Annals of Botany **102**: 675–684, 2008
doi:10.1093/aob/mcn150, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Morphology of Nectaries and Biology of Nectar Production in the Distylous Species *Fagopyrum esculentum*

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Received: 11 March 2008 Returned for revision: 28 April 2008 Accepted: 8 July 2008 Published electronically: 3 September 2008

• *Background and Aims* The mechanisms of floral nectar production in buckwheat (*Fagopyrum esculentum*, Polygonaceae), a distylous pseudo-cereal, have received relatively little attention, prompting an investigation of the factors that regulate this process. The aim was to perform a refined study of the structures that secrete nectar and of the internal and external parameters influencing nectar volumes and sugar concentrations.
• *Methods* In order to control environmental parameters, plants were cultivated in growth rooms under controlled conditions. The structure of nectaries was studied based on histological sections from flowers and flower buds. Nectar was extracted using glass micropipettes and the sugar concentration was measured with a hand refractometer. Sugar concentration in the phloem sap was measured using the anthrone method. To test the influence of photosynthesis on nectar production, different light and defoliation treatments were applied.
• *Key Results* Unicellular trichomes were located in the epidermis at the ventral part of eight nectary glands situated on the flower receptacle alternately with stamens. Vascular bundles consisting of both phloem and xylem were identified at the boundary between a multilayered nectary parenchyma and a sub-nectary parenchyma with chloroplasts. A higher volume of nectar in thrum morphs was observed. No other difference was found in morphology or in sugar supply to inflorescences between morphs. Nectar secretion was strongly influenced by plant age and inflorescence position. Nectar volumes were higher in the upper inflorescences and during the flowering peak. Light had a dual role, (1) acting directly on reproductive structures to trigger flower opening, which conditions nectar secretion, and (2) stimulating photosynthetic activity, which regulates nectar accumulation in open flowers.
• *Conclusions* In buckwheat, nectar is secreted by trichomes and probably proceeds, at least in part, from phloem sap. Nectar secretion is strongly influenced by floral morph type, plant age, inflorescence position and light.

Key words: Buckwheat, distyly, *Fagopyrum esculentum*, inflorescence position, morph comparisons, nectary histology, nectar sugar concentration, nectar volume, light intensity, organ biomass, phloem sap, plant age.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum*) is an annual multipurpose pseudo-cereal belonging to the family Polygonaceae. Buckwheat seeds contain proteins with a unique amino acid composition, antioxidants, trace elements, dietary fibres and several components with healing benefits (Krkošková and Mrázová, 2005). Seeds are used as human and cattle food. Buckwheat is also a dependable and high-yielding honey plant: honey production averages 70–100 kg ha^{-1} and reaches 150–300 kg ha^{-1} for the best cultivars (Campbell, 1997; Naumkin, 1998). Buckwheat is therefore attractive to beekeepers (McGregor, 1976; Marshall and Pomeranz, 1982; Goodman *et al.*, 2001). Sown with other annual nectar- and pollen-producing herbaceous plants, buckwheat can be used in non-cropped areas of impoverished farmland as food plants for insect pollinators (Carreck and Williams, 2002). Buckwheat also attracts beneficial insects that attack or parasitize aphids, mites and other pests and can be used for biocontrol (Bowman *et al.*, 1998).

Starting 1 month after sowing, flowering of buckwheat is profuse and long lasting; each plant produces several hundreds of flowers over 2–3 months (Quinet *et al.*, 2004). Inflorescences are compound racemes that are initiated acropetally in leaf axils (Quinet *et al.*, 2004). The main stem and axillaries end in a terminal cluster composed of several inflorescences. Buckwheat possesses a sexual dimorphism, with populations being equally composed of plants with pin flowers (long pistil and short stamens) and plants with thrum flowers (short pistil and long stamens) (Nagatomo and Adachi, 1985; Campbell, 1997; Quinet *et al.*, 2004). Flowers are open and fertile for only 1 d; they are strictly self-incompatible with obligate cross-pollination between pin and thrum flowers. In most countries, honeybees (*Apis mellifera*) are commonly considered to be the main pollinators (Elagin, 1953; McGregor, 1976; Hedtke and Pritsch, 1993; Björkman, 1995; Lee and Choi, 1997; Goodman *et al.*, 2001; Jacquemart *et al.*, 2007). They appear to be effective because they collect both types of pollen (pin and thrum) on a single trip and their foraging and prospecting behaviour, collecting both nectar and pollen, promotes frequent contacts with stigmas (Björkman, 1995; Jacquemart *et al.*, 2007).

Insect attraction, pollination and consequently seed set of buckwheat depend on nectar production (Tahir and Farooq,

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1988; Namai, 1990; Alekseyeva and Bureyko, 2000). Glands at the base of the ovary secrete nectar composed of sucrose, fructose and glucose. The two hexoses are the major components and total sugar concentration of flower nectar averages 55 % in the growth chamber (Cawoy *et al.*, 2006). In these controlled conditions, nectar secretion in a flower starts after opening (anthesis) and is constant during all the light period, even in pollinated flowers. Pin flowers produce less nectar than thrum flowers and are less visited by honeybees in the field (Cawoy *et al.*, 2006). Differences in nectar production between morphs in distylous species are unusual: Ornelas *et al.* (2004) and Teixeira and Machado (2004) reported that pin flowers of *Palicourea padifolia* and *Psychotria barbiflora* secreted more nectar than thrum flowers.

