 DOI: 10.1079/SSR200178

Buckwheat seed set *in planta* and during *in vitro* inflorescence culture: Evaluation of temperature and water deficit stress

Jolanta Slawinska[1,2] and Ralph L. Obendorf[1,*]

[1]Seed Biology, Department of Crop and Soil Sciences, Cornell University Agricultural Experiment Station, New York State College of Agriculture and Life Sciences, 617 Bradfield Hall, Cornell University, Ithaca, NY 14853–1901, USA; [2]Plant Physiology Department, Horticultural Faculty, Agricultural University, 31–425 Krakow, Al. 29 Listopada 54, Poland

Abstract

Common buckwheat (*Fagopyrum esculentum* Moench) plants produce many flowers, but fewer seeds. Seed set is highly variable among years, between plants, and during the period of flowering within a plant or raceme. Seasonal variations suggest that temperature and water-deficit stresses are important factors for seed set. The effects of mild temperature and water-deficit stresses on seed set and seed filling were determined *in planta* and *in vitro*. An *in vitro* method to culture matched sets of racemes from individual plants was used for precise comparisons between experimental treatments. Buckwheat racemes form new flowers continuously during several weeks *in planta*. Seed set resulting in yield occurs during the first 2–3 weeks of flowering and then rapidly declines *in planta* independently of mild stresses. Plants grown at 18°C have 40% increased seed set, set seeds over a longer duration, and produce 40% more dry matter per seed than plants grown at 25°C. Similar patterns occurred *in vitro*. A 3-day water-deficit stress during the first week of flowering reduced the number of seeds by 50% without a reduction in seed size and dry weight, or the number of flowers formed *in planta* or *in vitro*. The effect of water-deficit stress continued after rewatering and subsequently was expressed as a reduction in fertility in newly formed flowers, both *in planta* and *in vitro*. Mild temperature and water-deficit stresses affected both female and male components of seed set in common buckwheat, resulting in a persistent but non-additive reduction in sink strength.

Keywords: buckwheat, seed set, *Fagopyrum esculentum* Moench, *in vitro* inflorescence culture, temperature, water-deficit stress

* Correspondence
Fax: +1 607 255 2644
E-mail: rlo1@cornell.edu

Introduction

In the United States, commercial buckwheat (*Fagopyrum esculentum* Moench) production is essentially of one type, large-seeded (typically 32 mg per seed) common buckwheat, and cultivar, 'Mancan' or a related selection ('Manor'). Other types and cultivars are not widely produced nor commercially important. In New York State, buckwheat is seeded early in July, first flowers appear in about 4 weeks with approximately 14-h photoperiods (sunrise to sunset) during seed set, and plants are harvested soon after the fall equinox. In contrast to photoperiod-sensitive Japanese cultivars that exhibited delayed flowering at 14-h and 18-h photoperiods compared to 10-h photoperiods (Lachmann and Adachi, 1990), 'Mancan' was relatively insensitive to photoperiod; first flowers appeared about 4 weeks after seeding in the greenhouse (24°C day, 18°C night) at various times of the year, even when the natural photoperiods exceeded 14 h (Taylor and Obendorf, 2001). In New York State, average (over 30 years; Northeast Regional Climate Center) day temperatures were 25°C and 26°C and average night temperatures were 13°C and 15°C for Ithaca and Geneva, respectively, during the first 3 weeks of seed set in the field. Buckwheat plants produced many flowers but fewer seeds, and the period of flower formation continued after seed set (Obendorf *et al.*, 1993b; Björkman *et al.*, 1995a; Taylor and Obendorf, 2001). Seed set was highly variable among individual plants, from 2.8 to 52% of the flowers pollinated (Obendorf *et al.*, 1993b), and variable among years with seed set stopping more or less abruptly (Björkman *et al.*, 1995a). The causes of low seed set are unknown but have been attributed to high temperature, plant water stress, incompatibility caused by heterostylism, defective reproductive organs, failure of fertilization and embryo and/or endosperm abortion (Pomeranz, 1983; Adachi, 1990; Taylor and Obendorf, 2001). Seed set early in the

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

flowering period was 65% in a wet, cool season (1992) but only 25% in a dry, warm season (1993) (Björkman *et al.*, 1995a), suggesting that both temperature and water stress were important factors regulating seed set.

Buckwheat flowers are incomplete (lacking a corolla), perfect and heterostylous (Marshall and Pomeranz, 1982). Half of the plants have pin-type flowers with long styles and short filaments, and half of the plants have thrum-type flowers with short styles and long filaments. Flowers within each type are self- and cross-incompatible. Only legitimate cross-pollination, pin by thrum or thrum by pin, results in fertilization (Morris, 1952) in most cultivars of common buckwheat. In the field, buckwheat is pollinated by insect vectors, primarily (95%) honey bees (Björkman, 1995c). Greenhouse and growth chamber studies, in the absence of insect vectors, required hand pollination (Taylor and Obendorf, 2001). Flowers formed continuously for 7 weeks (4–11 weeks after seeding) on individual racemes *in planta* in the greenhouse (Taylor and Obendorf, 2001) and 14 days on raceme explants (Slawinska and Obendorf, 1993). Each flower was open for 1 day when pollinated (Taylor and Obendorf, 2001). About 20% of the flowers formed defective megagametophytes and were female sterile (Taylor and Obendorf, 2001); 80% of the flowers formed normal egg-sac components. Fertility was highest among the earliest appearing flowers (1–3 weeks *in planta*) on a raceme, whereas fertility declined rapidly in flowers that appeared later (>5 weeks *in planta*) in the greenhouse (Taylor and Obendorf, 2001). Lack of fertilization appeared to be the cause of the decline in fertility for late-appearing flowers, and the few that did set usually aborted (Taylor and Obendorf, 2001). The pattern was similar in the field (Björkman *et al.*, 1995a). A flower can produce a single achene (one-seeded fruit) at maturity (Obendorf *et al.*, 1993a).

In vitro culture of buckwheat racemes provides a method to reduce the very large number of observations required for *in planta* studies on seed set in buckwheat. Raceme explants (Slawinska and Obendorf, 1993) isolated from any one plant have identical genotype and, therefore, can be used to characterize precisely the influence of a single factor, such as temperature, on seed set. By sampling several plants ranging in response, a precise evaluation of treatments is possible with fewer observations.

The objectives of this study were to determine the effect of temperature and water-deficit stress on patterns of seed set and seed filling of common buckwheat, following legitimate hand pollination *in planta* and during *in vitro* culture of detached racemes.

