that it contains the highest amount of Eicosenoic Acid (A 20:1 fatty acid) of any known oil (Jolliff et al., 1981). Overall, C₂₀ and C₂₂ fatty acids comprise over 90% of the fatty acids in meadowfoam seed oil which is the highest amount known for any seed oil. Potential uses of meadowfoam oil include high quality waxes, lubricants, detergents, and plasticizers (Higgins et al., 1971). Meadowfoam vegetation is non-fibrous, and after threshing, only a small amount of fine residue remains. Meadowfoam does not present a residue problem like the currently grown grass crops.

Because of meadowfoam's adaptation to poorly drained soils, its potentially useful seed oil, and its low residue production, the New-Crops Project at Oregon State University has begun to develop meadowfoam into a commercial crop. The rate of meadowfoam commercialization appears to be related to the cost per unit oil. Increased oil yield per land area should lower oil cost; therefore, project research has focused on the development of high oil yielding cultivars and cultural practices. Two cultivars have been named and one released. Research has been conducted in the areas of seeding rates (Calhoun and

Crane, 1978), nitrogen fertilization (Calhoun and Crane, 1978; Pearson and Jolliff, 1986b), megagametophyte development (Franz and Jolliff, 1989), irrigation (Pearson and Jolliff, 1985, 1986a), and pollination (Jahns and Jolliff, 1989).

This thesis project is a continuation of the process of developing meadowfoam into a commercial crop. Two half-sib *L. floccosa* x *L. alba* lines, 85-765 and 85-729, and *L. alba* 'Mermaid' were evaluated and compared using yield component analysis, growth analysis, and ethanol-soluble carbohydrate and starch quantitation. The objectives were to characterize yield components, dry matter production and carbohydrate composition, and to relate these parameters to the relative oil yield performance of lines 85-765, 85-729 and Mermaid. Such knowledge may enhance the development of new higher oil yielding cultivars and cultural practices.

CHAPTER I

YIELD COMPONENTS IN THREE MEADOWFOAM LINES

ABSTRACT

Currently, the meadowfoam (*Limnanthes* R. Br. spp.) cultivar improvement program at Oregon State University relies on yield performance as a selection criteria. Future development of high yielding meadowfoam cultivars may be enhanced if relationships between oil yield, yield components, and agronomical, phenological, and morphological traits can be established. To identify such relationships, two half-sib *L. floccosa* x *L. alba* lines, 85-765 and 85-729, and 'Mermaid' meadowfoam were compared. The three lines were grown in solid stand in 1987-88 and 1988-89 at the Oregon State University Schmidt Farm on an Amity silt loam (fine-silty, mixed, mesic Argiaquic Xeric Agialboll). Line 85-765 produced the greatest oil yield in 1987-88. Seed weight of line 85-765 was 18 and 13% greater than lines 85-729 and Mermaid, respectively, and seed oil content was 18% greater than Mermaid. Both lines 85-765 and Mermaid produced greater oil yields than line 85-729 in 1988-89. Line 85-765 produced a 7% greater seed weight and Mermaid produced 55% more seeds per flower than line 85-729. Seed weight of line 85-765 was also 9% greater than Mermaid in 1988-89, but Mermaid produced 31% more seeds per flower than line 85-765. Seed weight differences were apparently due to variation in seed

growth rate and not seed growth duration. Differences in seeds per flower were not related to pollinator activity or flower phenology. Yields in this study were low compared to historical performances, and this may have prevented the three lines from fully expressing their relative oil yield capabilities. Both seed weight and seed number per flower were important yield components for determining the relative oil yield performance of lines 85-765, 85-729 and Mermaid.

INTRODUCTION

Meadowfoam is a new industrial oilseed crop first grown commercially on a farm-scale in 1984 in the Willamette Valley of Oregon. Meadowfoam oil is a unique source of two long chain (C_{20} and C_{22}) fatty acids (Jolliff et al., 1981). Potential uses include high quality waxes, lubricants, detergents and plasticizers (Higgins, et al. 1971). Meadowfoam commercialization may be enhanced by reducing cost per unit oil. Increased oil yield per land area currently seems to offer the most probable means to lowering costs. The prospects for improved oil yield through cultivar development and improved cultural practices at Oregon State University appear positive. However, resource limitations severely restrict the types of breeding and testing procedures used during the early stages of the development of this new crop. Yield component analysis has been and continues to be very useful in the development of meadowfoam. Such analysis can identify sources of variation in yield which can be capitalized by improving cultivars or cultural practices to result in higher oil yields (Fraser and Eaton, 1983).

Previous studies involving meadowfoam yield components have included studies of irrigation (Pearson and Jolliff, 1986a), planting dates and rates (Johnson et

al., 1980) and nitrogen fertilization (Pearson and Jolliff, 1986b). Yield components have also been used to determine breeding objectives (Jain and Abuelgasim, 1981), to differentiate populations into identifiable races or varieties (Brown et al., 1979), to characterize high yielding populations and to develop selection criteria for breeding programs (Krebs and Jain, 1985).

In this study, we propose to use yield component analysis to identify sources of yield variation in three meadowfoam lines developed at Oregon State University. In 1985-86 and 1986-87, the oil yield of a new *L. floccosa* x *L. alba* line, 85-765, was an average of 672 kg ha⁻¹ compared to 373 kg ha⁻¹ for 'Mermaid', a *L. alba* ssp. *alba* cultivar. During the same period, a half-sib line of 85-765, line 85-729, had an average oil yield of only 281 kg ha⁻¹. The occurrence of genetically related lines with such a wide disparity in performance presents an opportunity to further examine the determinants of meadowfoam oil yield in these progenies. High and low yielding closely related genetic lines can be compared to identify traits associated with yield; this technique was used successfully in rice (*Oryza sativa* L.) (Yoshida, 1972). The similar genetic background of the two half-sib meadowfoam lines may increase the probability of finding traits associated with yield. The objectives of this

study were to evaluate yield components that determine the relative oil yield performance of lines 85-765, 85-729, and Mermaid and to relate yield component differences to agronomical, morphological, and phenological traits.

MATERIALS AND METHODS

Lines 85-765 and 85-729 originated as half-sib F₅ spaced-plants from Oregon Limnanthes (ORL) 83-291. Oregon Limnanthes 83-291 originated as a spaced plant developed by two cycles of random mating between materials within a selection nursery using honey bees (*Apis mellifera* L.) as pollinators. The F₂ (or S₀) population of ORL 83-291 originated from a *L. floccosa* ssp. *grandiflora* x *L. alba* ssp. *alba* interspecific hybrid [OLC-2 x ORL 77-84] (Jolliff, et al., 1984). The extreme divergence of seed yields of lines 85-765 (high) and 85-729 (low) in solid stand was identified in 1986. Lines 85-765 and 85-729 are hereafter referred to as lines 765 and 729.

Field experiments were conducted in 1987-88 and 1988-89 at the Oregon State University Schmidt Farm (44°37'N, 123°13'W) on an Amity silt loam (fine-silty, mixed, mesic Argiaquic Xeric Agialboll). A fallow year preceded experimental plantings each season. Pre-planting soil levels of N, P, and K were determined in the first 30 cm. Nitrogen, P, and K levels were 5, 36, and 160 mg kg⁻¹ in 1987-88 and 6, 44, and 218 mg kg⁻¹ in 1988-89, respectively. Soil pH was 5.6 in 1987-88 and 5.8 in 1988-89. Fifty-four kg ha⁻¹ of N and 29 kg ha⁻¹ of P were pre-plant soil incorporated in both seasons. Fifty-six kg

ha⁻¹ of additional N were broadcast on 23 Feb. 1988 and on 28 Feb. 1989. The three lines were planted in 15-cm rows on 1 Oct. 1987 and 6 Oct. 1988. Planting rates in 1987-88 were adjusted to plant 251 viable seeds m⁻². In 1988-89, all three lines were planted at a 279 seeds m⁻² rate. Propachlor [2-chloro-N-(1-methylethyl)-N-phenylacetamide] was broadcast preemergence at 2.24 kg ha⁻¹ a.i. for weed control. In 1988-89, paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) was also broadcast preemergence at 0.56 kg ha⁻¹ a.i. Honey bee hives were placed next to the experimental plots during flowering at a density of approximately five hives ha⁻¹.

The experiment design was a randomized block with four replications. Individual plots were 3.6 by 6.1 m in 1987-88 and 5.5 by 6.1 m in 1988-89. Biomass and harvestable seed yield were determined by flail-harvesting (Carter Manufacturing, Brookston, IN) a 2.8 m² area on 15 July 1988 and a 5.6 m² area on 27 June 1989. The plot area used for determining biomass and harvestable seed yield was also used to determine lodging. Lodging was rated at last bloom and physiological maturity using a scoring system of 0 to 100 (plants completely flat). A stationary threshing machine (Kurt Pelz, Bonn-Bad Godesberg, West Germany) was used to remove seeds from the flail-harvested plant material. Resulting seeds were

cleaned with a Clipper cleaner (Ferrell-Ross, Saginaw, MI) and dried at 60°C until no further weight loss occurred. Two random 0.14 m² areas were vacuumed in each flail-harvested area to determine harvest loss. In 1988, the vacuum-collected samples were threshed and cleaned the same as the flail-harvested samples. In 1989, the vacuum-collected samples were hand threshed. Seed yield was determined by adding harvest loss to harvested yield.

A random sample of 1000 seeds was taken from the harvested yield to determine seed weight. A random three g sample of seeds was also taken from the harvested yield to determine seed oil content. Seed oil content was determined by pulsed nuclear magnetic resonance using a Bruker Minispec PC 120 equipped with an 18 mm RTa absolute probe head and a EDM 311 program module (Bruker Spectrospin Canada Limited, Milton, Ontario) at the New Crops Research Unit of the USDA-ARS Northern Regional Research Center, Peoria, IL.

A 0.1 m² area was randomly selected in each plot to determine flower phenology and flower numbers. Flowers open by 1100 h were removed and counted each day. Flowers were considered open when a honey bee could physically enter the flower. In both seasons, the reproductive parts of some flowers were damaged, apparently by a fruit fly (*Drosophilidae* family). The number of opened flowers that

had visually damaged reproductive parts were also counted each day. Open undamaged flowers were considered to be viable flowers and are referred to as such. Daily counts of viable flowers were summed to calculate the total number of viable flowers. The number of seeds produced per flower was calculated using the number of viable flowers and harvested seeds per plot. First, mid, and last bloom were considered to be the days that 1, 50, and 99% of the flowers had opened, respectively. After flowering was complete, the remaining unopened flower buds in the selected flower counting areas were removed and counted. Honey bee counts were made once daily during flowering in a marked 0.25 m² area in each plot between 1200 and 1300 h. All bees foraging in the marked areas at the first instant of observation were counted.

Seeds were considered physiologically mature (maximum seed dry weight and oil content) when flower moisture reached 440 g kg⁻¹ fresh weight (Currans and Grabe, 1987). Flower moisture was measured by randomly collecting a 474 cm³ sample of flowers from each plot and drying at 60°C until no further weight change occurred. Flower samples were collected on 1 and 8 July in 1988 and on 16 and 21 June in 1989. Interpolation between the two dates for each year was used to determine the day that flower moisture was 440 g kg⁻¹.

Data for 1987-88 and 1988-89 were combined for analysis of variance (Federer, 1955) unless error variances were not homogeneous. Year was considered a fixed affect. An F-test was used to test the equality of error variances (Snedecor and Cochran, 1980). Because of non-homogeneous error variances, yearly data for viable flowers per area, seeds per flower, and total biomass yield were not combined for analysis. Additionally, yearly dates of first, mid, and last bloom and physiological maturity were also not combined for analysis. Line means within years were compared using Fisher's Protected LSD. The experimentalwise alpha level was 0.05.

RESULTS AND DISCUSSION

The results of the analyses of variance with years combined are shown in Tables I.1 and I.2. The influence of lines on yield and yield components was not consistent over years as evidenced by significant year by line interactions (Fehr, 1987). In 1987-88, oil yield of line 765 was 31 and 52% greater than lines 729 and Mermaid, respectively (Table I.3). In 1988-89, Lines 765 and Mermaid produced oil yields 40 and 53% greater than line 729, respectively. With the exception of Mermaid, oil yields were lower in 1988-89 than in 1987-88. Compared to historical performance, oil yields in this study were low; Mermaid oil yield averaged 408 kg ha⁻¹ in replicated yield trials between 1983-84 and 1986-87.

The components of oil yield are seed oil content and seed yield (Table I.3). In 1987-88, seed oil contents of lines 765 and 729 were 18 and 15% greater than Mermaid. Line 765 also produced a 29 and 28% greater seed yield than lines 729 and Mermaid, respectively. In 1988-89, there were no differences in seed oil content among lines, but seed yields of lines 765 and Mermaid were 33 and 48% greater than line 729, respectively,. Like oil yields, seed yields in this study were low; Mermaid seed yield averaged 1283 kg ha⁻¹ in replicated yield trials between

1974-75 and 1986-87 with a range of 788 to 1760 kg ha⁻¹.