Although nectar is a significant parameter conditioning pollination and therefore seed set (Cresswell, 1999; Shafir *et al.*, 2003; Leiss *et al.*, 2004; Kudo and Harder, 2005), physiological approaches to its production in buckwheat have thus far been superficial and limited to basic weather and variety influences (Naumkin, 1998; Alekseyeva and Bureyko, 2000). Moreover, to our knowledge there are only two studies concerning nectary structure (De Craene and Akeroyds, 1988; De Craene and Smets, 1991) and no information about nectar secretion in this species. The aims of the present study were therefore to describe the structure of the nectaries and to investigate several exogenous and endogenous factors influencing nectar production in buckwheat. The objectives were: (1) to attempt to identify the origin of the difference in nectar production between morphs; (2) to determine whether nectar secretion was related to plant biomass, age of plants and inflorescence position on the plant; and (3) to evaluate the importance of light and photosynthetic activity for nectar production with different light or defoliation treatments. Experiments were carried out in controlled conditions as a large number of parameters can influence nectar production, and variable field conditions of temperature, air and soil humidity or irradiance make precise measurements of small quantities of nectar quite difficult (Pacini *et al.*, 2003; Pacini and Nepi, 2007).

MATERIALS AND METHODS

Plant material and growth conditions

Buckwheat (*Fagopyrum esculentum* Moench) seeds of the cultivar 'La Harpe' were obtained from 'Agri-Obtentions' (Guyancourt, France). This cultivar is a facultative short-day plant (Quinet *et al.*, 2004) developed by the INRA (Institut National de la Recherche Agronomique, Paris, France).

Plants were cultivated in the growth chambers of the Department of Biology of the Université catholique de Louvain (Louvain-la-Neuve, Belgium), in the absence of pollinators. Temperature was maintained at 23–24/17–19 °C (day/night) and relative humidity at 78 ± 5 %. Light was supplied by Philips HPIT 400-W lamps (Philips Lighting S.A., Brussels, Belgium). The day/night cycle was of 16/8 h and the photon flux density at the top of the canopy was maintained at 120 ± 20 μmol m^{-2} s^{-1}.

Plants were grown in peat compost, except for the experiment intended to investigate nectar production in relation to morphological features of morphs. In this case, plants were cultivated in a hydroponic system to allow direct access to the roots. Seeds sown in peat compost germinated within 3–4 d and, 8 d after sowing, the plants were singled planted into plastic pots (0·7 L). For hydroponic culture, seeds were germinated on rock wool and pre-treated with a fungicide (Rovral, 2·5 g L^{-1}). When 8 d old, seedlings of similar size were transferred to plastic containers (1·8 L) filled with a modified Yoshida's nutrient solution (Quinet *et al.*, 2004) adjusted to pH 6·5. Six seedlings per container were fixed in plugged holes of polystyrene plates floating on the nutrient solution, which was renewed every fortnight. To compensate for plant consumption and evaporation, the volume in each container was maintained by adding fresh Yoshida's nutrient solution once a week up to the 5th week and twice a week thereafter.

Axillary shoots were removed 4 weeks after sowing to facilitate plant accessibility and to homogenize plant architecture.

Histological study of nectaries

Flower buds and open flowers from pin and thrum morphs were collected. Buds were harvested 1 d before anthesis, and open flowers 2, 6 and 10 h after light switch on. Samples were fixed in FAA (70 % ethanol/acetic acid/formaldehyde, 18 : 1 : 1, by volume), dehydrated in a graded ethanol series and embedded in paraffin. Serial, 5-μm-thick, longitudinal sections were stained with haematoxylin-fast green and observed with a light microscope.

Estimation of nectar volumes and sugar concentrations

On the day of anthesis, nectar was extracted using 0·5-μL (for volume measurements) or 1-μL (for sugar concentration measurements) glass micropipettes (Hirschmann® Laborgeräte, Eberstadt, Germany).

For volume estimations, nectar was collected over 2 h between 0630 and 0930 h after light switch on, on the day of anthesis. The volume was estimated by measuring the length of the nectar column in the capillary tube.

For sugar concentration analyses, nectar samples (approximately 1 μL) were collected from flowers of all inflorescences, between 0930 and 1130 h after light switch on, on the day of anthesis. Sugar concentration was measured with a low-volume hand refractometer (Eclipse Handheld refractometer, Bellingham & Stanley Ltd, Tunbridge Wells, UK) and was expressed as a percentage of sucrose in nectar mass (w/w).

Organ biomass measurements

To determine whether nectar production is related to plant biomass or not, nectar was collected on all flowers in anthesis on the day when the flowering reached the last inflorescence at the top of the main stem, i.e. 7–9 weeks

after sowing. Plants were then divided into four parts: (1) laminas, (2) axes (main stem and petioles), (3) inflorescences and (4) roots. Cut parts were oven-dried for 5 d at 60 °C and weighed. For each morph, 25 plants grown in hydroponic culture were used.