Materials and methods

Culture of plants and raceme explants

Buckwheat (*Fagopyrum esculentum* Moench, 'Mancan' cultivar) plants were grown in a greenhouse at 24°C day (14 h) and 18°C night (10 h). Natural sunlight was supplemented for 14 h daily with approximately 740 μmol m^{-2} s^{-1} light from 1000 W Sylvania metal halide lamps. Single plants were grown in a greenhouse soil mix in 4-litre pots as described previously (Horbowicz and Obendorf, 1992). Pots were well watered, two to three times daily. Under these conditions, plants formed three to five primary branches. Synchronously developed axillary racemes from the upper four nodes of primary branches or second to fifth nodes of the main stem were used for *in planta* experiments or as explants. Terminal racemes were excluded. Ten to 12 raceme explants (Slawinska and Obendorf, 1993) were selected from four plants (two with pin-type flowers and two with thrum-type flowers) at 5–6 weeks after seeding. Raceme explants were removed by cutting the stem about 2 mm above the node of the raceme attachment and near the base of the internode below the node of attachment. The raceme explant was immediately placed in water and transported to the laboratory. The basal stem of the detached raceme explant was surface-sterilized for 10 min by immersion in a dilute solution of commercial bleach, 10% Clorox® (0.5% sodium hypochlorite final concentration) in water containing 0.02% Tween 80 as surfactant, and rinsed three times in sterile distilled water. The basal stem of the explant was recut and inserted into presterilized liquid medium in culture tubes. The culture medium contained half-strength MS salts (Murashige and Skoog, 1962) and B_5 vitamins (Gamborg *et al.*, 1968), sucrose at 2.5 g l^{-1}, glutamine at 0.1 g l^{-1}, kinetin ribose at 1 mg l^{-1}, and gibberellin at 1 mg l^{-1} (Slawinska and Obendorf, 1993). The medium was adjusted to pH 5.3 and sterilized by filtration. The detached raceme explants were incubated at 18 or 25°C under continuous cool-white fluorescent light at a flux density of 200 μmol m^{-2} s^{-1}. Groups of raceme explants were enclosed in clear plastic bags (pin and thrum separately) containing a reservoir of water to humidify (>90% RH) the atmosphere. Each day flowers were hand pollinated by legitimate cross-pollination, and the number of flowers pollinated and those forming seeds were recorded. After 7 days the basal stems were resterilized and freshly cut, and the raceme explants were transferred to fresh medium. Seeds (achenes) were harvested after 14 days in culture, seed set was calculated, and length, width,

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

and weight of seeds were determined after drying 7 days at 25°C and 30% RH.

Temperature

In planta

Sixteen 4-week-old plants from the greenhouse were transferred to growth chambers at either 18 or 25°C. To prevent accidental legitimate cross-pollination, plants with pin-type flowers and plants with thrum-type flowers were grown in separate chambers. All plants received 16 hours of fluorescent light daily at about 300 µmol $m^{-2} s^{-1}$. Four plants with pin-type flowers and four with thrum-type flowers were hand pollinated using legitimate cross-pollination, pin × thrum and thrum × pin, between plants within the same temperature regime. Newly opened flowers were pollinated, and flowers and seeds were counted three to four times each week on five tagged racemes of each plant. In some experiments, flowers on five racemes on each plant were pollinated with pollen from plants at 18°C, and flowers on another five racemes were pollinated with pollen from plants at 25°C.

In vitro *inflorescence culture*

Raceme explants were removed from 6-week-old buckwheat plants growing in the greenhouse. Five matched sets of raceme explants (10 racemes) were removed from each of four plants with pin-type flowers and four plants with thrum-type flowers. One raceme explant from each of the matched sets was cultured at constant 18°C and one at constant 25°C in culture medium at pH 5.3 (Slawinska and Obendorf, 1993). Newly opened flowers were hand pollinated daily by legitimate cross-pollination using pollen from flowers on racemes cultured at the same temperature as the receiving flowers.

In a series of experiments to test the effects of temperature on male versus female components, 10 matched sets (i.e. a set of four racemes from one plant distributed one to each of four treatments) of racemes (40 racemes) from plants with pin-type flowers and 10 matched sets of racemes from plants with thrum-type flowers were equally distributed across four treatments *in vitro*: (1) flowers on racemes at 18°C pollinated with pollen from racemes at 18°C; (2) flowers on racemes at 18°C pollinated with pollen from racemes at 25°C; (3) flowers on racemes at 25°C pollinated with pollen from racemes at 18°C; and (4) flowers on racemes at 25°C pollinated with pollen from racemes at 25°C. The number of flowers pollinated and the number of seeds set were recorded.

Water-deficit stress

In planta *experiment A (October to November 1991)*

Four plants, two with pin-type flowers and two with thrum-type flowers, were not watered for 3 days at 5 weeks after seeding (first week of flowering) and compared with four control plants in the greenhouse. After the 3-day water-deficit stress, resulting in severely wilted leaves (all blades vertical) and maximum recoverable stress (plants did not survive if stressed >3 days), all plants were thoroughly watered two or three times daily until termination of the experiment. Seed set was recorded on five racemes on each of the eight plants after legitimate cross-pollination of all newly opened flowers 3–4 times each week from 2 days after re-watering through 6 weeks after stress.

In planta *experiment B (December 1991 to February 1992)*

Another four plants (as in experiment A) were water-deficit stressed for 3 days during the first week of flowering and compared to four control plants. After the 3-day water-deficit stress (less severe than in experiment A as the youngest leaf blades were not vertical), all plants were watered thoroughly. Seed set was recorded on five racemes on each of the eight plants after legitimate cross-pollination of all newly opened flowers 3–4 times each week from 2 days after re-watering through 6 weeks after stress.

In vitro *raceme explant culture*

The same plants used for testing seed set *in planta* (experiment A) in the greenhouse were also used for *in vitro* experiments. Seed set on racemes of remaining branches *in planta* was unchanged by removal of branches for raceme explants. Five raceme explants were removed from each of the four control plants and from each of the four plants receiving the water-deficit treatment in the greenhouse. The 40 raceme explants were cultured at 18°C following the procedures, as described above.

Water-deficit stress effect on pollen source

Experiments were conducted to test the effect of water stress on pollen components versus female (egg sac) components in the regulation of seed set in buckwheat. Four plants, two with pin-type flowers and two with thrum-type flowers, were not watered for 3 days at 5 weeks after seeding in the greenhouse (during the first week of flowering) and compared with four control plants. After the 3-day water-deficit stress (as in experiment A above), all plants were watered thoroughly.

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

After the 3-day stress period, 12 raceme explants were removed from each of the four control plants, and 12 also from each of the four plants receiving the water-deficit treatment in the greenhouse. The raceme explants for all treatments were cultured at 18°C as described above. To test the effect of water stress on pollen, four pollination regimes were established: (1) flowers on racemes from control (Wet) plants were cross-pollinated with pollen of racemes from control plants (Wet × Wet); (2) flowers on racemes from control (Wet) plants were cross-pollinated with pollen of racemes from water-stressed plants (Wet × Dry); (3) flowers on racemes from water-stressed (Dry) plants were cross-pollinated with pollen of racemes from water-stressed plants (Dry × Dry); and (4) flowers on racemes from water-stressed (Dry) plants were cross-pollinated with pollen of racemes from control plants (Dry × Wet).