Seed weight and the number of seeds per area determine seed yield (Table I.3). Seed weight of line 765 was 12 and 11% greater than lines 729 and Mermaid, respectively, averaged across years. There were no differences between lines in seeds per area in 1987-88, but in 1988-89, mermaid produced 21 and 50% more seeds per area than lines 765 and 729, respectively.

Seeds per area combines the number of viable flowers per area and the number of seeds per flower. Both lines 765 and 729, the half-sib *L. floccosa* x *L. alba* hybrids, produced more total flower buds per area than Mermaid, the *L. alba* cv. (Table I.4). Due to insect damage and possibly other factors, only 29 and 43% of the flower buds in 1987-88 and 1988-89, respectively, were judged to be viable and able to set seed (Table I.4). The majority of the non-viable flowers were buds that senesced prior to bloom. The fraction of flower buds that senesce prior to bloom in the absence of insect damage is unknown. Lines did not differ in the percentage of viable flower buds, and insect damage was assumed to be equal across lines.

The number of viable flowers per area did not differ among lines in either season (Table I.5). Data for 1987-88 were quite variable (CV=47%) due to non-uniform insect damage. Although the number of viable flowers per area is

a factor in determining the number of seeds per area, they were not correlated ($n=24$). The low number of viable flowers per area contributed to the low seed yields observed in this study. In a N fertilizer study using Mermaid, Pearson and Jolliff (1986b) reported numbers of open flowers per area and seed yields ranging from 7340 m^{-2} and 761 $kg\ ha^{-1}$ to 17860 m^{-2} and 1722 $kg\ ha^{-1}$, respectively.

There were no differences between lines in the number of seeds per flower in 1987-88 (Table I.5). Again, the data were quite variable ($CV=59\%$) due to non-uniform insect damage affecting viable flower numbers. In 1988-89, mermaid produced 32 and 66% more seeds per flower than lines 765 and 729, respectively. Low seed number per flower also contributed to the low seed yields. Mermaid produced an average of 2.2 seeds per flower in the study by Pearson and Jolliff (1986b).

Environmental conditions can affect the number of seeds produced per flower. Air temperatures prior to and during the flowering period have been shown to be able to affect the number of seeds produced per flower in Mermaid (Franz and Jolliff, 1989). Moisture stress may also affect seed production (Brown, 1976; Pearson and Jolliff, 1986a). Within each year of this study, the three lines were exposed to the same environmental conditions during

flowering and seed growth. There were no differences between lines in the dates of first, mid, and last bloom and physiological maturity in either season (data not shown). Either there is a differential line response to environmental conditions during flowering, or the observed variation in the number of seeds per flower within years is due to other factors. Brown (1976) found no taxa by moisture stress interaction when moisture stress reduced seed production per plant in *L. floccosa* ssp. *floccosa*, *L. floccosa* ssp. *grandiflora* and *L. alba*.

In 1988-89, a concurrent study was conducted to evaluate megagametophyte fertilization and embryo abortion. The results from that study suggest that the greater production of seeds per flower by Mermaid in 1988-89 was due to greater megagametophyte fertilization (Franz, Oregon State University New Crops Project, unpublished data). Differences in fertilization may be due to differences in pollination. Because of protandry and a heavy sticky pollen, lines 765, 729 and Mermaid require pollinators for fertilization. It has been shown that increasing pollinator activity can increase the number of seeds per plant (Brown, 1976) and the number of seeds per flower (Jahns and Jolliff, 1989). Analysis of honey bee activity in this experiment showed no differences between seasons or lines (Table I.4); however,

precision was low (CV=48%). The number of bee visits per plot ranged from 2 to 18, and there were no consistent patterns between lines. The correlation between the number of seeds per flower and the number of bee visits was also not significant (n=24). These results may indicate that the observed differences in the number of seeds per flower were due to factors other than pollinator activity.

In addition to seeds per area, seed weight is a determinant of seed yield. Previous studies involving meadowfoam (Jain and Abuelgasim, 1981; Krebs and Jain, 1985) have suggested that seed weight increases as the length of the seed fill period increases. Using the time between mid bloom and physiological maturity as an estimate, there were no differences in the seed fill duration between seasons and lines (Table I.4) while there were differences in seed weight. There was also no correlation between seed fill duration and seed weight (n=24). Therefore, the data suggest that differential rates of individual seed weight accumulation among the lines resulted in the differential mature seed weights.

Lodging has been shown to reduce Mermaid meadowfoam seed weight (McGahuey, 1986). Line 765, which produced the greatest seed weights, had less lodging than Mermaid at last bloom, and both lines 729 and Mermaid at

physiological maturity (Table I.4). In 1987-88, lodging was correlated with seed weight at last bloom ($r=-0.75$, $n=12$) and at physiological maturity ($r=-0.60$, $n=12$); however, similar correlations in 1988-89 were not significant. Lodging scores indicate that lodging occurred later in 1988-89, and this may have reduced effects on seed weight.

Seed weight and seed oil content are sometimes positively correlated in meadowfoam. Johnson et al. (1980), using three *L. alba* collections, and Pierce and Jain (1977), using 35 collections which included four *Limnanthes*. spp., found positive correlations between seed weight and oil content. However, Pierce and Jain (1977) found no correlations across families in four of five populations where family parents were of the same species and collection. Brown et al. (1979) found no correlation between seed weight and oil content across 16 *L. alba* populations. In this study, seed weight and oil content were not correlated ($n=24$).

The low seed yields in this study resulted in low harvest indices (Table I.4); line 765, however, had a greater harvest index than lines 729 and Mermaid. Averaged over years, the harvest index of line 765 was 16.0 and 18.5% greater than line 729 and Mermaid, respectively. Biomass yields differed in 1987-88 but not

in 1988-89 (Table I.5). In 1987-88, lines 765 and Mermaid produced 25 and 20% greater biomass yields than line 729, respectively. Biomass yields were lower for all lines in 1988-89 than in 1987-88. Biomass yield was correlated with seed yield ($r=0.73$) and seed weight ($r=0.57$) but not with seed oil content or the number of viable flowers per area ($n=24$).

Significant year by line interactions were observed for oil yield, seed oil content, seed yield, seed weight and seeds per area (Table I.1). Although years were not combined for statistical analysis, the number of seeds produced per flower among lines was different between years as indicated by statistical significance within years (Table I.5). Weather differences between years may have been important. In 1989, temperatures were warmer prior to flowering, and precipitation was less during flowering (Fig. II.4). Flowering and maturity occurred earlier in 1989 than in 1988. The significant year by line interactions suggest that there may be differential responses by lines to environmental variation.

From previous performance, line 765 was expected to produce greater oil yields than lines 729 and Mermaid in this study. This occurred in 1987-88 but not in 1988-89. As mentioned previously, oil yields in this study were low compared to Mermaid yield trial results from the four

years prior to this study. Under growing conditions which result in low yields, it may be difficult to identify lines which are genetically superior for oil yield.

Both seed weight and the number of seeds produced per flower were important yield components for determining the relative oil yield performance of lines 765, 729 and Mermaid. Increased seed weight was apparently due to increased seed growth rate while increased megagametophyte fertilization apparently increased seed number per flower.

Table I.1. Summary of analyses of variance for oil yield, seed oil content, seed yield, seed weight and seed number per area with years 1987-88 and 1988-89 combined.

Line	df	Oil yield	Seed oil content	Seed yield	Seed weight	Seeds per area
		kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	mg	no. m ⁻²
Year	1	*	NS	*	*	NS
Line	2	**	*	**	**	*
Year x Line	2	**	*	*	*	*
CV (%)		15	6	13	3	12

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

Table I-2. Summary of analyses of variance for lodging scores at last bloom and physiological maturity, total flower buds per area, viable flower buds, seed fill duration, bee visits and harvest index with years 1987-88 and 1988-89 combined.

Source of variation	df	Lodging		Total flower buds per area	Viable flower buds	Seed fill duration	Bee visits	Harvest index
		Last bloom	Physiol. maturity					
		-----	score -----	no. m ⁻²	%	d	no.	%
Year	1	NS	NS	**	*	NS	NS	*
Line	2	**	*	**	NS	NS	NS	*
Year x Line	2	NS	NS	NS	NS	NS	NS	NS
CV (%)		59	46	11	33	15	48	13

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

Table I.3. Oil yield, seed oil content, seed yield, seed weight and seed number per area for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Line	Oil yield	Seed oil content	Seed yield	Seed weight	Seeds per area
	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	mg	no. m ⁻²
<u>1987-88</u>					
765	182	272	669	10.40	6554
729	139	266	520	8.85	5906
Mermaid	120	231	521	9.17	5707
<u>1988-89</u>					
765	127	263	485	8.78	5536
729	91	251	365	8.20	4465
Mermaid	139	256	541	8.08	6704
LSD (0.05)†	30	23	102	0.42	1100

†LSD for comparing lines within years.

Table I-4. Year means averaged across lines and line means averaged across years for lodging scores at last bloom and physiological maturity, total flower buds per area, viable flower buds, seed fill duration, bee visits, and harvest index for years 1987-88 and 1988-89 and lines 765, 729 and Mermaid.

Year or Line	Lodging		Total flower buds per area	Viable flower buds	Seed fill duration	Bee visits	Harvest index
	Last bloom	Physiol. maturity					
	----- score -----		no. m ⁻²	%	d	no.	%
<u>Year</u>							
1987-88	6.7	15.8	13810	29.1	30.7	6.83	9.0
1988-89	1.3	20.8	9583	42.6	30.3	7.25	10.7
F-test†	NS	NS	**	*	NS	NS	*
<u>Line</u>							
765	1.4	10.3	12229	35.0	29.8	7.00	10.9
729	3.7	23.6	12744	34.6	29.9	7.75	9.4
Mermaid	6.8	21.0	10116	38.0	31.8	6.38	9.2
LSD (0.05)‡	2.6	3.0	1418	NS	NS	NS	1.4

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

†F-test for comparing years.

‡LSD for comparing lines.

Table I.5. Viable flowers per area, seeds per flower and biomass yield for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Line	Viable flowers per area	Seeds per flower	Biomass yield
	no. m ⁻²	no.	kg ha ⁻¹
<u>1987-88</u>			
765	4070	1.9	7153
729	4320	1.6	5704
Mermaid	3480	1.8	6835
LSD (0.05)	NS†	NS	536
CV (%)	47	59	5
<u>1987-88</u>			
765	4253	1.3	4073
729	4033	1.1	3871
Mermaid	3865	1.7	5567
LSD (0.05)	NS	0.2	NS
CV (%)	10	9	20

†NS = not significant at P = 0.05.

CHAPTER II

DRY MATTER PRODUCTION, DEVELOPMENT, AND CARBOHYDRATE COMPOSITION IN THREE MEADOWFOAM LINES

ABSTRACT

The literature lacks information on meadowfoam (*Limnanthes R. Br. spp.*) growth, development and carbohydrate composition. This study was conducted to characterize meadowfoam growth and development, to quantify ethanol soluble carbohydrates and starch present in above-ground plant organs and to relate growth, development, and carbohydrate quantities to seed yield. Three lines, 85-765, 85-729 and 'Mermaid', with contrasting seed yield performance were grown in 1987-88 and 1988-89 at the Oregon State University Schmidt Farm on an Amity silt loam (fine-silty, mixed, mesic Argiaquic Xeric Agialboll). Organ dry weights and leaf area indices (LAI) were measured at 13 and 11 intervals after emergence in 1987-88 and 1988-89, respectively. Ethanol soluble carbohydrates and starch were quantified when stem weight peaked and at physiological maturity. There were no differences in predicted mean values of total above-ground and individual organ dry weights and LAI between lines in either season. In both seasons, total above-ground dry matter peaked between mid and last bloom. During flowering when stem weight peaked, stems and flowers contained 283 and 148 g kg⁻¹ ethanol soluble carbohydrates, respectively, averaged over lines and

years. LAI peaked prior to flowering and was less than 0.1 at last bloom each season while 35 and 40% of the seed fill period occurred after last bloom in 1987-88 and 1988-89, respectively, averaged across lines. Seed yield was correlated with the amount of potentially remobilizable carbohydrate accumulated when stem weight peaked ($r=0.54$). Thus, developing seeds obtain assimilates mainly from sources other than current leaf photosynthesis.