Analysis of total sugars from phloem sap supplying inflorescences

Nectar volume of one flower of the 4th inflorescence was estimated at full flowering (when all inflorescences of the plant had reached anthesis) on 25 10-week-old plants of each morph. Two days later, the inflorescences were excised and oven-dried for 5 d at 60 °C before being weighed.

Phloem sap harvest was begun immediately after excision of the inflorescences. To prevent arrest of sap flow, the tip of the peduncle was first re-cut under water and thereafter dipped in 2 mL of a 20 mM EDTA (ethylenediaminetetraacetic acid) solution buffered at pH 7·0 contained in an Eppendorf tube (Safe-lock tube 2 mL, Eppendorf, Hamburg, Germany). Evaporation of the EDTA solution was prevented by sealing the opening of the Eppendorf tube with a plastic foil that was perforated to introduce the tip of the peduncle. The Eppendorf tubes were fixed on sticks using sticky tape. Exudation began 1–2 h after light switch on and was stopped 8–9 h later. A Beckman DU640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) was used to measure the total sugar concentration using the anthrone method according to Yemm and Willis (1954). The results were expressed in mass of sugars per dry mass of the inflorescence.

Phenology of nectar secretion

Impacts of inflorescence position on the main stem and of plant age on nectar production were studied weekly by collecting nectar from flowers (one flower per day per inflorescence) that successively reached anthesis on four inflorescences of the main stem (1st, 4th, 7th and 10th, acropetally numbered), during the flowering period of 15 thrum plants. Total nectar production was estimated from the sum of the nectar volumes produced by flowers on these inflorescences (four flowers per day per plant). Thrum plants were chosen as they produce more accessible nectar in higher quantity (Cawoy *et al.*, 2006). In order to relate total nectar production by the four inflorescences to plant phenology, all flowers reaching anthesis on these inflorescences were counted weekly.

Light treatments and defoliations

To investigate the role of light and the influence of photosynthesis on nectar production, three different treatments were applied to entire plants, leaves and/or inflorescences to discriminate between potential effects through source and/or sink organs.

Entire plants in darkness. Five weeks after sowing, 15 thrum plants were transferred from the normal illuminated growth room (conditions as previously described) to a growth room in total darkness but under similar temperature and relative humidity conditions. Fifteen control plants were left in the illuminated growth room. Nectar volume of flowers of the 4th inflorescence was measured just before the transfer and over the following 7 d. For daily observations, plants deprived of light were briefly illuminated (1–3 min). The number of open flowers on the 4th inflorescence was also counted.

Inflorescences in darkness. Seven weeks after sowing, the 4th inflorescence of 12 thrum plants was deprived of light by enclosing it in an opaque plastic bag. Two control groups of 12 thrum plants each comprised plants with their 4th inflorescence either wrapped in transparent colourless plastic bags or kept in free atmosphere. The plastic bags were perforated with tiny holes on the edges to prevent water condensation. Nectar volume of flowers was measured before starting the inflorescence treatments and over the following 7 d. The number of open flowers on the 4th inflorescences was also recorded.

Plant defoliations. Seven weeks after sowing, 15 thrum plants were totally defoliated by sectioning leaf petioles at their base. Nectar was harvested from one flower of the 4th inflorescence just before defoliation and at intervals of 1–7 d over 39 d, for volume measurements (15 plants) and for sugar concentration estimations (six plants). The number of open flowers on the 4th inflorescence was also recorded. A control group consisted of 15 intact thrum plants. Height of both defoliated and control plants was measured at the end of the experiment.

Statistical analysis

Statistical analyses were performed using the SAS software (SAS system for Windows v9·1). Shapiro–Wilks normality tests were performed and no transformation of the raw data was required. Analyses of variance were performed with the general linear method procedures in SAS (one-way ANOVA). When group sizes were identical, differences between means were evaluated for significance by using the Student–Newman–Keuls test. When group sizes were different, the GT2 test was applied. Correlation and simple regression analyses were performed to compare: (1) the volume of nectar produced by one flower of the 4th inflorescence and carbohydrate supply to this inflorescence; and (2) flower production per week and total nectar secretion estimated from the sum of nectar produced by the four inflorescences 1 d per week. In order to examine the relationship between organ weights and floral nectar secretion, multiple linear regressions with forward selection (50 %) were performed and the parametric correlation coefficients were calculated. Means are given with their standard errors.

RESULTS

Morphology and structure of nectaries

At flower anthesis, glands situated on the receptacle, at the base of the ovary alternately with the filaments of the eight stamens, secreted drops of a non-protected nectar (Fig. 1A). During the day, the drops converged to form a continuous layer. After removal of gynoecium and stamens, a top view of the flower revealed the eight yellow protruding glands organized in a circle (Fig. 1B). In front view, trichomes were observed at the lower face of the nectary glands (Fig. 1C).