Results

Temperature

In planta

New flowers formed through 7 weeks after the first flower appeared on plants at 25°C (Fig. 1A) and then ceased (data not shown). Plants at 18°C continued to form new flowers (Fig. 1A) after the seventh week of flowering, when plants were 11 weeks old (data not shown). The rate of flower production slowed at 25°C from 3 weeks after the first flower (Fig. 1A). Formation of new seeds ceased after 4 weeks of flowering at 25°C, whereas at 18°C, new seeds formed through the sixth week after first flower, when plants were 10 weeks old (Fig. 1B). At 18°C, 7.7 seeds formed per raceme, similar to that *in vitro* (Fig. 1D), but at 25°C only 3.6 seeds formed per raceme (Fig. 1B). In two experiments, seeds matured at 18°C averaged 47–49 mg dry weight per seed, compared to 31–33 mg per seed at 25°C (Table 1) and to 32 mg seed for the certified seed stocks used for this study. Racemes on plants at 25°C produced 38–73% fewer seeds, and seeds accumulated 29–35% less dry matter than seeds on plants at 18°C with pollen from plants at 18°C. In a third experiment (data not shown), dry weight was 47 ± 3 mg per seed and 5.4 ± 0.2 mg per embryo at 18°C but 32 ± 3 mg per seed and 5.7 ± 0.5 mg per embryo at 25°C. The increase in seed dry weight at 18°C was in the endosperm because embryo weights were not different. The lower frequency of seed set in experiment 1 (Table 1) reflects a lower seed set during weeks 4–7 of flowering than during weeks 1–3. In another experiment (data not shown), seeds matured on plants at 20°C averaged 44 ± 4 mg dry weight per seed (n = 119).

Raceme explants

Because raceme explants enclosed in clear plastic bags containing a reservoir of water to humidify the atmosphere (>90% RH) resulted in 1.6 times as many seeds per raceme, all raceme explants for *in vitro* experiments reported herein were enclosed in plastic bags, and were removed from bags daily for pollination. Newly opened flowers increased in number until 10 days in culture at both 18 and 25°C, with about two-thirds as many flowers accumulating at 25°C (26 flowers) as at 18°C (33 flowers) (Fig. 1C). Formation of new seeds at 25°C ceased abruptly after about 7 days in culture, whereas at 18°C new seeds formed through 11 days (Fig. 1D). The number of seeds per raceme was 7.4 at 18°C and 2.5 at 25°C. Average seed length and width were similar at both temperatures when harvested after 14 days in culture (data not shown).

Effect of temperature on pollen source

To determine whether the 25°C temperature affected the female parts of the flower or the pollen, flowers on plants growing at 18 and 25°C were pollinated with pollen from plants at 18 and 25°C in two experiments. Experiment 1 was conducted during winter conditions of very low humidity (10–50% RH), and Experiment 2 was performed during summer months of higher humidity (>50% RH). Hand pollinations resulted in 30–40 pollen grains per flower (Taylor and Obendorf, 2001), an amount typical in field pollinations by insects (Björkman, 1995b), and assured that pollen delivery was not limiting, since seed set was maximal with 10–30 pollen grains per flower (Namai, 1990; Namai and Takeyama, 1992; Björkman, 1995a).

The number of flowers per raceme was the same in all treatments (Fig. 2A,B). Within the first 3 weeks of flowering, 73% of seeds were set at both 18°C and 25°C, confirming previous observations (Taylor and Obendorf, 2001) that the first 3 weeks of flowering are the most important for seed set (compare Fig. 2C '18 × 18' and Fig. 2D '25 × 25'). Plants at 18°C set 36% more seeds than plants at 25°C with pollen from plants at 18°C (Fig. 2C, D). However, when flowers on plants at 18°C were pollinated with pollen from flowers on plants at 25°C, seed set was reduced 45% compared to pollen from plants at 18°C (Fig. 2C), indicating the involvement of a major pollen–temperature factor. When flowers on plants at 25°C were pollinated with pollen from plants at 18°C, seed set was the same as with pollen from plants at 25°C (Fig. 2D). If the reduced seed set between 18 and 25°C plants was totally due to a pollen-source–temperature factor, pollen from plants at 18°C should have produced more seeds. Therefore, a temperature of

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

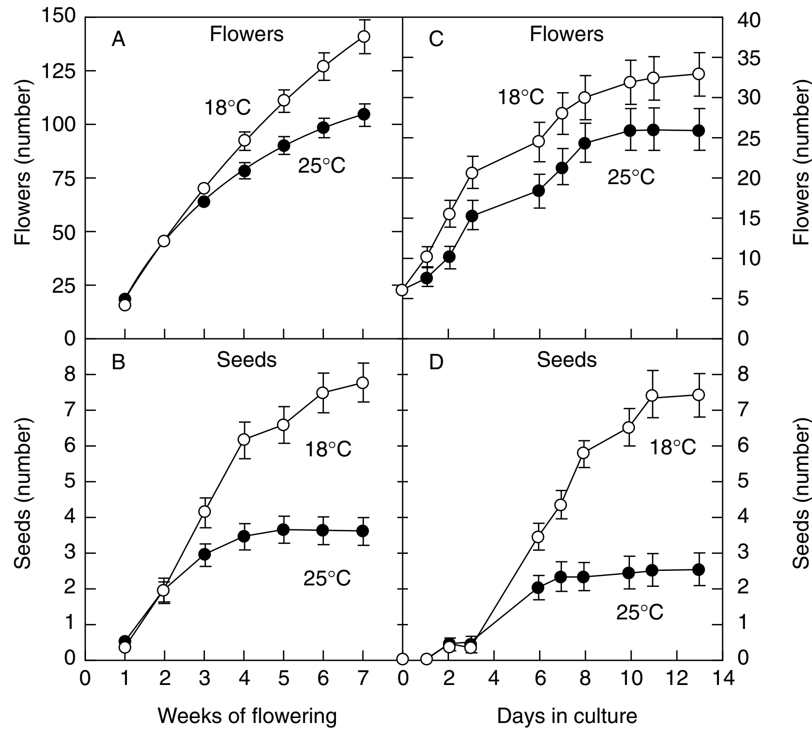

Figure 1. Accumulated number of flowers pollinated (A) and seeds formed (B) per raceme *in planta* at 18 and 25°C as a function of weeks of flowering (experiment 1). Accumulated number of flowers pollinated (C) and seeds set (D) *in vitro* on raceme explants cultured at 18 and 25°C as a function of days in culture. Values are mean ± SE for 40 racemes (eight replicates).