INTRODUCTION

Meadowfoam, a new industrial oilseed crop, is a unique source of two long chain (C_{20} and C_{22}) fatty acids (Jolliff et al., 1981). High quality waxes, lubricants, detergents and plasticizers are among the potential uses of meadowfoam oil (Higgins et al., 1971). An increased oil yield per land area appears to be necessary to increase the rate of meadowfoam commercialization. To continue the development of new cultivars and improved agronomic practices, a comprehensive understanding of meadowfoam growth and development is needed (Evans, 1975).

Meadowfoam is grown as a winter annual in the Mediterranean climate of the Willamette Valley of Oregon. After fall seeding, the plant forms a rosette from which flowering stems arise in late winter and spring. Previous studies have shown that growth and development characteristics can influence meadowfoam yield. Jain and Abuelgasim (1981) observed that, within *L. alba* and *L. douglasii*, early flowering populations had the highest seed yield. They also found contrasting plant types associated with higher yield. In producing seed yields greater than *L. alba* populations, *L. douglasii* var. *nivea* flowered early and exhibited higher harvest indices while *L. douglasii* var. *sulphurea* flowered late and exhibited delayed

senescence. Krebs and Jain (1985) sampled biomass and leaf area index (LAI) at four dates and found that higher yields were associated with rapid initial growth and leaf development in *L. douglasii*, while delayed biomass accumulation and less leaf area appeared more favorable to seed production in *L. alba*. We have found that seed yield is positively correlated with biomass production measured at maturity in two *L. floccosa* x *L. alba* lines and *L. alba* 'Mermaid'. While these studies show that growth and development characteristics can influence meadowfoam yield, a comprehensive season-long study to fully characterize meadowfoam growth and development has yet to be reported.

In addition to growth and development characteristics, little is known about the carbohydrate composition of meadowfoam dry matter and the importance of ethanol soluble carbohydrates and starch for meadowfoam seed yield. During the seed fill period, meadowfoam leaf area is very low or non-existent, and stem and flower (less seed) weights decline (unpublished data, New Crops Research Project, Oregon State University). Using I_2KI staining, we have found starch in meadowfoam leaves, stems and flower buds in samples taken at early flowering but not in samples taken at physiological maturity. These observations may indicate that seed yield is enhanced by

the remobilization of ethanol soluble carbohydrates and starch accumulated in other plant parts.

Thus, the objectives of this study were to characterize season-long meadowfoam above-ground dry matter production and development, to quantify ethanol soluble carbohydrates and starch present in leaves, stems, and flowers and to relate dry matter production, development and carbohydrate quantities to seed yield. The study used two half-sib *L. floccosa* x *L. alba* lines, 85-765 and 85-729, and Mermaid meadowfoam. These lines represent a range of seed yield performance with lines 85-765 and 85-729 producing relatively high and low yields, respectively.

MATERIALS AND METHODS

Field experiments were conducted in 1987-88 and 1988-89 at the Oregon State University Schmidt Farm (44°37'N, 123°13'W) on an Amity silt loam (fine-silty, mixed, mesic Argiaquic Xeric Agialboll). A fallow year preceded experimental plantings. Pre-planting soil levels of N, P, and K were determined in the first 30 cm. Nitrogen, P, and K levels were 5, 36, and 160 mg kg⁻¹ in 1987-88 and 6, 44, and 218 mg kg⁻¹ in 1988-89, respectively. Soil pH was 5.6 in 1987-1988 and 5.8 in 1988-89. Fifty-four kg ha⁻¹ of N and 29 kg ha⁻¹ of P were pre-plant soil incorporated each season. Fifty-six kg ha⁻¹ of additional N were broadcast on 23 Feb. in 1988 and on 28 Feb. in 1989.

Lines 85-765, 85-729 (hereafter referred to as lines 765 and 729) and Mermaid were planted in 15 cm rows on 1 Oct. 1987 and on 6 Oct. 1988. Planting rates in 1987-88 were adjusted to plant 251 viable seeds m⁻². In 1988-89, all three lines were planted at a 279 seeds m⁻² rate. Propachlor [2-chloro-N-(1-methylethyl)-N-phenylacetamide] was broadcast preemergence at 2.24 kg ha⁻¹ a.i. for weed control. In 1988-89, paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) was also broadcast preemergence at 0.56 kg ha⁻¹ a.i. Honey bee (*Apis mellifera* L.) hives were placed next to the experimental plots during flowering at

a density of approximately five hives ha^{-1} .

The experiment design was a randomized block with four replications. Individual plots were 3.6 by 6.1 m in 1987-88 and 5.5 by 6.1 m in 1988-89. Emergence was measured in a 0.3 m^2 area selected at random in each plot. Plants were considered emerged when the cotyledons were visible. The day of 50% emergence, 13 and 14 Nov. in 1987 and 1988, respectively, was considered the day of average emergence. These dates were used to calculate days after emergence (DAE).

A 0.1 m^2 area was selected at random in each plot to determine flower phenology and flower numbers. Flowers open by 1100 h were removed and counted each day. Flowers were considered open when a honey bee could physically enter the flower. First, mid, and last bloom were considered to be the days that 1, 50, and 99% of the flowers had opened, respectively.

Seeds were considered to be physiologically mature (maximum seed dry weight and oil content) when flower moisture reached 440 g kg^{-1} fresh weight (Currans and Grabe, 1987). Flower moisture was measured by collecting a 474 cm^3 sample of flowers at random from each plot and drying at 60°C until no further weight change occurred. Flower samples were collected 231 and 238 DAE in 1988 and 214 and 219 DAE in 1989. Interpolation between the two

samples each year was used to determine the day that flower moisture was 440 g kg^{-1} .

To evaluate dry matter accumulation and partitioning, plant samples were taken at random at 57, 71, 96, 110, 127, 138, 152, 166, 180, 194, 208, 222, and 240 DAE in 1987-88. Sample size was 0.15 m^2 , made up of adjacent 0.10 and 0.05 m^2 samples. Plants were divided into leaves (including petiole), stems (including crown), flowers, and seeds. Samples were dried at 60°C until no further weight change occurred. All abscised plant material in the sample area was included in the samples. Dry weights of leaves, stems, flowers and seeds were summed to determine total above-ground dry weight. Leaf area was measured in the 0.10 m^2 sample using an area meter (LI 3100, Li Cor, Lincoln, NE). Only leaves that were more than 50% green were included in leaf area. Samples were also taken at 202, 215, and 229 DAE to measure flower and seed dry matter only. Sample size was 0.05 m^2 for sampling at 202 and 215 DAE and 0.10 m^2 for sampling at 229 DAE. In 1988-89, 0.10 m^2 plant samples were taken at 71, 98, 112, 121, 133, 147, 161, 175, 189, 203, and 220 DAE and divided into plant organs as in 1987-88. Leaf area was also determined from these samples. Additional 0.10 m^2 samples to measure flower and seed dry matter only were taken at 182, 196, and 210 DAE.

Seed yield was measured by machine-harvesting a 2.8 m² area in 1987-88 and a 5.6 m² area in 1988-89 in each plot. Two randomly selected 0.14 m² areas were vacuumed in each machine harvested area to determine harvest loss. Seed yield was determined by adding harvest loss to machine-harvested yield.

Functional growth analysis was used to evaluate above-ground dry matter accumulation. Polynomial regression equations were fitted to natural logarithm transformations of total above-ground dry weight, organ dry weight, and LAI to describe changes over time (Hunt, 1982). Through analyses of covariance, observed variations in leaf and stem dry weights and leaf area from samples taken 57 to 127 DAE in 1987-88 and 71 DAE in 1988-89 were partly attributable to variations in sample plant numbers. Using the error regression coefficients, adjusted means were calculated for these samples (Steel and Torrie, 1980). The adjusted means were used in fitting the polynomial regression equations. Both the significance of the highest order regression term and visual analysis of plots of residuals versus predicted values were used to select regression equations. Second-order equations were chosen for flower and seed dry weight. Third-order equations were chosen for total above-ground dry weight and LAI. Fourth order equations

were chosen for leaf dry weight. Fifth order equations were chosen for stem dry weight. Coefficients of determination exceeded 0.91 for all equations. Ninety-five percent confidence intervals for predicted mean values were calculated for each regression equation, and these were used to determine if regression equations were significantly different from each other within a season (Clawson et al., 1986).

Crop growth rate (CGR) and net assimilation rate (NAR) were derived from regression equations for dry weight and LAI (Hunt, 1982) where

$$\text{CGR} = \frac{1}{P} \frac{dW}{dT} \text{ and } \text{NAR} = \frac{1}{L} \frac{dW}{dT}$$

with P equal to land area, W equal to dry weight, L equal to leaf area and T equal to time. Units for CGR and NAR were $\text{g m}^{-2} \text{ land area day}^{-1}$ and $\text{g m}^{-2} \text{ leaf area day}^{-1}$, respectively. Differences in CGR and NAR were inferred to be significant if differences in regression equations for dry weight and LAI were significant (Clawson et al., 1986; Cox and Andrade, 1988).

Ethanol soluble carbohydrates and starch were quantified at two times: at the observed peak of stem dry weight and at physiological maturity. In 1987-88, plants from the 0.05 m^2 samples used to evaluate dry matter accumulation and partitioning were used to provide samples for carbohydrate analysis. From preliminary studies, stem

weight was expected to peak during flowering. Plants sampled during flowering and at physiological maturity (194, 208, 222, and 240 DAE) were divided into leaves, stems, and flowers immediately after removal from the field and then microwaved (Sharp R-7280, Sharp Electronics Corp., Mahwah, New Jersey) at high power to denature enzymes. Sampling began at 1300 h. Leaves and flowers were microwaved for 30 sec., and stems were microwaved for 120 sec. Samples were then dried at 60°C. To obtain samples for carbohydrate analysis in 1988-89, 0.05 m² samples were taken adjacent to the 0.10 m² samples used to evaluate dry matter accumulation and partitioning during flowering and at physiological maturity (175, 189, 203, and 220 DAE). As in 1987-88, the plants were divided into leaves, stems, and flowers and then microwaved. Seeds were removed from flowers prior to carbohydrate analysis.

Ethanol soluble carbohydrates and starch were quantified according to the procedure of Hassid and Neufeld (1964), with steps added to quantify ethanol soluble carbohydrates. Dried leaves, stems, and flowers less seeds were first ground to pass through a 115 mesh screen. Twenty five to 50 mg samples were used for analysis. Ethanol soluble carbohydrates were extracted using 5 ml of 80°C 80% (v/v) ethanol. After 10 min., the samples were centrifuged at 5000 x g for 10 min., and the

supernatant was decanted and saved. This extraction was repeated once. The combined supernatants were made up to known volume and clarified by adding 0.2 ml of lead acetate (McCready, 1970). Excess lead was removed by the addition of 1 ml of sodium oxalate. The solution was filtered, and ethanol soluble carbohydrates were quantified by the anthrone method. A 0.5 ml sample aliquot was added to 5 ml of anthrone reagent (Hassid and Neufeld, 1964). Sample tubes were placed in a boiling water bath for 12 min. and then cooled in an ice bath. After cooling, absorbance was measured at 620 nm. Ethanol soluble carbohydrate amounts were calculated using a standard curve from D-glucose.

The tissue remaining after ethanol extraction was used to quantify starch. Starch was extracted with perchloric acid and purified using the procedure of Hassid and Neufeld (1964). Extracted and purified starch was quantified by the anthrone method using the same procedure as used for ethanol soluble carbohydrates. Starch amounts were calculated by multiplying values obtained from a D-glucose standard curve by 0.9. All samples were run in duplicate and averaged for statistical analysis. Leaf, stem and flower dry weights were multiplied by ethanol soluble carbohydrate and starch concentrations to estimate the amount of potentially remobilizable carbohydrate

present when stem weight peaked. Starch was converted to its glucose equivalent for calculating potentially remobilizable carbohydrate amounts.

RESULTS

Dates of first, mid, and last bloom and physiological maturity did not differ between lines in either season. In 1987-88, first, mid, and last bloom and physiological maturity occurred 186, 203, 223 and 234 DAE, respectively, averaged across lines. In 1988-89, first, mid and last bloom and physiological maturity occurred 172, 185, 203, and 215 DAE, respectively.

There were no differences in predicted amounts of total above-ground dry matter for the three lines in either season (Fig. II.1). Each season, predicted total above-ground dry matter peaked between mid and last bloom at 852 and 655 g m⁻² in 1987-88 and 1988-89, respectively, averaged across lines (Fig. II.1). At physiological maturity, predicted total above-ground dry matter was 32 and 45% less than peak predicted values in 1987-88 and 1988-89, respectively.

Due to nonsignificant differences in predicted total above-ground dry matter between the three lines, line differences in CGR were inferred to be also nonsignificant (Fig. II.2). Crop growth rates reached maximum values between 170 and 173 DAE, fifteen days before first flower, in 1987-88 and 170 and 171 DAE, two days before first flower, in 1988-89. Peak CGR was 12.1 g m⁻² day⁻¹ in 1987-

88 and $13.5 \text{ g m}^{-2} \text{ day}^{-1}$ in 1988-89 averaged across lines (Fig. II.2).