In histological longitudinal sections, the floral nectaries had the shape of a hook (Fig. 1D). They consisted of a modified monolayered epidermis covering a specialized multilayered parenchyma called the nectary parenchyma (Nepi, 2007) that filled the hook and extended up to the ovary base. A parenchyma with few chloroplasts and vascular bundles, the sub-nectary parenchyma (Nepi, 2007), constituted the underlying tissue (Fig. 1I, K).

The nectary epidermis comprised two types of cells. Alive unicellular trichomes, rich in cytoplasm, occupied the ventral part of the hook whereas the other epidermis cells, square or rectangular in section, generally appeared cytoplasm-free, with walls in the process of suberization (Fig. 1E, F). In the flower buds, the cells of the nectary parenchyma were small and contained a dense granular cytoplasm, a large nucleus and small vacuoles (Fig. 1G). During nectar exudation, the cytoplasm became less dense and vacuoles increased in volume (Fig. 1H). Vascular bundles were observed at the boundary between the specialized and the sub-nectary parenchyma (Fig. 1I–K). They

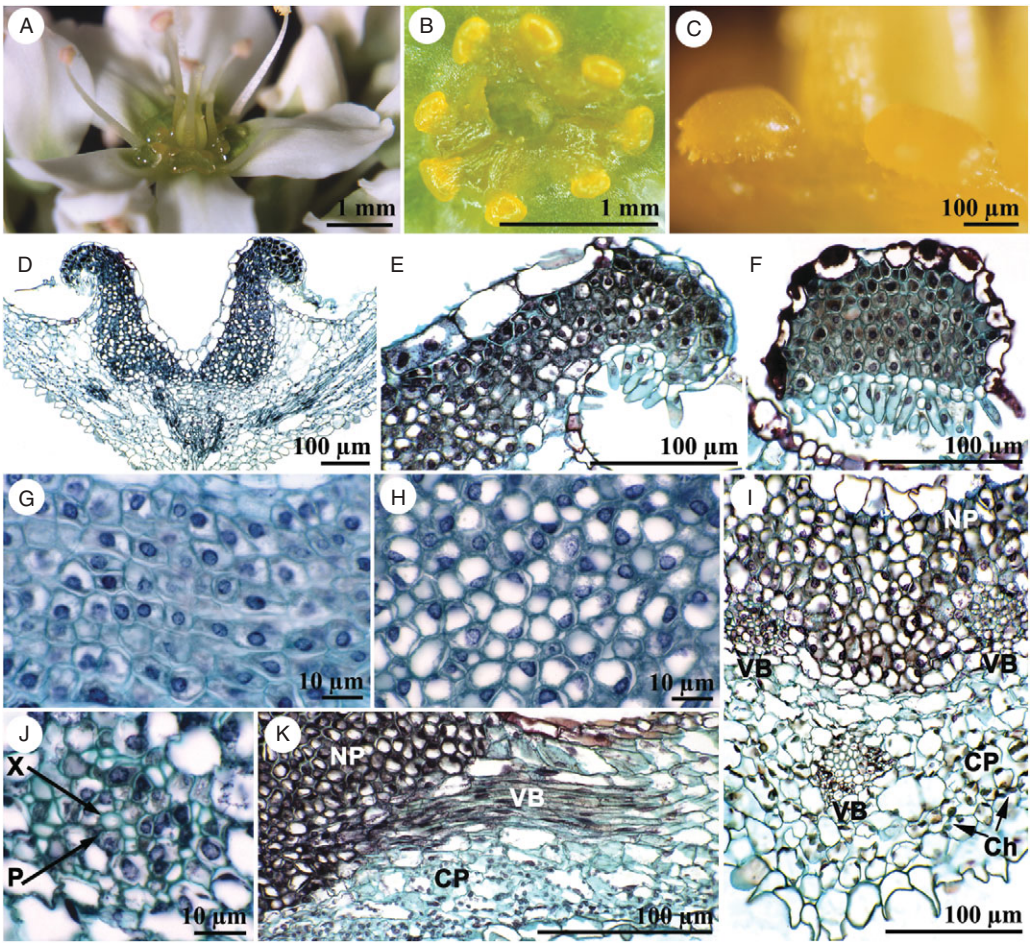

FIG. 1. Morphology of the buckwheat nectary and histology of the nectary tissues. (A) Thrum flower in anthesis that secretes exposed nectar-forming drops on the receptacle. (B) Top view of a flower after removal of gynoecium and stamens showing eight yellow protruding glands organized in a circle. (C) Front macroscopic view of a nectary with trichomes at the ventral face. (D) Longitudinal section of a flower. The hook-shaped nectary consists of a specialized multilayered parenchyma, the nectary parenchyma (NP), covered by a monolayered epidermis. (E) Longitudinal section in a nectary: the epidermis of the ventral face consists of unicellular secretory trichomes. No secretory cells can be observed elsewhere. (F) Transversal section of a nectary. Trichomes are visible over the entire width of the ventral face of the nectary. (G) Longitudinal section in the nectary parenchyma before nectar exsudation. The vacuoles are small and the nucleus is in the middle of the cell. (H) Longitudinal section in the nectary parenchyma during nectar exsudation. The cells are vacuolated and the nucleus is applied to the wall. (I) Longitudinal section in a flower. Two vascular bundles (VB) at the base of the nectary parenchyma (NP) and one in the sub-nectary parenchyma (CP) are visible. Chloroplasts (Ch) are visible in the sub-nectary parenchyma. (J) Longitudinal section in a vascular bundle composed of phloem (P) and xylem (X). (K) Longitudinal section in a flower showing a vascular bundle (VB) which crosses the sub-nectary parenchyma (CP) in a tepal and reaches the nectary parenchyma (NP).

consisted of both phloem and xylem (Fig. 1J) and were connected to the vasculature of the other floral organs. No difference in nectary histology was observed between the two floral morphs.