Table 1. Frequency of seed fill *in planta*, seeds per raceme, dry weight per seed, and length and width of seeds from flowers on plants grown at 18 or 25°C after legitimate cross-pollination with pollen from plants grown at 18 or 25°C. Seeds per raceme and dry weight, length and width of individual mature seeds (achenes) are expressed as mean ± SE (40 racemes, eight replications in experiment 1; eight replications for seeds per raceme and five or six replications for other columns in experiment 2)

Temperature (°C)		Filled seeds				
Flower	Pollen	Frequency, *N* (%)	Seeds per raceme, *N*	Dry weight, mg (%)	Length, mm	Width, mm
Experiment 1						
18	18	176/204 (86)	5.0 ± 0.6	46.5 ± 0.8 (100)	7.2 ± 0.1	5.7 ± 0.1
18	25	122/160 (76)	3.7 ± 0.5	46.7 ± 1.0 (100)	7.0 ± 0.1	6.0 ± 0.1
25	18	98/136 (72)	3.1 ± 0.5	32.9 ± 0.7 (71)	6.7 ± 0.1	4.8 ± 0.1
25	25	112/163 (69)	3.4 ± 0.5	33.2 ± 0.5 (71)	6.7 ± 0.1	4.4 ± 0.1
Experiment 2						
18	18	195/196 (99)	7.5 ± 1.6	48.7 ± 0.8 (100)	6.0 ± 0.1	5.3 ± 0.1
18	25	112/112 (100)	4.7 ± 0.4	46.7 ± 1.0 (96)	5.9 ± 0.1	5.1 ± 0.1
25	18	57/57 (100)	2.0 ± 0.4	31.3 ± 1.0 (64)	6.5 ± 0.1	4.5 ± 0.1
25	25	73/79 (92)	2.9 ± 0.5	32.6 ± 0.8 (67)	6.5 ± 0.1	4.4 ± 0.1

Experiment 1: low humidity (10–50% RH) winter; pollinated first–seventh week of flowering (4–11 weeks after seeding).
Experiment 2: high humidity (50–90% RH) summer; pollinated first–third week of flowering (4–7 weeks after seeding).

25°C, above the optimum temperature of 18°C, affects both female *and* male parts of the flower, and results in reduced seed set. This pattern was confirmed in a second experiment conducted under summer conditions of higher humidity. The number of filled seeds per raceme was significantly higher ($P < 0.05$) on plants at 18°C than at 25°C when pollinated with pollen from plants at 18°C, but seed set was not significantly different with pollen from plants at 25°C (Table 1, experiment 2). Since the pattern was similar

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

under higher humidity (50–90% RH) summer conditions and during the very low humidity (10–50% RH) conditions of winter, it is not likely that the observed differences are caused solely by early shed of pollen at 25°C before pollination.

Using raceme explants *in vitro* (>90% RH), both flower formation and seed set stopped abruptly after the first week of culture at 25°C (Fig. 3B, D). A reserve of explants at 25°C provided pollen to continue pollinations in the '18 × 25' treatment (Fig. 3A, C). In contrast to the *in planta* experiment (Fig. 2C), seed set on raceme explants at 18°C was independent of pollen-source–temperature, showing equal seed set after crosses with pollen from racemes at 18 or 25°C (Fig. 3C). The raceme explant experiment was repeated several times (data not shown). Reduced seed set using pollen from racemes at 25°C occurred only when seed set was low in all treatments.

Water-deficit stress

Experiment A (*in planta*)

The number of flowers per raceme was similar for control and water-deficit-stressed plants (Fig. 4A). Seed set was markedly reduced (60%) on plants receiving the water-deficit-stress treatment, from 18 seeds per raceme on control plants to 7 seeds per raceme on the water-stressed plants 4–6 weeks after stress (Fig. 4B). Seed size and mass were similar for stressed and control treatments. Average seed dry weight for control plants was 35.8 ± 1.4 mg per seed, compared to 31.0 ± 2.1 mg per seed for plants receiving a water-deficit stress. Of the seeds initiated, control plants aborted 3.9 ± 0.9 seeds per raceme, compared to 5.0 ± 1.1 seeds aborted per raceme after water-deficit stress. Leaves of the water-stressed plants started the senescence process (yellowing) 8 days sooner than the leaves of the control plants.

Experiment B (*in planta*)

The number of flowers per raceme was similar at 47.4 ± 3.0 for the control and 40.0 ± 3.1 for the water-stressed plants (data not shown). Seed set was reduced after the water-deficit treatment, but the reduction was less striking than in experiment A. The control plants produced 9.7 ± 1.0 seeds per raceme, compared to 5.6 ± 1.0 seeds per raceme on the water-stressed plants at 6 weeks after water stress. Control plants produced 7.5 ± 0.9 filled seeds and 2.2 ± 0.4 aborted (not filled) seeds per raceme, compared to 5.3 ± 1.0 filled seeds and 0.3 ± 0.1 aborted (not filled)

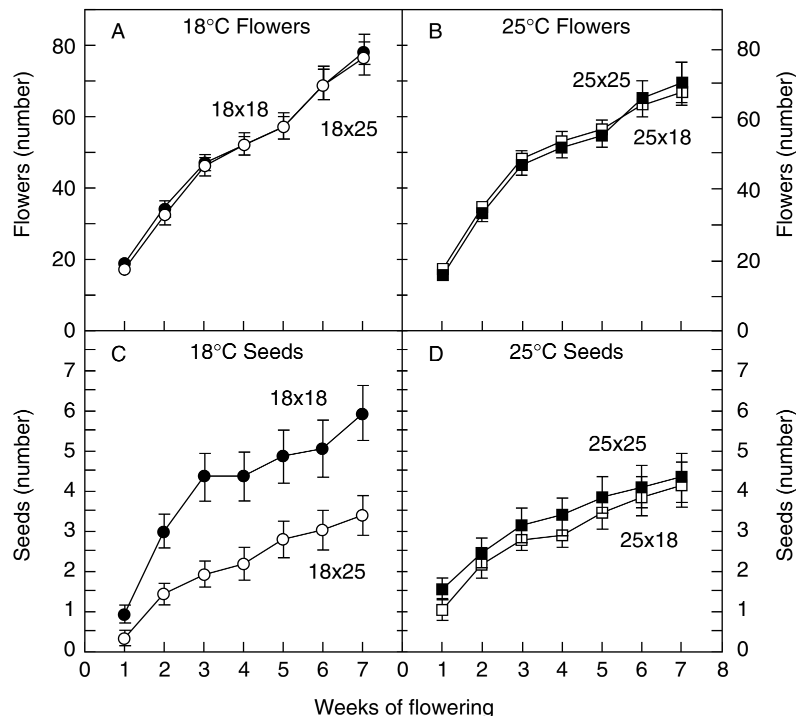

Figure 2. Accumulated number of flowers pollinated and seeds formed per raceme *in planta* (experiment 2). Racemes on plants grown at 18°C pollinated with pollen from plants grown at 18 or 25°C (A, C). Racemes on plants grown at 25°C pollinated with pollen from plants grown at 18 or 25°C (B, D). Values are mean ± SE for 40 racemes (eight replicates).