Like total above-ground dry matter, there were no differences among lines in predicted LAI in either season (Fig. II.1). Averaged over lines, predicted LAI peaked at 5.0 and 3.1 in 1987-88 and 1988-89, respectively. Peaks occurred 162 to 163 DAE, 23 days before first flower, in 1987-88 and 159 to 160 DAE, 12 days before first flower, in 1988-89. Both rosette leaves and leaves arising from flowering stems contribute to the LAI of meadowfoam. Observed LAI at 166 DAE in 1987-88 was composed of 53% rosette leaf area averaged across lines (data not shown). At 161 DAE in 1988-89, rosette leaf area made up 45% of total leaf area (data not shown). When LAI peaked, rosette leaf area was declining, and flowering stem leaf area was increasing (data not shown).

Net assimilation rate (Fig. II.2) exhibited two similar peaks in 1987-88. The first peak occurred 86 to 91 DAE and a second peak at 195 to 199 DAE, an average of 6 days before mid bloom. Only Mermaid exhibited two peaks in 1988-1989 with the first peak occurring 113 DAE and the second higher peak at 187 DAE, 2 days after mid bloom. After an initial rise and subsequent plateau, NAR of lines 765 and 729 exhibited one peak, one and two days after mid bloom, respectively, in 1988-89. Peak NAR during

flowering averaged $4.5 \text{ g m}^{-2} \text{ day}^{-1}$ in 1987-88 and $9.1 \text{ g m}^{-2} \text{ day}^{-1}$ in 1988-89 (Fig. II.2). Due to nonsignificant differences between lines for total above-ground dry weight and LAI, line differences in NAR were also considered nonsignificant.

The predicted accumulated dry matter of leaves, stems, flowers, and seeds also did not differ between lines either season (Fig. II.3). Stems comprised the majority of the meadowfoam plants accumulated above-ground dry matter when total above-ground dry matter peaked during flowering. In 1987-88, observed total above-ground dry matter peaked at 208 DAE (Fig. II.1) and was composed of 62% stem, 21% leaf, 15% flower and 2% seed dry matter averaged across lines (Fig. II.3). In 1988-89, stems, leaves, flowers, and seeds comprised 54, 28, 15, and 3%, respectively, of total above-ground dry matter at the observed peak at 189 DAE. Line differences in the CGR's of leaves, stems, flowers, and seeds (not shown) were not significant due to nonsignificant differences between lines for predicted accumulated leaf, stem, flower and seed dry matter.

Seed yields of the machine harvested areas were 669, 520 and 521 kg ha^{-1} in 1987-88 and 485, 365 and 541 kg ha^{-1} in 1988-89 for lines 765, 729 and Mermaid, respectively. Seed yield of line 765 was significantly ($P < 0.05$) greater

than lines 729 and Mermaid in 1987-88 (SE=36). In 1988-89, seed yields of both lines 765 and Mermaid were significantly greater than line 729 (SE=29).

Observed values for stem weight (Fig. II.3) peaked at 208 and 189 DAE in 1987-88 and 1988-89, respectively, and leaf, stem, and flower samples from these dates were analyzed to quantify ethanol soluble carbohydrates and starch. Samples taken at 240 DAE, six days after physiological maturity, and 220 DAE, five days after physiological maturity, in 1987-88 and 1988-89, respectively, were also analyzed. Ethanol soluble carbohydrate concentrations in leaves, stems, and flowers were several fold higher than starch in both seasons and sampling times (Tables II.1 and II.2). Ethanol soluble carbohydrate and starch concentrations were higher in samples taken at the observed peak of stem weight than at physiological maturity. For stems, ethanol soluble carbohydrate and starch concentrations were 24 and 37 times higher when stem weight peaked than at physiological maturity, averaged across years and lines. Stems contained the highest concentration of ethanol soluble carbohydrates. When stem weight peaked, stem ethanol soluble carbohydrate concentration was an average of 354 and 92% higher than that of leaves and flowers, respectively.

DISCUSSION

Peak predicted above-ground dry matter accumulation was 30% greater in 1987-88 than in 1988-89 averaged across lines (Fig. II.1). This may have been related to the longer growth duration and greater LAI in that year. Growth duration was 7% greater, and peak predicted LAI was 60% greater in 1987-88 than in 1988-89. Presumably higher accumulated intercepted light energy in 1987-88 resulted in greater above-ground dry matter production (Charles-Edwards et al., 1986).

Unlike peaks of LAI and CGR which occurred at the same dates both seasons, net dry matter accumulation terminated earlier in 1988-89 than in 1987-88. The earlier termination of positive CGR in 1988-89 might have been a response to the earlier flowering period in 1988-89 than in 1987-88. In both seasons, predicted above-ground dry matter peaked and CGR fell to zero between mid and last bloom. In contrast, Jain and Abuelgasim (1981) and Krebs and Jain (1985), in studies conducted in northern California, observed that CGR increased during flowering indicating continued dry matter accumulation.

Higher maximum and minimum temperatures prior to flowering during April (Fig. II.4) may have contributed to the earlier bloom period in 1988-89. Early warm spring

conditions were found to quickly terminate vegetative growth in northern California (Higgins et al., 1971). Flowering in meadowfoam is also related to the occurrence of late spring and summer drought (Gentry and Miller, 1965; Arroyo, 1973). However, accumulated precipitation prior to bloom in April was similar in each season (Fig. II.4). Photon flux density has also been found to affect time of flowering in growth chamber studies (unpublished data, New Crops Research Project, Oregon State University).

The termination of above-ground dry matter accumulation between mid and last bloom occurs as LAI rapidly declines during flowering. Predicted LAI at mid bloom was 0.8 in 1987-88 and 1.0 in 1988-89 averaged across lines. At last bloom, predicted LAI's for all three lines were less than 0.1 in both 1987-88 and 1988-89. Leaf senescence prior to and during flowering in meadowfoam may be related to the nitrogen demands of reproductive growth (Sinclair and De Wit, 1975). Meadowfoam seed has been reported to contain 150 to 250 g kg⁻¹ crude protein (Gentry and Miller, 1965). McGahuey (1986) found that plant above-ground N peaked 6 days prior to flowering and then declined under N fertility conditions similar to those used in this experiment.

Substantial above-ground dry matter accumulation occurred while LAI declined. From the time that predicted LAI started to decline until the time that net above-ground dry matter production ceased, predicted above-ground dry matter increased an average of 88 and 126% in 1987-88 and 1988-89, respectively. Due to LAI declining while CGR either increased or declined at a rate less than the decline in LAI, NAR exhibited a sharp increase during flowering (Fig. II.2).

The increase in NAR may indicate an increase in photosynthetic efficiency due to an increased demand for photoassimilates to support growing reproductive structures. Increased soybean (*Glycine max* (L.) Merrill) NAR during the seed growth period has been attributed to this (Koller et al., 1970). A decrease in root growth rate and a subsequent gain in above-ground growth rate or photosynthesis by plant parts other than leaves can also cause increases in NAR (Watson, 1952).

Photosynthesis does occur in meadowfoam flower buds and stems (unpublished data, New Crops Research Project, Oregon State University). Using well-developed flower bud clusters with a few flowers already at anthesis, flower buds exhibited photosynthetic rates that were 29% of the rates observed for leaves present on flowering stems on an area basis. Stems exhibited photosynthetic rates that

were 4% of the rates observed for leaves present on flowering stems. Flower bud photosynthesis may account for the sharp rises observed in NAR during flowering.

With LAI's of less than 0.1 at last bloom, developing seeds obtain assimilates from sources other than leaves. After last bloom, seed growth continued for 11 and 12 days until physiological maturity in 1987-88 and 1988-89, respectively, averaged across lines. Using the time between mid bloom and physiological maturity as an estimate, the entire seed fill period was an average of 31 and 30 days long in 1987-88 and 1988-89, respectively. The observed declines in accumulated seed dry matter (Fig. II.3) were apparently due to the loss of mature seeds during the sampling process. Similar declines in seed dry matter were not observed in a study where seed dry matter was measured on a per seed basis (Curran and Grabe, 1987).

In addition to possible flower photosynthesis, ethanol soluble carbohydrates and starch present in leaves, stems and flowers may provide assimilates for seed growth. The observed increases in seed dry weight (Fig. II.3) while total above-ground dry weight decreased (Fig. II.1) indicate that seed growth may be due to remobilization. The declines in accumulated stem and flower dry matter (Fig. II.3) appear to be due to losses

in ethanol soluble carbohydrates and starch (Tables II.1 and II.2). There was no observed abscission of flowers or stems that would account for dry matter loss. Machine-harvested seed yield was significantly ($P < 0.01$) correlated with the amount of potentially remobilizable carbohydrate (data not shown) accumulated when stem weight peaked during flowering with $r = 0.54$ ($n = 24$).

In summary, dry matter production, development, and ethanol soluble carbohydrate and starch concentrations were similar in lines 765, 729, and Mermaid; however, several important trends related to growth, development and yield were identified. In both seasons of this study, dry matter accumulation terminated between mid and last bloom. LAI rapidly declined during flowering and was less than 0.1 by last bloom. Evidence found in this study indicates that developing seeds obtain assimilates from sources other than current leaf photosynthesis. Peaks in NAR during flowering may indicate that organs other than leaves contribute photosynthetically in meadowfoam. During flowering, stems and flowers were found to contain 295 and 162 g kg⁻¹ dry weight of ethanol soluble carbohydrates and starch, respectively, averaged over years and lines. These carbohydrates may be remobilized for seed fill.

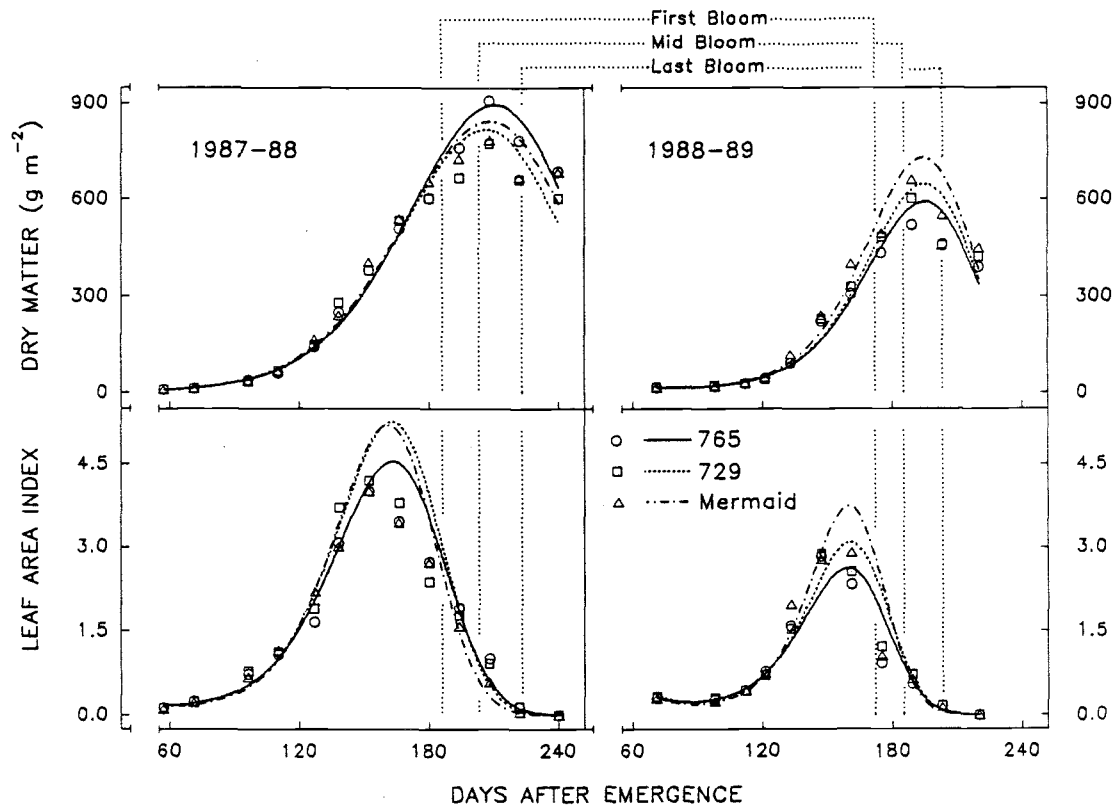


Figure II.1. Seasonal patterns of total above-ground dry matter accumulation and leaf area index for lines 765, 729 and Mermaid. Data points represent observed values and lines indicate predicted mean values derived from regression equations.