Nectar production in relation to plant organ biomass and phloem sap supply to inflorescences

Dry weight of inflorescences, axes (main stem and petioles), laminas and roots were similar in the two morphs whereas nectar production by flowers from the 4th inflorescence was statistically higher in thrum than in pin plants (Tables 1 and 2). However, no difference in the relative sugar concentration supplied from phloem sap to the 4th inflorescence was observed between the two morphs (Table 2).

Nectar production varied among plants of the same morph. However, there was no significant relationship between the volume of nectar produced by flowers and carbohydrate supply to inflorescences (thrum: $r^2 = 0{\cdot}0281$, $F = 0{\cdot}67$, $P = 0{\cdot}4229$; pin: $r^2 = 0{\cdot}0813$, $F = 2{\cdot}04$, $P = 0{\cdot}1671$), nor between nectar production by plants and their organ dry weight (thrum: $r^2 = 0{\cdot}0491$, $F = 1{\cdot}19$, $P = 0{\cdot}2873$; pin: $r^2 = 0{\cdot}0407$, $F = 0{\cdot}98$, $P = 0{\cdot}3333$).

Phenology of nectar production

One month after sowing, nectar production by the plant began with the first open flowers which appeared on the first inflorescence (acropetally numbering) (Fig. 2A). Flowering progressed acropetally from raceme to raceme, up to the terminal cluster. The flowering peak, i.e. when the largest number of flowers at anthesis in a week was recorded on the plant (four inflorescences: 1st, 4th, 7th and 10th, acropetally numbered), occurred 3–4 weeks later (55–64 d from sowing, Fig. 2B). Thereafter, the number of flowers at anthesis slowly decreased. After 3 months of cultivation, flowering stopped in some of the first inflorescences. Most plants stopped flowering after 4–5 months of cultivation; the four inflorescences produced between 645 and 2424 flowers per plant.

At flowering peak, flowers in the upper inflorescences were the most productive (Fig. 2A). Except for the first inflorescence ($F = 0{\cdot}12$, $P = 0{\cdot}7267$), nectar volume per

TABLE 1. *Number of flowers in anthesis, nectar volume and organ biomass [roots, axes (main stem and petioles), inflorescences and laminas] as a function of flower morph of buckwheat plants, on the day when flowering reached the last inflorescence at the top of the main stem*

Measured parameter	Thrum plants	Pin plants	*F*	*P*
Number of flowers in anthesis per plant	29·44 ± 2·13	27·84 ± 1·81	0·33	0·5705
Nectar volume (μL):				
per plant	2·27 ± 0·17	1·61 ± 0·12	10·48	0·0021*
per flower	0·08 ± 0·01	0·06 ± 0·01	18·90	<0·0001*
Organ dry weight (mg)				
Roots	370 ± 31	378 ± 31	0·03	0·8678
Laminas	653 ± 35	683 ± 35	0·36	0·5508
Axes: main stem and petioles	1693 ± 118	1688 ± 99	0·01	0·9735
Inflorescences	203 ± 9	187 ± 12	1·01	0·3204

Plants were grown in a hydroponic system under controlled conditions. Nectar was collected on all flowers in anthesis. Data are presented as means ± s.e.; $n = 25$ plants per morph.

* $P < 0{\cdot}05$ (one-way ANOVA).

TABLE 2. *Nectar volumes per flower on the 4th inflorescence and total sugars in the phloem sap collected at the tip of the peduncle after removal of the inflorescence and an 8–9-h period of exudation, as a function of floral morph of buckwheat*

Measured parameter	Thrum plants	Pin plants	*F*	*P*
Nectar volume (μL per flower)	0·17 ± 0·03	0·12 ± 0·02	24·53	<0·0001*
Total sugars in phloem sap (mg g^{-1} of inflorescence dry weight)	10·62 ± 1·73	10·52 ± 1·68	0·01	0·9660

Plants were grown in peat compost under controlled conditions. Sugar content of phloem sap is reported to 4th inflorescence dry weight. Data are presented as means ± s.e.; $n = 25$ plants per morph.

* $P < 0{\cdot}05$ (one-way ANOVA).