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

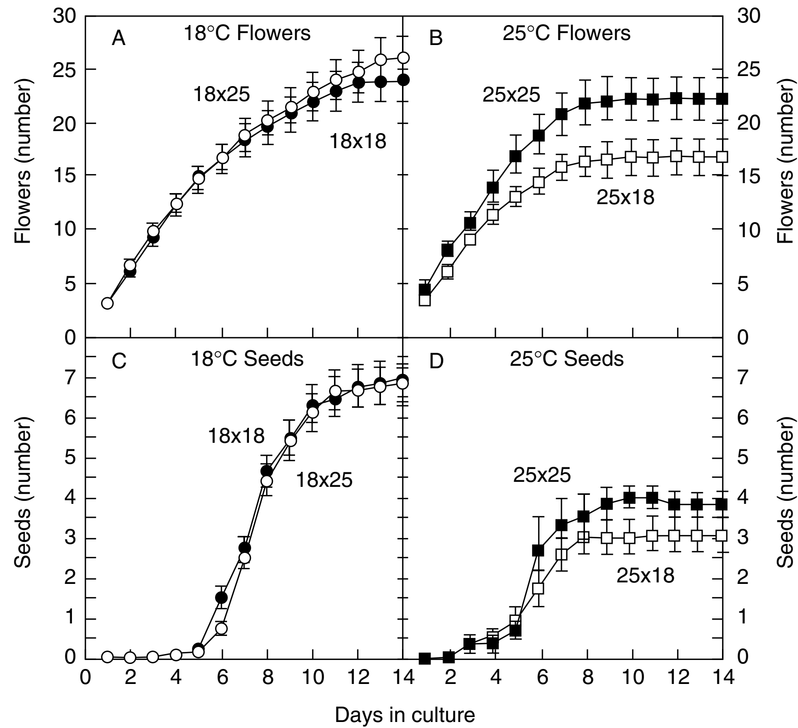

Figure 3. Accumulated number of flowers pollinated and seeds set per raceme explant *in vitro*. Raceme explants cultured at 18°C and pollinated with pollen from raceme explants cultured at 18 or 25°C (A, C). Raceme explants cultured at 25°C and pollinated with pollen from raceme explants cultured at 18 or 25°C (B, D). Values are mean ± SE for 20 racemes.

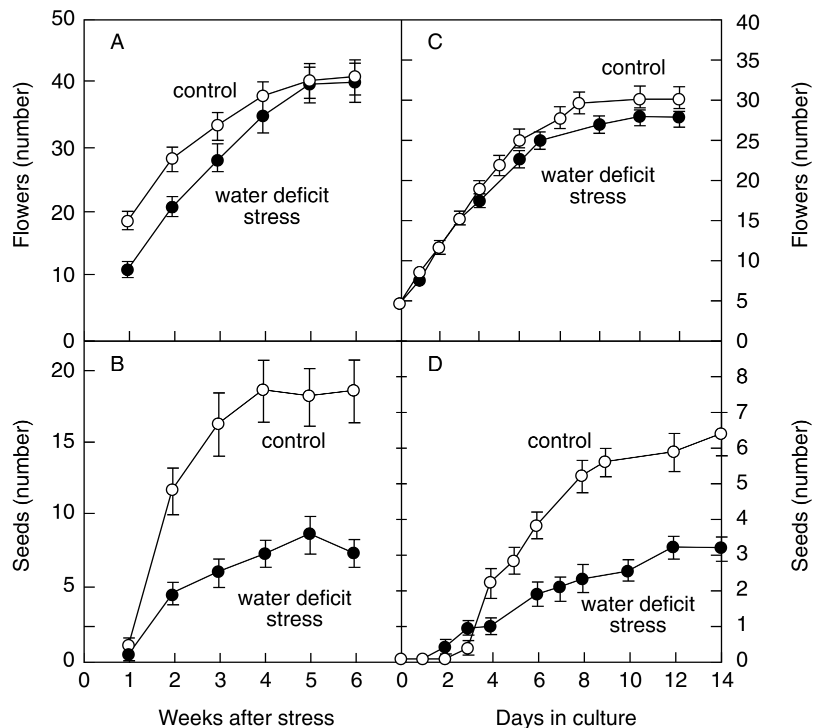

Figure 4. Accumulated number of flowers pollinated (A) and seeds formed (B) per raceme *in planta* on control plants and on plants after a 3-day water-deficit stress as a function of weeks after stress (experiment A). Flowers pollinated (C) and seeds set (D) on raceme explants *in vitro* from control plants and from plants after a 3-day water-deficit stress as a function of days in culture. Raceme explants were cultured at 18°C. Values are mean ± SE for 20 racemes.

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

seeds per raceme on plants receiving the water-deficit-stress treatment. Seed size and mass were similar on stress-treated and control plants and comparable to experiment A. Average seed dry weight for filled seeds on control plants was 34.6 ± 1.1 mg per seed, compared to 32.0 ± 0.9 mg per filled seed on plants after the water-deficit-stress treatment. Lengths and widths of the filled seeds were 7.5 ± 0.1 mm length and 5.0 ± 0.1 mm width for seeds on control plants and 7.6 ± 0.1 mm length and 5.2 ± 0.1 mm width after the water-deficit-stress treatment. Leaves of the water-deficit-stressed plants started the senescence process (yellowing) at about the same time as the leaves of the control plants.

*Experiment A (*in vitro*)*

In vitro experiments using raceme explants showed similar trends in flower formation and seeds per raceme as *in planta* experiments in growth chambers. Detached raceme explants from both control and water-stressed plants produced flowers at the same rate and duration throughout development (Fig. 4C). Seed set was reduced 50% on the racemes from water-stressed plants 4–14 days in culture (Fig. 4D).

Water-deficit-stress effect on pollen source

Three additional experiments provided raceme explants for *in vitro* reciprocal pollinations at 18°C between racemes of control and water-deficit-stressed plants. In the first experiment, the temperature was cool (24°C) during the period of water stress *in planta*. Flower production was the same on explants at 18°C in each treatment (Fig. 5A, B). Seed set was greater on racemes from control plants (Fig. 5C, Wet × Wet, 11–14 days) than on racemes from water-stressed plants (Fig. 5D, Dry × Wet) when pollen was from control plant racemes at 18°C. However, when pollinated with pollen of racemes from water-stressed plants, seed set was the same (Fig. 5C, Wet × Dry and Fig. 5D, Dry × Dry).