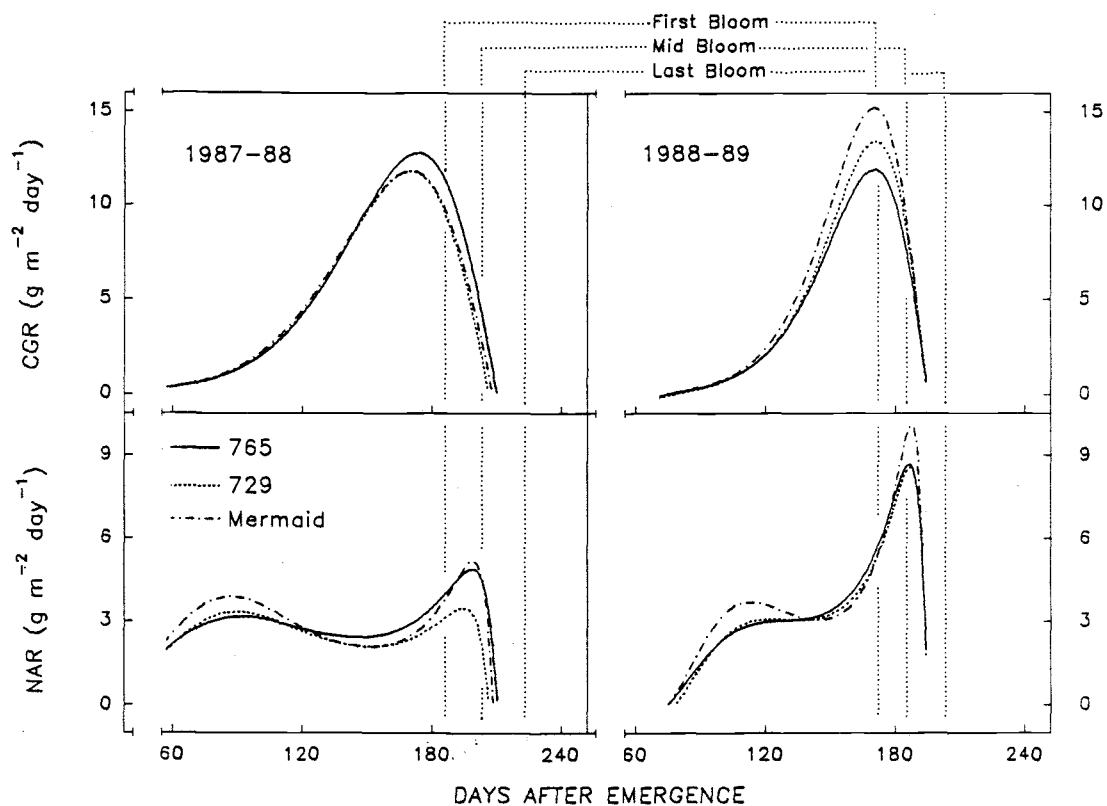


Figure II.2. Seasonal patterns of crop growth rate (CGR) and net assimilation rate (NAR) for lines 765, 729 and Mermaid.

Figure II.3. Seasonal patterns of leaf, stem, flower, and seed dry matter accumulation for lines 765, 729 and Mermaid. Data points represent observed values and lines indicate predicted mean values derived from regression equations.

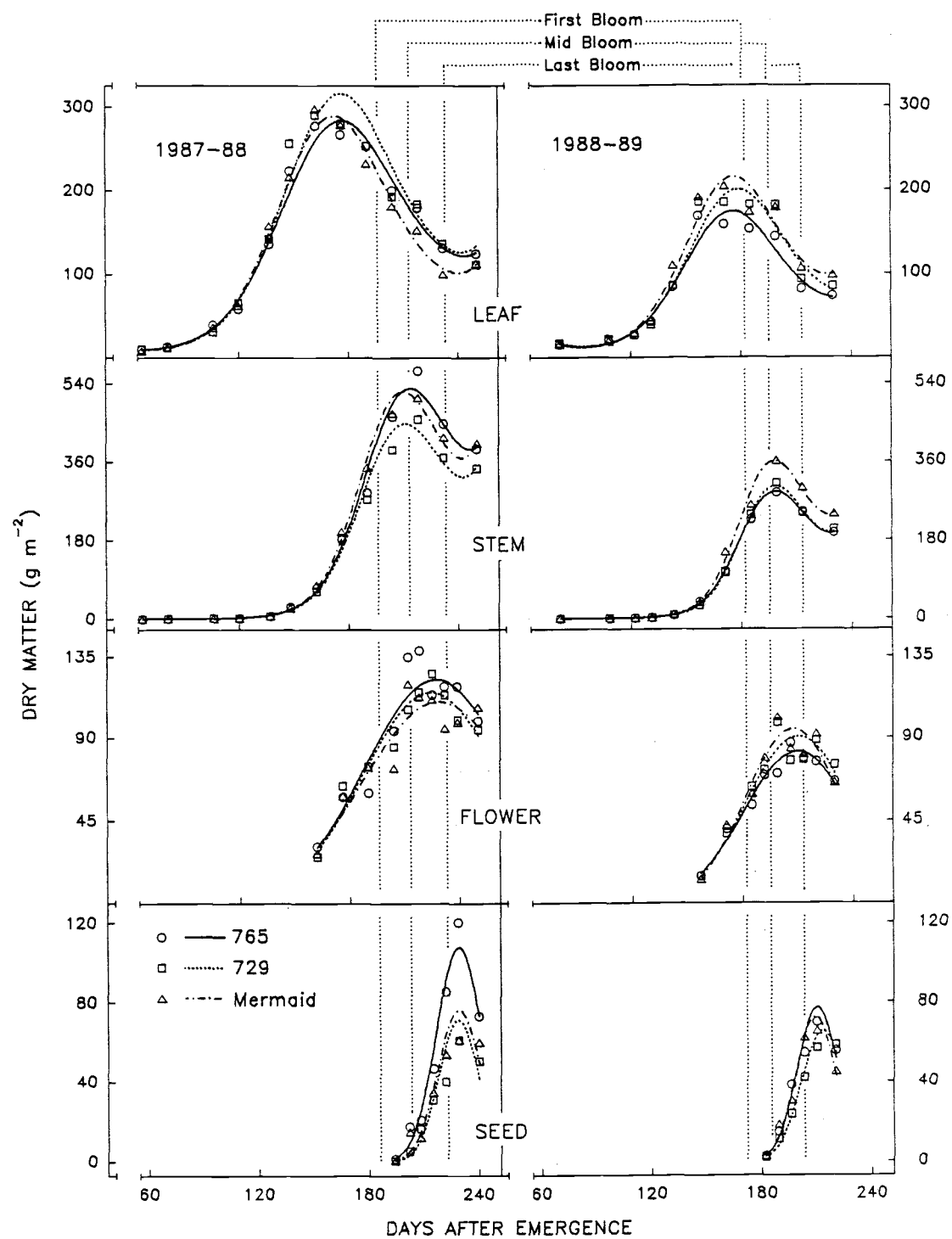


Figure II.3

Table II.1. Leaf, stem and flower ethanol soluble carbohydrate and starch concentrations from samples taken at the observed peak in stem weight in 1987-88 and 1988-89 for lines 765, 729 and Mermaid.

Line	Leaves		Stems		Flowers	
	Ethanol soluble carbohydrates	Starch	Ethanol soluble carbohydrates	Starch	Ethanol soluble carbohydrates	Starch
----- g kg ⁻¹ dry weight -----						
<u>1987-88</u>						
765	68.8	4.6	302.1	23.9	144.8	21.6
729	48.8	2.8	273.7	10.9	163.5	16.8
Mermaid	58.3	3.2	291.3	10.6	153.9	15.9
LSD (0.05)	NS†	1.7	16.7	6.8	NS	NS
CV (%)	26	11	3	26	8	36
<u>1988-89</u>						
765	68.0	1.7	262.0	7.3	130.4	10.2
729	65.0	1.5	299.2	8.6	155.2	9.3
Mermaid	65.2	2.0	270.1	8.6	138.8	10.0
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	19	32	7	28	8	13

†NS = not significant at the 0.05 probability level.

Table II.2. Leaf, stem and flower ethanol soluble carbohydrate and starch concentrations from samples taken at physiological maturity in 1987-88 and 1988-89 for lines 765, 729 and Mermaid.

Line	Leaves		Stems		Flowers	
	Ethanol soluble carbohydrates	Starch	Ethanol soluble carbohydrates	Starch	Ethanol soluble carbohydrates	Starch
----- g kg ⁻¹ dry weight -----						
<u>1987-88</u>						
765	11.4	1.2	40.4	0.8	20.2	1.3
729	12.6	1.2	42.7	0.6	20.6	1.4
Mermaid	12.0	1.2	47.7	0.7	20.3	1.6
LSD (0.05)	NS†	NS	NS	NS	NS	NS
CV (%)	9	19	31	16	6	17
<u>1988-89</u>						
765	13.2	1.6	22.0	0.8	17.3	1.3
729	12.9	1.7	25.4	1.9	19.9	1.8
Mermaid	12.9	1.4	19.4	0.8	16.6	1.1
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	9	30	45	44	16	26

†NS = not significant at the 0.05 probability level.

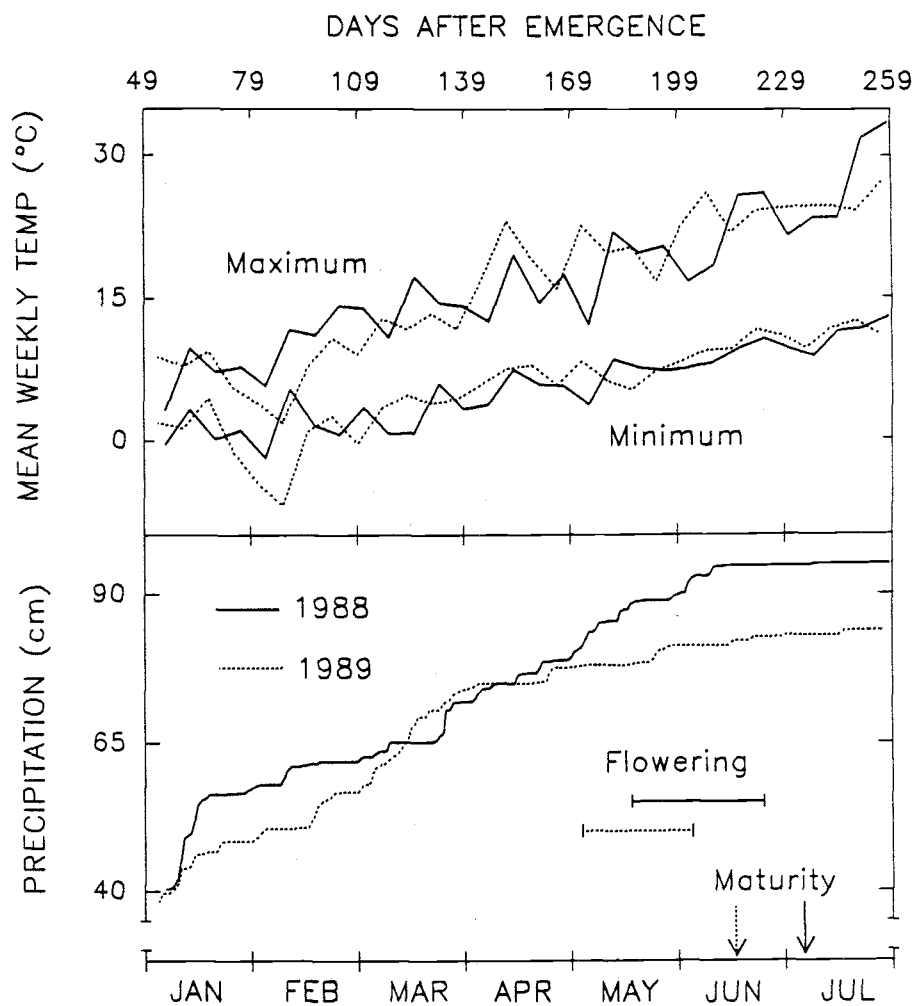


Figure II.4. Weekly averages of maximum and minimum temperatures and precipitation accumulated from 1 Oct. for the months of Jan. to July for 1988 and 1989 measured at the National Weather Service Corvallis Station located at the Oregon State Univ. Crop Science Field Laboratory, Corvallis, OR.

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APPENDICES

Appendix Table I.1. Analyses of variance for oil yield, seed oil content, seed yield, 1000-seed weight, and seeds per area for lines 765, 729, and Mermaid with years 1987-88 and 1988-89 combined.