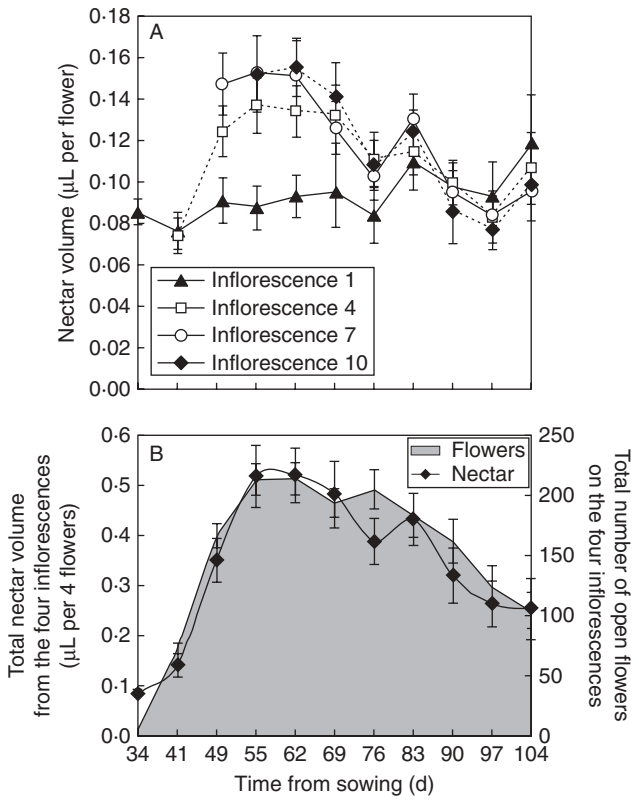

FIG. 2. Weekly production of nectar and flowers by thrum buckwheat plants. Inflorescences are numbered acropetally. Data shown are means ± s.e. ($n = 15$ plants). (A) Nectar volume per flower of 1st, 4th, 7th or 10th inflorescence. (B) Number of flowers reaching anthesis per week on 1st, 4th, 7th and 10th inflorescence and sum of nectar production per flower of 1st, 4th, 7th and 10th inflorescence.

TABLE 3. *Effects of a dark treatment applied to whole thrum buckwheat plants on the number of newly open flowers and nectar volumes per flower on the 4th inflorescence*

Measured parameter and treatment	Time of data collection: Before transfer to dark	Time of data collection: One day after transfer to dark	*F*	*P*
Number of flowers in anthesis per day				
Control plants	3·53 ± 0·40	3·86 ± 0·36	0·38	0·5428
Dark-treated plants	3·33 ± 0·42	1·27 ± 0·33	14·88	0·0006*
Nectar volume (μL per flower)				
Control plants	0·14 ± 0·01	0·13 ± 0·01	0·41	0·526
Dark-treated plants	0·13 ± 0·01	0·03 ± 0·01	32·27	<0·0001*

Data are presented as means ± s.e. Before dark treatment, $n = 15$ for both control and dark treatments; 1 d after the start of the dark treatment, $n = 15$ for controls and $n = 9$ for dark treatment.

* $P < 0·05$ (one-way ANOVA).

flower varied during a plant's life (inflorescence 4: $F = 3·07$, $P = 0·0023$; inflorescence 7: $F = 3·66$, $P = 0·0008$; inflorescence 10: $F = 3·95$, $P = 0·0006$) (Fig. 2A). Nectar production per plant was linked to the number of flowers at anthesis, as demonstrated by a positive relationship between the number of flowers reaching anthesis weekly on the four inflorescences and the total nectar production estimated from the sum of the nectar volumes produced by flowers on these inflorescences ($y = 0·0291 + 0·0021x$, $r^2 = 0·9109$, $F = 92·00$, $P < 0·0001$) (Fig. 2B).

Light treatments and defoliations

Entire plants in darkness. Transferring plants from light to darkness significantly decreased the number of flowers at anthesis as well as nectar secretion by the few flowers that opened (Table 3). After 1 d in complete darkness, 60 % of plants showed some newly open flowers on the 4th inflorescence. On following days, there was no further anthesis and nectar was not produced by the flowers that remained closed. After 1 week without light, inflorescences became senescent and leaves turned yellow.

Inflorescences in darkness. Comparing control inflorescences either kept in free atmosphere or enclosed in a transparent colourless plastic bag with inflorescences wrapped in an opaque plastic bag demonstrated that darkness rapidly disturbed inflorescence functioning. After just 1 d in the dark, the percentage of inflorescences with newly open flowers was decreasing (both controls: 100 %; light protected: 67 %). The number of open flowers per inflorescence was significantly reduced (Table 4). Corolla unfolding of the few flowers that opened in darkness was often incomplete but nectar secretion by these flowers was not affected; however, as illustrated by control inflorescences wrapped in a transparent bag, nectar secretion increased in a confined atmosphere (Table 4). On the following days, no further flowers opened on light-protected inflorescences and the experiment was stopped after 7 d.