In a second experiment (data not shown), the period of water stress *in planta* coincided with hot, sunny weather. When the drought stress occurred at elevated temperatures (28–30°C), the effect of source of pollen was reduced following reciprocal crosses on *in vitro* raceme explants at 18°C. In a third experiment (data not shown), the water-stress period *in planta* coincided with very hot weather (32–35°C), and seed

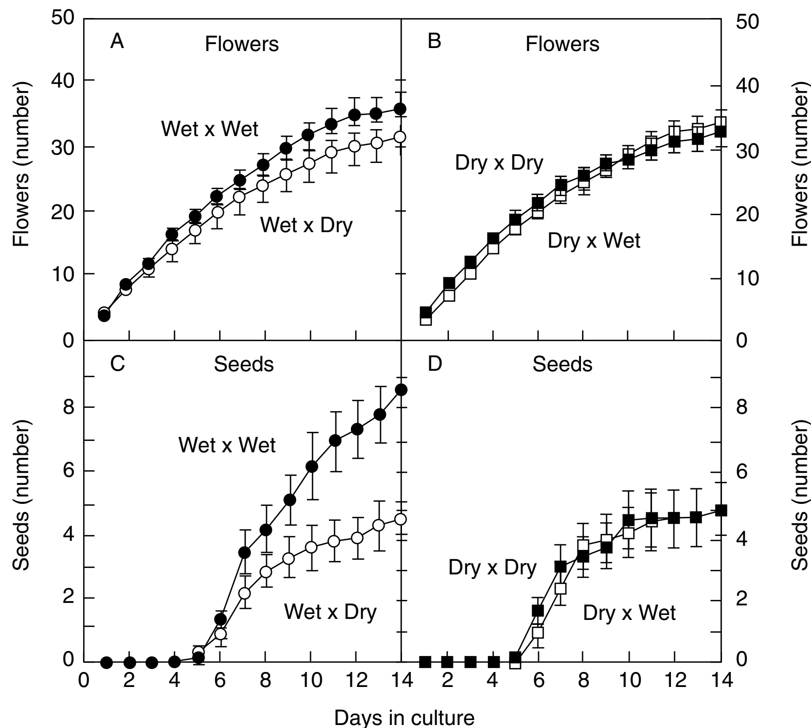

Figure 5. Accumulated number of flowers pollinated (A, B) and seeds formed (C, D) per raceme explant *in vitro* from control plants and from plants after a 3-day water-deficit stress as a function of days in culture. Flowers on raceme explants from control plants were pollinated with pollen from raceme explants from control plants (Wet × Wet) or with pollen from raceme explants from water-stressed plants (Wet × Dry) (A, C). Flowers on raceme explants from plants after a 3-day water-deficit stress were pollinated with pollen from raceme explants from control plants (Dry × Wet) or with pollen from raceme explants from water-stressed plants (Dry × Dry) (B, D). All raceme explants were cultured at 18°C. Values are mean ± SE for 20 racemes.

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

set on *in vitro* raceme explants at 18°C was identical in all four treatments. *In planta* experiments with reciprocal crosses between control and water-deficit-stressed plants were abandoned due to elevated temperatures in 1993.

Discussion

The optimum temperature for seed set in buckwheat is 18°C. Flowering was markedly delayed when plants were grown at 15°C (also reported by Lachmann and Adachi, 1990), and the experiment was discontinued. In experiments with raceme explants *in vitro* for 14 days, seed set was 4.5 ± 0.5 seeds per raceme (37 ± 3 flowers) at 20°C (data not shown), 4.4 seeds per raceme (36 flowers) at 15°C night/25°C day (data not shown), and 2.5 ± 0.5 (Fig. 1D) and 3.8 ± 0.3 (Fig. 3D) seeds per raceme at 25°C. These values were lower than 7.4 ± 0.7 (Fig. 1D), 7.0 ± 0.6 (Fig. 3C) and 8.5 ± 0.9 (Fig. 5C) seeds per raceme on raceme explants *in vitro* at 18°C, and 7.8 ± 0.6 seeds per raceme *in planta* at 18°C (Fig. 1C), thereby establishing 18°C as the optimum temperature for seed set for experiments reported herein. Buckwheat plants at 18°C produce the same number of flowers during the first 3 weeks of flowering, the period when nearly all seeds are set, as plants growing at 25°C, but plants at 25°C produce only half as many seeds per raceme as plants at 18°C. At 25°C, seed set stops abruptly after 3 to 4 weeks of flowering *in planta*, whereas at 18°C, seeds were set for a longer period of time (Fig. 1C). A strikingly similar pattern was observed with *in vitro* culture of detached raceme explants (Fig. 1D). The frequency of flowers judged as not fertilized increased as plants aged (Taylor and Obendorf, 2001), resulting in decreased seed set with increased plant age (Obendorf *et al.*, 1993b; Björkman *et al.*, 1995a). High temperatures may also contribute to degeneration of egg-sac components, leading to lack of fertilization of the egg and central cell nucleus (Guan and Adachi, 1992, 1994). At 24°C day (14 h) and 18°C night (10 h), about 20% of the flowers had abnormal egg sac components at anthesis, and this proportion remained constant during the first 5 weeks of flowering *in planta* (Taylor and Obendorf, 2001).

Taken together, these observations underscore the acute temperature sensitivity of processes leading to seed set at above-optimum temperatures. This difference cannot be explained by availability of photosynthates because the raceme explants received identical nutrient medium and all racemes were cultured at above 90% RH. A small improvement in seed set was observed when raceme explants were cultured at 25°C day and 15°C night (data not shown), typical of a field environment. However, at 15°C reproductive development is delayed (Lachmann and Adachi, 1990). The limiting factor is not night temperature alone, but appears to be an accumulated temperature effect over a period of days, as demonstrated in cowpea (Ahmed and Hall, 1993): a heat stress 7–9 days before anthesis in cowpea resulted in low pollen viability, a pattern consistent with our results.

Seeds on plants at 18°C accumulated 40% more dry weight per seed than seeds on plants at 25°C (Table 1). This effect was independent of seeds per raceme (3.1–7.5 at 18°C; 2.0–3.4 at 25°C), therefore not limited by assimilate supply in these experiments, and independent of pollen source. In mixed multiple-donor pollinations, variations in pollen performance were observed among pollen donor plants, but donors had equal success in single-donor pollinations (Björkman *et al.*, 1995b). For experiments reported herein, reciprocal crosses were with single-donor plants. Embryo dry weight was the same at 18 and 25°C (Horbowicz *et al.*, 1998), and therefore, the increased seed weight at 18°C was due to increased endosperm deposition. Seed production at 18°C should increase both the milling fraction for buckwheat flour and the quality of seed germplasm. Maturation at 18°C also increases the concentration of soluble carbohydrates, especially fagopyritol B1, in the embryo (Horbowicz *et al.*, 1998).