Source of variation	df	MS	F	P value
<u>Oil Yield</u>				
Year	1	4676.5163	8.94	0.0243
Block(Year)	6	522.9129	1.41	0.2891
Line	2	3157.4304	8.49	0.0050
Year x Line	2	3225.1521	8.67	0.0047
Error	12	371.8367		
<u>Seed oil content</u>				
Year	1	0.00166667	<0.00	0.9738
Block(Year)	6	1.42611111	0.63	0.7017
Line	2	11.96291667	5.32	0.0222
Year x Line	2	8.89541667	3.95	0.0480
Error	12	2.25027778		
<u>Seed yield</u>				
Year	1	68149.8101	10.53	0.0176
Block(Year)	6	6472.3133	1.49	0.2627
Line	2	37612.0302	8.64	0.0047
Year x Line	2	24403.4023	5.61	0.0191
Error	12	4352.9081		
<u>1000-seed weight</u>				
Year	1	7.55554817	6.51	0.0433
Block(Year)	6	1.15973472	15.79	0.0001
Line	2	2.77881012	37.84	0.0001
Year x Line	2	0.48317079	6.58	0.0118
Error	12	0.07344335		
<u>Seeds per area</u>				
Year	1	1425317.95	0.69	0.4379
Block(Year)	6	2065181.61	4.06	0.0187
Line	2	2405319.79	4.72	0.0307
Year x Line	2	3394641.19	6.67	0.0113
Error	12	509195.79		

Appendix Table I.2. Analyses of variance for lodging at last bloom and physiological maturity, total flower buds per area, and viable flower buds for lines 765, 729, and Mermaid with years 1987-88 and 1988-89 combined.

Source of variation	df	MS	F	P value
<u>Lodging--last bloom</u>				
Year	1	176.0416667	3.28	0.1199
Block(Year)	6	53.5972222	9.74	0.0005
Line	2	59.5729167	10.82	0.0021
Year x Line	2	15.8229167	2.88	0.0955
Error	12	5.5034722		
<u>Lodging--physiol. maturity</u>				
Year	1	145.041667	0.43	0.5365
Block(Year)	6	337.652778	4.81	0.0101
Line	2	401.791667	5.73	0.0179
Year x Line	2	44.291667	0.63	0.5486
Error	12	70.152778		
<u>Total flower buds per area</u>				
Year	1	107230537.5	15.21	0.0080
Block(Year)	6	7050493.1	4.16	0.0171
Line	2	15508850.0	9.16	0.0038
Year x Line	2	3346850.0	1.98	0.1812
Error	12	1693372.2		
<u>Viable flower buds</u>				
Year	1	0.10860943	8.13	0.0291
Block(Year)	6	0.01335448	1.93	0.1560
Line	2	0.00280166	0.41	0.6755
Year x Line	2	0.00035036	0.05	0.9508
Error	12	0.00690968		

Appendix Table I.3. Analyses of variance for seed fill duration, bee visits, and harvest index for lines 765, 729, and Mermaid with years 1987-88 and 1988-89 combined.

Source of variation	df	MS	F	P value
<u>Seed fill duration</u>				
Year	1	1.0416667	0.20	0.6720
Block(Year)	6	5.2638889	0.27	0.9415
Line	2	10.0416667	0.51	0.6122
Year x Line	2	12.7916667	0.65	0.5388
Error	12	19.6388889		
<u>Bee visits</u>				
Year	1	1.0416667	0.03	0.8608
Block(Year)	6	31.0972222	2.76	0.0634
Line	2	3.7916667	0.34	0.7207
Year x Line	2	18.2916667	1.62	0.2376
Error	12	11.2638889		
<u>Harvest index</u>				
Year	1	0.0017625	8.85	0.0248
Block(Year)	6	0.0001992	1.30	0.3300
Line	2	0.0007495	4.88	0.0282
Year x Line	2	0.0002902	1.89	0.1938
Error	12	0.0001537		

Appendix Table I.4. Analyses of variance for viable flowers per area, seeds per flower, and biomass yield for lines 765, 729, and Mermaid in 1987-88.

Source of variation	df	MS	F	P value
<u>Viable flowers per area</u>				
Block	3	1778111.111	0.52	0.6812
Line	2	744133.333	0.22	0.8091
Error	6	3389344.444		
<u>Seeds per flower</u>				
Block	3	0.08101171	0.08	0.9710
Line	2	0.10771585	0.10	0.9061
Error	6	1.07466767		
<u>Biomass yield</u>				
Block	3	22381.721	0.23	0.8704
Line	2	2318217.171	24.13	0.0014
Error	6	96084.812		

Appendix Table I.5. Analyses of variance for viable flowers per area, seeds per flower, and biomass yield for lines 765, 729, and Mermaid in 1988-89.

Source of variation	df	MS	F	P value
<u>Viable flowers per area</u>				
Block	3	405066.667	2.33	0.1735
Line	2	151075.000	0.87	0.4657
Error	6	173575.000		
<u>Seeds per flower</u>				
Block	3	0.20216350	12.24	0.0057
Line	2	0.38618770	23.38	0.0015
Error	6	0.01651600		
<u>Biomass yield</u>				
Block	3	115731.006	0.14	0.9322
Line	2	3431626.590	4.16	0.0736
Error	6	825138.122		

Appendix Table I.6. Analyses of variance for time of first, mid, and last bloom and physiological maturity for lines 765, 729, and Mermaid in 1987-88.

Source of variation	df	MS	F	P value
<u>First bloom</u>				
Block	3	3.2222222	0.75	0.5617
Line	2	5.0833333	1.18	0.3695
Error	6	4.3055556		
<u>Mid bloom</u>				
Block	3	32.9722222	1.39	0.3350
Line	2	39.5833333	1.66	0.2663
Error	6	23.8055556		
<u>Last bloom</u>				
Block	3	2.3055556	1.41	0.3295
Line	2	0.7500000	0.46	0.6532
Error	6	1.6388889		
<u>Physiol. maturity</u>				
Block	3	21.4166667	8.86	0.0127
Line	2	4.7500000	1.97	0.2205
Error	6	2.4166667		

Appendix Table I.7. Analyses of variance for time of first, mid, and last bloom and physiological maturity for lines 765, 729, and Mermaid in 1988-89.

Source of variation	df	MS	F	P value
<u>First bloom</u>				
Block	3	1.00000000	4.00	0.0701
Line	2	0.58333333	2.33	0.1780
Error	6	0.25000000		
<u>Mid bloom</u>				
Block	3	0.66666667	0.06	0.9802
Line	2	9.25000000	0.80	0.4926
Error	6	11.58333333		
<u>Last bloom</u>				
Block	3	1.22222222	1.91	0.2287
Line	2	1.75000000	2.74	0.1428
Error	6	0.63888889		
<u>Physiol. maturity</u>				
Block	3	1.63888889	2.95	0.1203
Line	2	1.00000000	1.80	0.2441
Error	6	0.55555556		

Appendix Table I.8. Time of first, mid, and last bloom and physiological maturity for lines 765, 729, and Mermaid in 1987-88 and 1989-99.

Line	First Bloom	Mid Bloom	Last Bloom	Physiological Maturity
days after emergence				
<u>1987-88</u>				
765	186.0	206.0	223.5	234.5
729	187.0	203.5	223.0	234.0
Mermaid	185.0	200.0	223.5	232.5
LSD (0.05)	NS†	NS	NS	NS
CV (%)	1.1	2.4	0.6	0.7
<u>1988-89</u>				
765	172.0	184.0	203.0	215.0
729	172.0	187.0	203.0	216.0
Mermaid	172.5	184.0	204.0	215.0
LSD (0.05)	NS	NS	NS	NS
CV (%)	0.3	1.8	0.4	0.4

†NS = not significant at $P = 0.05$.

Day of emergence 1987-88 = 13 November 1987.

Day of emergence 1988-89 = 14 November 1988.

Appendix Table II.1. Analyses of variance for the regression of days after emergence on $\ln(\text{total above-ground dry weight})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	3	9.90283	0.99
	error	9	0.01451	
729	model	3	9.49195	0.99
	error	9	0.03183	
Mermaid	model	3	9.99090	0.99
	error	9	0.01954	
<u>1988-89</u>				
765	model	3	6.07385	0.99
	error	7	0.03189	
729	model	3	6.21209	0.98
	error	7	0.04369	
Mermaid	model	3	6.77187	0.99
	error	7	0.03937	

Appendix Table II.2. Analyses of variance for the regression of days after emergence on $\ln(\text{leaf area index})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	3	14.87585	0.98
	error	9	0.08480	
729	model	3	18.94498	0.97
	error	9	0.20206	
Mermaid	model	3	24.99495	0.98
	error	9	0.13351	
<u>1988-89</u>				
765	model	3	15.44035	0.97
	error	7	0.19032	
729	model	3	17.25954	0.98
	error	7	0.16796	
Mermaid	model	3	25.23712	0.97
	error	7	0.37295	

Appendix Table II.3. Analyses of variance for the regression of days after emergence on $\ln(\text{leaf dry weight})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	4	3.77483	0.99
	error	8	0.00981	
729	model	4	3.95173	0.99
	error	8	0.01472	
Mermaid	model	4	3.93210	0.99
	error	8	0.00728	
<u>1988-89</u>				
765	model	4	1.85788	0.98
	error	6	0.02344	
729	model	4	2.03010	0.97
	error	6	0.04350	
Mermaid	model	4	2.23774	0.98
	error	6	0.02698	

Appendix Table II.4. Analyses of variance for the regression of days after emergence on $\ln(\text{stem dry weight})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	5	16.6941	0.99
	error	7	0.0325	
729	model	5	15.9202	0.99
	error	7	0.0629	
Mermaid	model	5	16.4122	0.99
	error	7	0.0258	
<u>1988-89</u>				
765	model	5	13.1800	0.99
	error	5	0.0056	
729	model	5	13.4407	0.99
	error	5	0.0062	
Mermaid	model	5	14.2552	0.99
	error	5	0.0075	

Appendix Table II.5. Analyses of variance for the regression of days after emergence on $\ln(\text{flower dry weight})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	2	0.95367	0.93
	error	7	0.02069	
729	model	2	0.92636	0.94
	error	7	0.01642	
Mermaid	model	2	0.81818	0.91
	error	7	0.02309	
<u>1988-89</u>				
765	model	2	1.18562	0.98
	error	6	0.00934	
729	model	2	1.35189	0.97
	error	6	0.01393	
Mermaid	model	2	1.55449	0.97
	error	6	0.01785	

Appendix Table II.6. Analyses of variance for the regression of days after emergence on $\ln(\text{seed dry weight})$ for lines 765, 729, and Mermaid in 1987-88 and 1988-89.

Line	Source of variation	df	MS	R ²
<u>1987-88</u>				
765	model	2	6.35078	0.96
	error	4	0.11798	
729	model	2	10.04233	0.97
	error	4	0.14724	
Mermaid	model	2	7.79938	0.91
	error	4	0.36638	
<u>1988-89</u>				
765	model	2	3.84407	0.99
	error	3	0.03273	
729	model	2	4.41078	0.99
	error	3	0.04384	
Mermaid	model	2	3.53363	0.98
	error	3	0.05995	

Appendix Table II.7. Polynomial regression model describing the relationship of $\ln(\text{total above-ground dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	0.793798	0.607669	1.306	0.2238
dae	0.011528	0.014560	0.792	0.4489
dae ²	0.000298	0.000106	2.826	0.0199
dae ³	-1.033E-6	2.4E-7	-4.373	0.0018
<u>729</u>				
constant	0.692457	0.900089	0.769	0.4614
dae	0.013026	0.021567	0.604	0.5608
dae ²	0.000298	0.000156	1.905	0.0892
dae ³	-1.065E-6	3.5E-7	-3.042	0.0140
<u>Mermaid</u>				
constant	0.015494	0.705322	0.022	0.9830
dae	0.027937	0.016900	1.653	0.1327
dae ²	0.000197	0.000123	1.611	0.1417
dae ³	-8.47E-7	2.7E-7	-3.090	0.0129

dae = days after emergence.

Appendix Table II.8. Polynomial regression model describing the relationship of $\ln(\text{total above-ground dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	8.795993	1.733415	5.074	0.0014
dae	-0.186816	0.039767	-4.698	0.0022
dae ²	0.001728	0.000288	6.010	0.0005
dae ³	-4.274E-6	6.6E-7	-6.490	0.0003
<u>729</u>				
constant	9.98261	2.028843	4.920	0.0017
dae	-0.212632	0.046544	-4.568	0.0026
dae ²	0.001907	0.000337	5.668	0.0008
dae ³	-4.663E-6	7.7E-7	-6.049	0.0005
<u>Mermaid</u>				
constant	9.169158	1.925991	4.761	0.0021
dae	-0.199203	0.044184	-4.508	0.0028
dae ²	0.001849	0.000319	5.787	0.0007
dae ³	-4.590E-6	7.3E-7	-6.272	0.0004

dae = days after emergence.