Plant defoliations. Defoliated plants produced fewer open flowers than controls (27·5 ± 2·2 vs. 37·1 ± 2·8, $F = 7·11$, $P = 0·0126$). The number of open flowers per day decreased significantly in defoliated plants after day 25 (Fig. 3A). Following defoliation, nectar secretion by flowers slowly decreased and stabilized 19 d later (Fig. 3B). After 5 d, the volume of nectar secreted per flower was 35–50 % higher in control plants than in defoliated plants (Fig. 3B). Similarly, sugar concentration in nectar of defoliated plants decreased and stabilized 14 d after defoliation (Fig. 3C). Sugar concentration of nectar was 30–40 % higher in controls than in defoliated plants. When the defoliation experiment was discontinued, 40 d after leaf excisions, there was usually no newly open flower on the defoliated plants. These defoliated plants became senescent and were significantly smaller than the control plants (164 ± 6 vs. 190 ± 7 cm, $F = 9·37$, $P = 0·0048$).

TABLE 4. *Effects of a dark treatment applied to the 4th inflorescence of thrum buckwheat plants on the number of newly open flowers and nectar volumes per flower on this inflorescence*

Measured parameter and treatment	Time of data collection: Before dark application	Time of data collection: One day after dark application	*F*	*P*
Number of flowers in anthesis per day				
Control inflorescences in free atmosphere	4·17 ± 0·21	3·91 ± 0·48	0·22	0·6395
Control inflorescences in transparent bag	3·75 ± 0·41	4·25 ± 0·33	0·90	0·3520
Dark-treated inflorescences in opaque bag	4·50 ± 0·45	1·67 ± 0·48	18·37	<0·0001*
Nectar volume (μL per flower)				
Control inflorescences in free atmosphere	0·13 ± 0·01	0·12 ± 0·01	0·47	0·4978
Control inflorescences in transparent bag	0·13 ± 0·01	0·20 ± 0·03	4·21	0·0523
Dark-treated inflorescences in opaque bag	0·13 ± 0·01	0·15 ± 0·02	0·25	0·6197

Data are presented as means ± s.e. Before dark treatment, $n = 12$ for the three treatments; after 1 d of dark treatment, $n = 12$ for both controls in free atmosphere or in transparent bag and $n = 8$ for dark treatment.

* $P < 0·05$ (one-way ANOVA).