A temporary water-deficit stress during the first week of flowering reduced the number of seeds formed following pollination after the water stress, but not the size and weight of seeds or the number of flowers formed. After rewatering water-deficit-stressed plants, a burst of new flowers appeared (Fig. 4A). Flowers that formed after recovery from a water-deficit stress had a lower frequency of initiating seed development than flowers forming on control plants (Fig. 4B). Leaves on water-stressed plants started the senescence process (yellowing) sooner than leaves on control plants. This observation implies that the supply of photosynthate may become limiting earlier during the seed development phase of the stressed plants. In maize, reproductive failure at low water potential has been attributed to a decreased sucrose flux and an altered carbohydrate metabolism (Boyle *et al.*, 1991; Zinselmeier *et al.*, 1995). In buckwheat, the ability of flowers to use available nutrients appeared more limiting to seed set than the supply of photosynthates. The fact that the effect of water-deficit stress during the first week of flowering cannot be reversed by feeding racemes on liquid nutrient culture implies that something other than availability of photosynthates limits seed set following water-deficit stress. While an altered carbohydrate metabolism was not addressed in the present study, in other experiments (data not shown) sucrose concentrations in buckwheat ovary/ovule tissues were high at –1 to +3 days before/after anthesis and

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

independent of subsequent seed set. Since both the control and water-stressed racemes were cultured at 18°C, defective development of the egg-sac components, or other female structures, was the most likely cause of the lowered seed set. Water-deficit stress affected maize (*Zea mays* L.) ovaries (Zinselmeier *et al*., 1995) and may also affect pollen (Barnabás, 1985). Buckwheat ovules are positioned with the micropyle to the top, in line with convergence of the three stylar canals (Obendorf *et al*., 1993a). Misalignment of the micropyle and stylar canals caused by differential shrinking of ovule and ovary tissues was proposed as a reason for reduced fertility in flowers appearing on ageing plants (Obendorf *et al*., 1993b; Taylor and Obendorf, 2001). A similar misalignment in flowers forming after a water-deficit stress could result in reduced fertility.

Increasing plant age reduced seed set in newly opened flowers (Obendorf *et al*., 1993b; Taylor and Obendorf, 2001) in both water-deficit-stressed and control plants (Fig. 4B). Fewer seeds initiated development late in the flowering period. Those seeds that do initiate late in the flowering period usually had aborted ovules (Taylor and Obendorf, 2001) and failed to fill with seed storage reserves in the embryo and endosperm. The water-deficit-stress information may be a different signal than the age-stress information, but both result in reduced fertility of newly opened flowers that form subsequently. A 3-day water-deficit stress during the first week of flowering was sufficient to reduce the number of seeds by 50% without a reduction in seed size or dry matter accumulation per seed. Both male and female components limited reproductive success (non-additive) at above-optimal temperature or after water-deficit stress. Either stress resulted in a persistent reduction in seed set and sink strength. Avoidance of heat or water-deficit stresses during the early period of flowering was essential to maximizing seed set and yield potential in common buckwheat. Under the conditions described herein, flowers that formed late in the period of flowering contributed little to seed set and to yield potential.

Acknowledgements

Funded in part by grants from Minn-Dak Growers, Ltd, The Birkett Mills, Japan Buckwheat Millers Association, and Kasho Company Limited. J.S. was funded in part by a fellowship grant from The Kosciuszko Foundation. We thank W.L. DeMinck, P. Clement, K. Burridge, M. Lo, R. Touranont, S.M. Downer, C. Kaplan, B.R. Hudson and M.B. Obendorf for assistance with some of the experiments, including hand pollination of about 100,000 flowers.

References

Adachi, T. (1990) How to combine the reproductive system with biotechnology in order to overcome the breeding barrier in buckwheat. *Fagopyrum* **10**, 7–11.

Ahmed, F.E. and Hall, A.E. (1993) Heat injury during early floral bud development in cowpea. *Crop Science* **33**, 764–767.

Barnabás, B. (1985) Effect of water loss on germination ability of maize (*Zea mays* L.) pollen. *Annals of Botany* **55**, 201–204.

Björkman, T. (1995a) The effect of pollen load and pollen grain competition on fertilization success and progeny performance in *Fagopyrum esculentum*. *Euphytica* **83,** 47–52.

Björkman, T. (1995b) The effectiveness of heterostyly in preventing illegitimate pollination in dish-shaped flowers. *Sexual Plant Reproduction* **8,** 143–146.

Björkman, T. (1995c) Role of honey bees (Hymenoptera: Apidae) in the pollination of buckwheat in eastern North America. *Journal of Economic Entomology* **88,** 1739–1745.

Björkman, T., Rathburn, K. and Pearson, K.J. (1995a) The progression of female fertility in buckwheat through the flowering season. pp. 437–441 *in* Matano, T.; Ujihara, A. (Eds) *Current advances in buckwheat research*. Asahi Matsumoto City, Japan, Shinshu University Press.

Björkman, T., Samimy, C. and Pearson, K.J. (1995b) Variation in pollen performance among plants of *Fagopyrum esculentum*. *Euphytica* **82,** 235–240.

Boyle, M.G., Boyer, J.S. and Morgan, P.W. (1991) Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potential. *Crop Science* **31,** 1246–1252.

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**, 151–158.

Guan, L.M. and Adachi, T. (1992) Reproductive deterioration in buckwheat (*Fagopyrum esculentum*) under summer conditions. *Plant Breeding* **109**, 304–312.

Guan, L.M. and Adachi, T. (1994) Ultrastructural changes of the mature embryo sac in buckwheat (*Fagopyrum esculentum*) as a result of high temperature exposure. *Cytologia (Tokyo)* **59**, 237–248.

Horbowicz, M. and Obendorf, R.L. (1992) Changes in sterols and fatty acids of buckwheat endosperm and embryo during seed development. *Journal of Agricultural and Food Chemistry* **40**, 745–750.

Horbowicz, M., Brenac, P. and Obendorf, R.L. (1998) Fagopyritol B1, *O*-α-D-galactopyranosyl-(1→2)-D-*chiro*-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. *Planta* **205,** 1–11.

Lachmann, S. and Adachi, T. (1990) Studies on the influence of photoperiod and temperature on floral traits in buckwheat (*Fagopyrum esculentum* Moench) under controlled stress conditions. *Plant Breeding* **105**, 248–253.