Appendix Table II.9. Polynomial regression model describing the relationship of $\ln(\text{leaf area index})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	0.679919	1.469259	0.463	0.6545
dae	-0.112555	0.035205	-3.197	0.0109
dae ²	0.001476	0.000255	5.785	0.0003
dae ³	-4.627E-6	5.7E-7	-8.101	0.0001
<u>729</u>				
constant	1.696999	2.267996	0.748	0.4734
dae	-0.143257	0.054344	-2.636	0.0271
dae ²	0.001758	0.000394	4.463	0.0016
dae ³	-5.402E-6	8.8E-7	-6.126	0.0002
<u>Mermaid</u>				
constant	1.877698	1.843537	1.019	0.3350
dae	-0.156611	0.044173	-3.545	0.0063
dae ²	0.001923	0.000320	6.007	0.0002
dae ³	-5.959E-6	7.2E-7	-8.315	0.0001

dae = days after emergence.

Appendix Table II.10. Polynomial regression model describing the relationship of $\ln(\text{leaf area index})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	15.432828	4.234613	3.644	0.0082
dae	-0.483812	0.097147	-4.980	0.0016
dae ²	0.004366	0.000702	6.216	0.0004
dae ³	-1.192E-5	1.61E-6	-7.411	0.0001
<u>729</u>				
constant	18.446076	3.978028	4.637	0.0024
dae	-0.558679	0.091261	-6.122	0.0005
dae ²	0.004949	0.000660	7.500	0.0001
dae ³	-1.334E-5	1.51E-6	-8.825	0.0001
<u>Mermaid</u>				
constant	21.997793	5.927830	3.711	0.0075
dae	-0.662659	0.135991	-4.873	0.0018
dae ²	0.005868	0.000983	5.967	0.0006
dae ³	-1.584E-5	2.25E-6	-7.031	0.0002

dae = days after emergence.

Appendix Table II.11. Polynomial regression model describing the relationship of $\ln(\text{leaf dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	7.635884	1.441774	5.296	0.0007
dae	-0.240618	0.047505	-5.065	0.0010
dae ²	0.003475	0.000538	6.452	0.0002
dae ³	-0.000017	2.533E-6	-6.877	0.0001
dae ⁴	2.881E-8	4.223E-9	6.821	0.0001
<u>729</u>				
constant	9.623659	1.765872	5.450	0.0006
dae	-0.307389	0.058185	-5.283	0.0007
dae ²	0.004224	0.00066	6.404	0.0002
dae ³	-0.000021	3.102E-6	-6.710	0.0002
dae ⁴	3.416E-8	5.173E-9	6.605	0.0002
<u>Mermaid</u>				
constant	8.781381	1.241446	7.074	0.0001
dae	-0.291049	0.040905	-7.115	0.0001
dae ²	0.004164	0.000464	8.980	<0.0001
dae ³	-0.000021	2.181E-6	-9.660	<0.0001
dae ⁴	3.532E-8	3.637E-9	9.712	<0.0001

dae = days after emergence.

Appendix Table II.12. Polynomial regression model describing the relationship of $\ln(\text{leaf dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	29.596232	5.527947	5.354	0.0017
dae	-0.884681	0.174944	-5.057	0.0023
dae ²	0.010018	0.001965	5.097	0.0022
dae ³	-0.000046	9.355E-6	-4.888	0.0027
dae ⁴	7.314E-8	1.602E-8	4.565	0.0038
<u>729</u>				
constant	30.014959	7.530979	3.986	0.0072
dae	-0.882594	0.238334	-3.703	0.0101
dae ²	0.009836	0.002678	3.674	0.0104
dae ³	-0.000044	0.000013	-3.464	0.0134
dae ⁴	6.941E-8	2.183E-8	3.180	0.0191
<u>Mermaid</u>				
constant	32.603672	5.931169	5.497	0.0015
dae	-0.984612	0.187704	-5.246	0.0019
dae ²	0.011172	0.002109	5.298	0.0018
dae ³	-0.000051	0.00001	-5.103	0.0022
dae ⁴	8.249E-8	1.719E-8	4.799	0.0030

dae = days after emergence.

Appendix Table II.13. Polynomial regression model describing the relationship of $\ln(\text{stem dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-11.842889	7.642588	-1.550	0.1652
dae	0.532313	0.317081	1.679	0.1371
dae ²	-0.00968	0.004919	-1.968	0.0897
dae ³	0.000084	0.000036	2.322	0.0532
dae ⁴	-3.207E-7	1.256E-7	-2.554	0.0379
dae ⁵	4.46E-10	1.68E-10	2.660	0.0325
<u>729</u>				
constant	-17.330437	10.641138	-1.629	0.1474
dae	0.756558	0.441487	1.714	0.1303
dae ²	-0.013109	0.006848	-1.914	0.0972
dae ³	0.000109	0.00005	2.163	0.0673
dae ⁴	-4.071E-7	1.748E-7	-2.328	0.0527
dae ⁵	5.62E-10	2.34E-10	2.405	0.0471
<u>Mermaid</u>				
constant	-18.736738	6.808726	-2.752	0.0284
dae	0.823125	0.282485	2.914	0.0225
dae ²	-0.01424	0.004382	-3.250	0.0141
dae ³	0.000117	0.000032	3.661	0.0081
dae ⁴	-4.403E-7	1.119E-7	-3.936	0.0056
dae ⁵	6.08E-10	1.49E-10	4.068	0.0048

dae = days after emergence.

Appendix Table II.14. Polynomial regression model describing the relationship of $\ln(\text{stem dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-17.362275	11.708601	-1.483	0.1982
dae	0.95416	0.461231	2.069	0.0934
dae ²	-0.019548	0.006954	-2.811	0.0375
dae ³	0.000179	0.00005	3.541	0.0166
dae ⁴	-7.227E-7	1.767E-7	-4.089	0.0095
dae ⁵	1.068E-9	2.40E-10	4.445	0.0067
<u>729</u>				
constant	-41.903645	12.383235	-3.384	0.0196
dae	1.884515	0.487807	3.863	0.0118
dae ²	-0.032981	0.007355	-4.484	0.0065
dae ³	0.000271	0.000053	5.087	0.0038
dae ⁴	-1.031E-6	1.869E-7	-5.518	0.0027
dae ⁵	1.466E-9	2.54E-10	5.768	0.0022
<u>Mermaid</u>				
constant	-25.660712	13.560664	-1.892	0.1170
dae	1.271709	0.534189	2.381	0.0631
dae ²	-0.024295	0.008054	-3.017	0.0295
dae ³	0.000213	0.000058	3.647	0.0148
dae ⁴	-8.435E-7	2.047E-7	-4.121	0.0092
dae ⁵	1.232E-9	2.78E-10	4.425	0.0069

dae = days after emergence.

Appendix Table II.15. Polynomial regression model describing the relationship of $\ln(\text{flower dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-10.608830	2.495837	-4.251	0.0038
dae	0.141987	0.025810	5.501	0.0009
dae ²	-0.000327	0.000066	-4.969	0.0016
<u>729</u>				
constant	-11.565848	2.223064	-5.202	0.0012
dae	0.152177	0.022991	6.619	0.0003
dae ²	-0.000355	0.000059	-6.055	0.0005
<u>Mermaid</u>				
constant	-9.098599	2.636677	-3.451	0.0107
dae	0.126293	0.027267	4.632	0.0024
dae ²	-0.000289	0.000070	-4.157	0.0043

dae = days after emergence.

Appendix Table II.16. Polynomial regression model describing the relationship of $\ln(\text{flower dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-19.136742	2.174568	-8.800	0.0001
dae	0.235844	0.023906	9.865	0.0001
dae ²	-0.000591	0.000065	-9.085	0.0001
<u>729</u>				
constant	-20.388160	2.655472	-7.678	0.0003
dae	0.248938	0.029193	8.527	0.0001
dae ²	-0.000622	0.000079	-7.840	0.0002
<u>Mermaid</u>				
constant	-24.681447	3.006758	-8.209	0.0002
dae	0.296437	0.033055	8.968	0.0001
dae ²	-0.000752	0.000090	-8.362	0.0002

dae = days after emergence.

Appendix Table II.17. Polynomial regression model describing the relationship of $\ln(\text{seed dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1987-88.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-165.630439	29.393582	-5.635	0.0049
dae	1.486864	0.271866	5.469	0.0054
dae ²	-0.003245	0.000626	-5.181	0.0066
<u>729</u>				
constant	-214.543161	32.836955	-6.534	0.0028
dae	1.913607	0.303714	6.301	0.0032
dae ²	-0.004184	0.000670	-5.979	0.0039
<u>Mermaid</u>				
constant	-184.840409	51.799025	-3.568	0.0234
dae	1.651881	0.479097	3.448	0.0261
dae ²	-0.003606	0.001104	-3.267	0.0309

dae = days after emergence.

Appendix Table II.18. Polynomial regression model describing the relationship of $\ln(\text{seed dry weight})$ and days after emergence for lines 765, 729, and Mermaid in 1988-89.

Independent Variable	Coefficient	Std. Error	t-value	Sig. Level
<u>765</u>				
constant	-177.274976	20.225386	-8.765	0.0031
dae	1.729165	0.201882	8.565	0.0033
dae ²	-0.004116	0.000502	-8.196	0.0038
<u>729</u>				
constant	-159.180084	23.408142	-6.800	0.0065
dae	1.535191	0.233651	6.570	0.0072
dae ²	-0.003607	0.000581	-6.206	0.0084
<u>Mermaid</u>				
constant	-184.889670	27.371365	-6.755	0.0066
dae	1.811189	0.273211	6.629	0.0070
dae ²	-0.004335	0.000680	-6.379	0.0078

dae = days after emergence.

Appendix Table II.19. Rosette leaf dry weight means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
----- g dry weight m ⁻² -----				
<u>1987-88</u>				
57	ls‡	9.7	10.4	8.6
71	ls	13.5	12.5	12.6
96	ls	39.4	31.3	36.2
110	ls	58.5	66.0	63.0
127	ls	134.5	141.6	156.3
138	obs§	195.0	234.4	182.5
152	obs	191.8	228.7	212.1
166	obs	157.5	184.7	175.4
180	obs	127.8	138.4	113.4
194	obs	87.5	100.9	92.6
208	obs	63.3	88.3	68.8
222	obs	54.4	64.2	43.3
240	obs	11.8	7.3	7.7
<u>1988-89</u>				
71	ls	15.6	17.3	15.7
98	obs	19.8	22.1	19.7
112	obs	27.6	27.3	29.1
121	obs	43.1	39.0	44.2
133	obs	79.0	80.1	104.1
147	obs	113.5	127.9	124.3
161	obs	86.3	99.8	117.1
175	obs	68.6	80.2	79.9
189	obs	72.3	80.0	92.2
203	obs	35.1	32.0	57.1
220	obs	45.2	48.4	66.6

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡ls = least squares mean.

§obs = observed mean.

Appendix Table II.20. Stem leaf dry weight means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
----- g dry weight m ⁻² -----				
<u>1987-88</u>				
127	ls‡	1.3	0.4	1.5
138	obs§	28.4	21.5	33.3
152	obs	85.1	60.9	85.4
166	obs	109.2	95.7	102.7
180	obs	124.8	114.6	118.8
194	obs	112.5	91.5	88.6
208	obs	115.6	95.4	84.0
222	obs	76.8	72.1	56.9
240	obs	113.0	104.2	105.0
<u>1988-89</u>				
133	obs	5.8	6.4	6.1
147	obs	55.5	58.0	67.2
161	obs	73.2	85.2	87.7
175	obs	84.9	102.2	94.2
189	obs	72.3	102.1	87.6
203	obs	47.3	61.5	50.3
220	obs	28.1	36.5	32.1

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡ls = least squares mean.

§obs = observed mean.

Appendix Table II.21. Stem dry weight means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
----- g dry weight m ⁻² -----				
<u>1987-88</u>				
57	ls‡	0.6	0.5	0.6
71	ls	0.7	0.7	0.8
96	ls	1.9	2.5	2.3
110	ls	2.7	2.4	2.9
127	ls	7.6	7.2	8.4
138	obs§	27.7	25.9	25.9
152	obs	72.5	65.2	79.2
166	obs	183.4	187.2	201.4
180	obs	290.1	274.4	348.1
194	obs	464.8	388.4	472.3
208	obs	570.8	458.4	507.8
222	obs	448.4	369.9	417.6
240	obs	390.1	345.3	403.6
<u>1988-89</u>				
71	ls	0.5	0.4	0.4
98	obs	0.6	0.7	0.6
112	obs	1.4	1.1	1.3
121	obs	2.5	2.6	2.8
133	obs	8.6	8.6	10.2
147	obs	38.9	30.3	38.2
161	obs	108.3	105.9	153.4
175	obs	227.9	238.6	261.2
189	obs	289.4	310.6	361.8
203	obs	245.8	243.2	301.2
220	obs	196.4	205.1	240.6

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡ls = least squares mean.

§obs = observed mean.

Appendix Table II.22. Flower dry weight means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
----- g dry weight m ⁻² -----				
<u>1987-88</u>				
152	obs‡	31.0	25.5	27.5
166	obs	58.3	64.3	58.9
180	obs	60.6	74.7	74.4
194	obs	94.1	85.6	73.6
202	obs	135.3	106.0	120.2
208	obs	138.6	115.7	113.0
215	obs	114.1	125.8	111.5
222	obs	118.8	113.9	95.6
229	obs	118.8	100.0	99.0
240	obs	99.6	94.7	107.1
<u>1988-89</u>				
147	obs	14.8	14.8	12.9
161	obs	40.5	37.9	42.7
175	obs	53.3	63.2	59.5
182	obs	69.5	72.4	79.2
189	obs	70.7	98.5	101.4
196	obs	87.1	77.4	84.3
203	obs	78.7	78.1	81.2
210	obs	76.9	88.6	92.1
220	obs	66.0	75.1	65.4

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡obs = observed mean.

Appendix Table II.23. Seed dry weight means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
----- g dry weight m ⁻² -----				
<u>1987-88</u>				
194	obs‡	1.6	0.3	0.5
202	obs	17.7	5.3	15.0
208	obs	21.1	16.8	12.3
215	obs	47.0	31.1	35.0
222	obs	85.7	40.5	54.1
229	obs	120.5	61.3	61.5
240	obs	73.4	50.5	60.2
<u>1988-89</u>				
182	obs	2.6	1.8	2.7
189	obs	14.8	11.2	18.4
196	obs	38.4	23.6	30.7
203	obs	54.5	41.9	62.2
210	obs	69.8	56.9	65.5
220	obs	55.4	58.3	45.2

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡obs = observed mean.

Appendix Table II.24. Rosette leaf area index means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
-- m ² leaf area m ⁻² ground area --				
<u>1987-88</u>				
57	ls‡	0.132	0.136	0.111
71	ls	0.256	0.237	0.231
96	ls	0.729	0.782	0.672
110	ls	1.074	1.122	1.154
127	ls	1.635	1.877	2.168
138	obs§	2.654	3.394	2.598
152	obs	2.641	3.353	2.676
166	obs	1.759	2.052	1.884
180	obs	0.750	0.654	0.505
194	obs	0.104	0.192	0.115
<u>1988-89</u>				
71	ls	0.310	0.329	0.293
98	obs	0.271	0.305	0.242
112	obs	0.435	0.440	0.439
121	obs	0.771	0.704	0.756
133	obs	1.493	1.438	1.862
147	obs	1.842	1.792	1.774
161	obs	1.006	1.181	1.361
175	obs	0.083	0.178	0.116
189	obs	0.008	0.007	0.003
203	obs	0.000	0.001	0.000

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡ls = least squares mean.

§obs = observed mean.

Appendix Table II.25. Stem leaf area index means for lines 765, 729 and Mermaid in 1987-88 and 1988-89.

Days after emergence†	Mean	Line		
		765	729	Mermaid
-- m ² leaf area m ⁻² ground area --				
<u>1987-88</u>				
127	ls‡	0.018	0.016	0.023
138	obs§	0.425	0.331	0.422
152	obs	1.373	0.860	1.338
166	obs	1.700	1.745	1.556
180	obs	1.985	1.724	2.213
194	obs	1.793	1.594	1.469
208	obs	1.015	0.923	0.591
222	obs	0.138	0.156	0.053
240	obs	0.004	0.001	0.000
<u>1988-89</u>				
133	obs	0.091	0.092	0.112
147	obs	0.985	1.084	1.006
161	obs	1.325	1.383	1.542
175	obs	0.843	1.039	0.942
189	obs	0.546	0.708	0.638
203	obs	0.168	0.163	0.136
220	obs	0.001	0.001	0.000

†Day of emergence 1987-88 = 13 November, 1987. Day of emergence 1988-89 = 14 November, 1988.

‡ls = least squares mean.

§obs = observed mean.

Appendix Table II.26. Analyses of variance for leaf, stem and flower ethanol soluble carbohydrate concentrations from samples taken at the observed peak in stem weight in 1987-88 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	297.555304	1.32	0.3514
Line	2	400.444510	1.78	0.2471
Error	6	224.904144		
<u>Stems</u>				
Block	3	760.022882	8.12	0.0156
Line	2	822.833805	8.79	0.0165
Error	6	93.567108		
<u>Flowers</u>				
Block	3	163.907780	1.09	0.4223
Line	2	350.783167	2.33	0.1779
Error	6	150.294833		

Appendix Table II.27. Analyses of variance for leaf, stem and flower starch concentrations from samples taken at the observed peak in stem weight in 1987-88 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	6.919120	44.36	0.0002
Line	2	3.507999	22.49	0.0016
Error	6	0.155987		
<u>Stems</u>				
Block	3	32.015831	2.10	0.2015
Line	2	229.313129	15.05	0.0046
Error	6	15.237006		
<u>Flowers</u>				
Block	3	43.309634	1.00	0.4563
Line	2	40.570982	0.93	0.4438
Error	6	43.488248		

Appendix Table II.28. Analyses of variance for leaf, stem and flower ethanol soluble carbohydrate concentrations from samples taken at the observed peak in stem weight in 1988-89 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	211.900479	1.30	0.3572
Line	2	11.100699	0.07	0.9348
Error	6	162.800330		
<u>Stems</u>				
Block	3	592.014407	1.41	0.3299
Line	2	1533.693032	3.64	0.0922
Error	6	421.316992		
<u>Flowers</u>				
Block	3	56.734129	0.41	0.7537
Line	2	633.610804	4.55	0.0628
Error	6	139.326800		

Appendix Table II.29. Analyses of variance for leaf, stem and flower starch concentrations from samples taken at the observed peak in stem weight in 1988-89 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	0.076100	0.24	0.8677
Line	2	0.263899	0.82	0.4839
Error	6	0.321334		
<u>Stems</u>				
Block	3	5.926911	1.17	0.3974
Line	2	2.189414	0.43	0.6686
Error	6	5.081752		
<u>Flowers</u>				
Block	3	2.047675	1.36	0.3422
Line	2	1.006422	0.67	0.5475
Error	6	1.508712		

Appendix Table II.30. Analyses of variance for leaf, stem and flower ethanol soluble carbohydrate concentrations from samples taken at physiological maturity in 1987-88 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	1.916768	1.63	0.2794
Line	2	1.260023	1.07	0.4003
Error	6	1.176900		
<u>Stems</u>				
Block	3	305.362130	1.68	0.2702
Line	2	54.941731	0.30	0.7504
Error	6	182.300128		
<u>Flowers</u>				
Block	3	15.436711	9.26	0.0114
Line	2	0.207776	0.12	0.8851
Error	6	1.667794		

Appendix Table II.31. Analyses of variance for leaf, stem and flower starch concentrations from samples taken at physiological maturity in 1987-88 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	0.198473	3.94	0.0722
Line	2	0.002501	0.05	0.9520
Error	6	0.050409		
<u>Stems</u>				
Block	3	0.014973	1.19	0.3886
Line	2	0.042264	3.37	0.1044
Error	6			
<u>Flowers</u>				
Block	3	0.166925	2.68	0.1408
Line	2	0.060797	0.97	0.4299
Error	6	0.062368		

Appendix Table II.32. Analyses of variance for leaf, stem and flower ethanol soluble carbohydrate concentrations from samples taken at physiological maturity in 1988-89 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	1.678750	1.24	0.3749
Line	2	0.127190	0.09	0.9116
Error	6	1.353913		
<u>Stems</u>				
Block	3	182.074420	1.83	0.2420
Line	2	35.525307	0.36	0.7135
Error	6	99.437959		
<u>Flowers</u>				
Block	3	12.060412	1.53	0.3008
Line	2	12.097094	1.53	0.2899
Error	6	7.892595		

Appendix Table II.33. Analyses of variance for leaf, stem and flower starch concentrations from samples taken at physiological maturity in 1988-89 for lines 765, 729, and Mermaid.

Source of variation	df	MS	F	P value
<u>Leaves</u>				
Block	3	0.384614	1.72	0.2609
Line	2	0.156048	0.70	0.5333
Error	6	0.223106		
<u>Stems</u>				
Block	3	0.878738	3.37	0.0959
Line	2	1.719655	6.59	0.0306
Error	6	0.260802		
<u>Flowers</u>				
Block	3	0.363463	2.79	0.1317
Line	2	0.489847	3.76	0.0874
Error	6	0.130256		

Appendix III. Procedure for the quantitation of ethanol soluble carbohydrates and starch.

SAMPLE PREPARATION

1. Grind plant material in a Wiley Mill using a 40-mesh screen.
2. Further grind in a mortar and pestle until plant material passes through a 115-mesh screen.
3. Dry material prior to analysis at 38°C.

EXTRACTION OF ETHANOL SOLUBLE CARBOHYDRATES

1. Reagent: 80% ethanol.
2. Extract ethanol soluble sugars from a 25 to 50 mg sample aliquot with 5 ml of 80°C 80% (v/v) ethanol for 10 minutes.
3. Centrifuge at 5000 x g for 10 minutes and decant supernatant.
4. Repeat extraction and centrifugation (steps two and three).
5. Combine supernatants and make up to known volume with water (25 to 50 ml).
6. Save non-ethanol soluble material for starch extraction.

CLARIFICATION OF ETHANOL EXTRACT

1. Reagents: saturated lead acetate (3 to 4 scoops in 100 ml water) and saturated sodium oxalate (3 to 4 scoops in 100 ml water).
2. Add 0.2 ml of saturated lead acetate to the ethanol extract.
3. After 25 minutes, add 1 ml of saturated sodium oxalate.
4. After 10 minutes, filter through washed Whatman #42 filter paper.
5. Proceed to quantitation of carbohydrates using anthrone reagent.

EXTRACTION AND PURIFICATION OF STARCH

1. Reagents: 52% perchloric acid, 20% sodium chloride, I_2KI solution (add 7.5 g I_2 and 7.5 g KI to a small amount of water and grind in a mortar; bring volume up to 250 ml and filter), 2% ethanolic sodium chloride (350 ml 95% ethanol, 80 ml water, and 50 ml 20% sodium chloride diluted to 500 ml), and 0.25N ethanolic sodium hydroxide (350 ml 95% ethanol, 100 ml water, and 25 ml 5N sodium hydroxide diluted to 500 ml).
2. Add 5 ml of water to the non-ethanol soluble material and place in a boiling water bath for 15 minutes.

Remove and cool to room temperature.

3. Extract starch by adding 6.5 ml of 52% perchloric acid while stirring. Use water bath to keep temperature at 25°C.
4. After 20 minutes, centrifuge at 5000 x g for 10 minutes and decant supernatant.
5. Repeat extraction and centrifugation (steps two to four). Skip boiling water bath in step two during second extraction.
6. Combine supernatants and make up to known volume with water (25 ml).
7. Filter solution through a small amount of glass wool.
8. Transfer a 10 ml aliquot into a conical centrifuge tube, and add 5 ml of 20% sodium chloride and 2 ml of I₂KI reagent.
9. After standing for 20 minutes, centrifuge at 5000 x g for 15 minutes, and carefully remove supernatant with a pipette.
10. Suspend precipitate in 5 ml of 2% ethanolic sodium chloride, centrifuge at 5000 x g for 15 minutes, and remove supernatant with a pipette.
11. Repeat step ten.
12. Add 2 ml of 0.25N ethanolic sodium hydroxide to the precipitate. Gently shake until blue color disappears.

13. Wash starch with 5 ml of 2% ethanolic sodium chloride, centrifuge at 5000 x g for 15 minutes, and remove supernatant with a pipette.
14. Repeat step thirteen.
15. Add approximately 5 ml of hot water to the starch. After the starch is dissolved, bring to a known volume.
16. Proceed to quantitation of carbohydrates using anthrone reagent.

ANTHRONE ESTIMATION OF CARBOHYDRATES

1. Anthrone reagent: add 175 mg of anthrone per 100 ml of 72% sulfuric acid. Glucose standards: 5, 25, 50, 100, and 150 μ g anhydrous D-glucose per ml.
2. Make up fresh anthrone reagent each day. Place on stirrer until anthrone is dissolved and then place on ice.
3. Transfer 0.5 aliquots of samples and D-glucose standards into glass test tubes.
4. Dispense 5 ml of anthrone reagent into each test tube and mix.
5. Place test tubes in a boiling water bath for 12 minutes.

6. Remove test tubes from boiling water bath and place in an ice water bath.
7. Measure absorbance of samples and standards against a reagent blank (water plus anthrone reagent) at 620 nm.
8. Use standards to generate a standard curve for D-glucose. Use D-glucose standard curve to determine carbohydrate concentration of samples.