Marshall, H.G. and Pomeranz, Y. (1982) Buckwheat: description, breeding, production, and utilization. *Advances in Cereal Science and Technology* **5**, 157–210.

Morris, M.R. (1952) Cytogenetic studies on buckwheat. Genetic and cytological studies of compatibility in

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

relation to heterostyly in common buckwheat, *Fagopyrum sagittatum*. *Journal of Heredity* **42,** 85–89.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco cultures. *Physiologia Plantarum* **15**, 473–497.

Namai, H. (1990) Pollination biology and reproductive ecology for improving genetics and breeding common buckwheat, *Fagopyrum esculentum* (1). *Fagopyrum* **10**, 23–46.

Namai, H. and Takeyama, A. (1992) Effects of the number of compatible pollen grains deposited on a stigma lobe of each flower to seed set percentage, seed weight and seed yield in common buckwheat. pp. 149–156 *in* Lin, R.; Zhou, M.; Tao, Y.; Li, J.; Zhang, Z. (Eds) *Proceedings of the 5th international symposium on buckwheat, 20–26 August 1992, Taiyuan, China*. Beijing, Agriculture Publishing House.

Obendorf, R.L., Horbowicz, M. and Taylor, D.P. (1993a) Structure and chemical composition of developing buckwheat seed. pp. 244–251 *in* Janick, J.; Simon, J.E. (Eds) *New crops*. New York, John Wiley and Sons.

Obendorf, R.L., Horbowicz, M., Taylor, D.P. and Slawinska, J. (1993b) Buckwheat seed development and regulation of seed set. pp. 39–46 *in* Côme, D.; Corbineau, F. (Eds) *Proceedings of the fourth international workshop on seeds: Basic and applied aspects of seed biology, Angers, France, 20–24 July 1992*. Paris, ASFIS.

Pomeranz, Y. (1983) Buckwheat: Structure, composition, and utilization. *CRC Critical Reviews in Food Science and Nutrition* **19**, 213–258.

Slawinska, J. and Obendorf, R.L. (1993) Buckwheat seed set and development by *in vitro* inflorescence culture. pp. 61–66 *in* Côme, D.; Corbineau, F. (Eds) *Proceedings of the fourth international workshop on seeds: Basic and applied aspects of seed biology, Angers, France, 20–24 July 1992*. Paris, ASFIS.

Taylor, D.P. and Obendorf, R.L. (2001) Quantitative assessment of some factors limiting seed set in buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae). *Crop Science* **41** (in press).

Zinselmeier, C., Westgate, M.E., Schussler, J.R. and Jones, R.J. (1995) Low water potential disrupts carbohydrate metabolism in maize (*Zea mays* L.) ovaries. *Plant Physiology* **107,** 385–391.

Received 5 July 2000
accepted after revision 25 April 2001
© CAB *International* 2001

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press

Grassland Ecophysiology and Grazing Ecology

Edited by **G Lemaire**, *INRA, France*, **J Hodgson**, *Massey University, New Zealand*, **A Moraes**, *Universidade Federal do Parana*, **P C de F Cavalho** and **C Nabinger**, *Universidade Federal do Rio Grande do Sul, Brazil*

October 2000 **432 pages** **Hardback**
ISBN 0 85199 452 0
£65.00 (US$120.00)

Readership: Plant ecology/physiology, grassland science, animal science.

The sustainability and ecophysiology of grazing systems are of growing importance. This research level book presents edited, key papers from the International Symposium on Grassland Ecophysiology and Grazing Ecology held in Curitiba, Brazil in August 1999. It considers how plants within grasslands respond to and are adapted to grazing animals.

This title:
- Is of worldwide interest, covering both temperate and tropical pastures
- Includes contributors and leading authorities from North and South America, Europe and Australasia

Contents:

- Sustainability of grazing systems: goals, concepts and methods, *J Hodgson and S C da Silva*

Part I: Environmental constraints and plant responses to defoliation
- Effects of nitrogen and water supply on N and C fluxes and partitioning in defoliated swards, *F Gastal and J L Durand*
- An integrated view of C and N uses in leaf growth zones of defoliated grasses, *H Schnyder, R Schäufele, R de Visser and C J Nelson*
- Effects of grazing on the roots and rhizosphere of grasses, *L A Dawson, S J Grayston and E Paterson*
- Reserve formation and recycling of carbon and nitrogen during regrowth of defoliated plants, *B Thornton, P Millard and U Bausenwein*

Part II: Morphogenesis of pasture species and adaptation to defoliation
- Shoot morphological plasticity of grasses: leaf growth *vs.* tillering, *C J Nelson*
- Tiller dynamics of grazed swards, *C Matthew, S G Assuero, C K Black and N R Sackville Hamilton*
- Effect of nitrogen on some morphogenetical traits of temperate and tropical perennial forage grasses, *P Cruz and M Boval*
- Modelling the dynamics of temperate grasses and legumes in cut mixtures, *J F Soussana and A Oliveira Machado*

Part III: Plant-Animal interactions
- Plant-animal interactions in complex plant communities: from mechanism to modelling, *I J Gordon*
- Modeling spatial aspects of plant-animal interactions, *E A Laca*
- Defoliation patterns and herbage intake on pastures, *M H Wade and P C de Carvalho*
- Selective grazing on grass-legume mixtures in tropical pastures, *C E Lascano*

Part IV: Sustainable grazing management of natural pastures
- Leaf tissue turnover and efficiency of herbage utilization, *G Lemaire and M Agnusdei*
- Dynamics of heterogeneity in a grazed sward, *A J Parsons, P Carrère and S Schwinning*
- Soil-plant-animal interactions and impact on nitrogen and phosphorus cycling and re-cycling in grazed pasture, *S C Jarvis*
- Sustainable management of pasture and rangelands, *J Stuth and G E Maraschin*

Part V: Problems of Animal Production Related to Pastures in Subtropical and Temperate Regions of South America
- *Campos* in Southern Brazil, *C Nabinger, A. de Moraes, and G E Maraschin*
- *Campos* in Uruguay, *E J Berretta, D F Risso, F Montossi, and G Pigurina*
- Argentina's Humid Pampa, *V A Deregibus*

For further information or to order please contact CABI *Publishing*, UK or an exclusive CABI *Publishing* distributor in your area.
Please add £2.50 per book postage and packing (excluding UK).

CABI Publishing, CAB International, Wallingford, Oxon, OX10 8DE,UK
Tel:+44(0)1491 832111 Fax:+44(0)1491 829292 Email:orders@cabi.org
CABI Publishing, CAB International, 10 East 40th Street, Suite 3203, New York, NY 10016, USA
Tel:+1 212 481 7018 Fax:+1 212 686 7993 Email:cabi-nao@cabi.org

https://doi.org/10.1079/SSR200178 Published online by Cambridge University Press