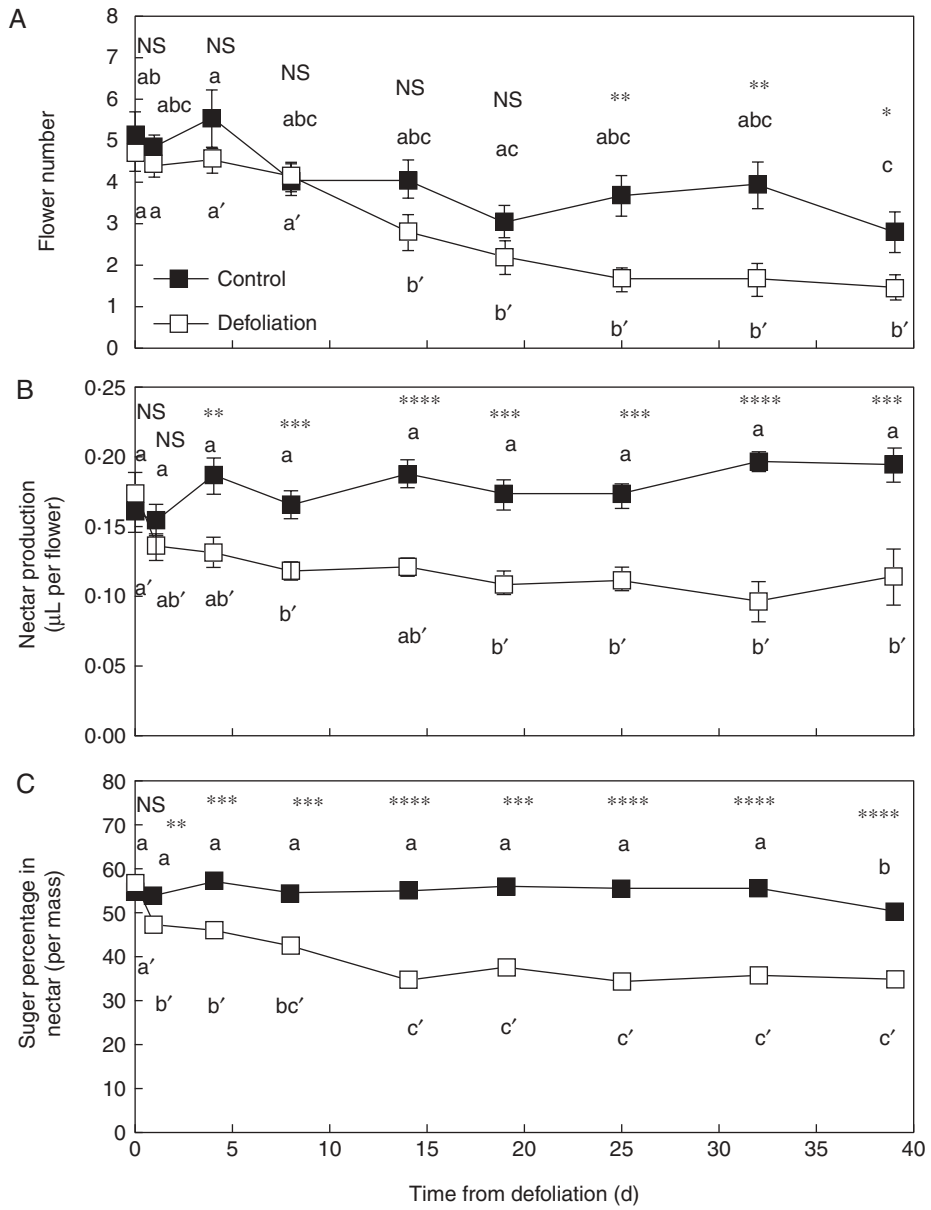

FIG. 3. Number of newly open flowers, nectar volume and sugar concentration as a function of time from defoliation of the whole thrum buckwheat plants; defoliated and control plants as indicated. Data shown are means ± s.e. Significant differences between treatments, at a given time, are indicated by asterisks (one-way ANOVA, statistical significance: n.s. = not significant; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$; ****$P < 0{\cdot}0001$) while significant differences between periods of a treatment are indicated by different letters ($P \geq 0{\cdot}05$). (A) Daily number of open flowers on the 4th inflorescence ($n = 15$). (B) Nectar volume per flower from the 4th inflorescence ($n = 15$). (C) Nectar sugar concentration expressed as a percentage of sucrose in nectar mass (w/w). Nectar samples were collected from flowers of all inflorescences ($n = 6$).

DISCUSSION

Nectary structure and nectar secretion

In buckwheat, nectar secretion of the receptacular nectaries started after flower opening. Nectar drops accumulated all around the nectaries, which appeared as eight hook-shaped protrusions located on the receptacle between the stamens. These protusions were called globular stalked nectaries by Da Craene and Akeroyds (1988). The nectary epidermis mediates nectar release in a majority of plant species (Fahn, 1988; Pacini *et al.*, 2003). In buckwheat, it was composed of suberized cells, except at the ventral face of the hook, which consisted of alive unicellular hairs. The location of nectar in the flowers of buckwheat, the nature of the epidermis cells, and the absence of modified stomata and lysigenous cavities to eliminate nectar support the view that the nectar is secreted through the trichomes (Fahn, 1988; De Craene and Smets, 1991). Such a mode of secretion with one-celled secretory hairs has been observed in Dipsacales, Orchidaceae and Tropaeolaceae (Bernardello, 2007). Epidermis that includes nectar-secreting structures can be related to epidermal nectaries

in general but in some groups, such as Polygonaceae, trichomes or papillae are related to mesenchymatic nectaries and are located in the epidermis of the nectaries (De Craene and Smets, 1991; Bernardello, 2007). The techniques used in the present study did not allow identification of the route by which the nectar passes through the cuticle of the cells, a barrier that may be crossed either through pores, by permeation or after cuticle rupture (Fahn, 1979).

In buckwheat, the multilayered nectary parenchyma, located beneath the epidermis, was supplied with water and nutrients by vascular bundles made up of phloem and xylem and connected to the vascular system of the other floral organs. Nectar sugars are derived from the photosynthetic activity of the nectary itself and/or of other floral and/or vegetative parts, generally close to the flower (Pacini *et al.*, 2003; Pacini and Nepi, 2007). Nectar assimilates originating from tissues situated outside the nectary are undoubtedly unloaded from the phloem (Fahn, 2000; Pacini and Nepi, 2007). The source of nectar sugars may be immediate photosynthesis or may require temporary starch storage in nectary amyloplasts before nectar secretion (Pacini *et al.*, 2003). No plasts were observed in the nectary parenchyma or in the epidermis whereas the sub-nectary parenchyma contained chloroplasts and vascular bundles. Therefore, the pre-nectar probably proceeded, at least partially, directly from the phloem sap and enzymes present in the nectary parenchyma partially hydrolysed the pre-nectar sucrose into glucose and fructose prior to secretion (Fahn, 1988; Pacini and Nepi, 2007). This idea is supported by the fact that buckwheat nectar consists of the three sugars mentioned above (Cawoy *et al.*, 2006). Sub-nectary parenchyma might also contribute to nectar sucrose production. Further observations under UV light to identify the presence of chloroplasts and with IKI (iodine–potassium–iodine), which stains starch grains in amyloplasts, are required (Nepi, 2007). These should help to validate our hypothesis that there are neither chloroplasts nor amyloplasts in the nectary parenchyma of buckwheat. Moreover, it would be of interest to look for amyloplasts in younger flower buds as these can disappear before anthesis (Peng *et al.*, 2004).

Nectar production by floral morphs

Previous studies have clearly demonstrated that the two morphs of buckwheat do not differ in the number of racemes, cymes and flowers they produce (Quinet *et al.*, 2004; Cawoy *et al.*, 2006). The present study showed that plants of both floral morphs were similar as regards root, axis (main stem and petioles), lamina and inflorescence weights. However, as reported by Cawoy *et al.* (2006), thrum flowers had a higher production of nectar than pin flowers. This difference in production averaged 30 % during the first part of the flowering period. Nevertheless, the sugar composition of nectar was identical in the two morphs.

According to Pacini *et al.* (2003), the quantity of nectar secreted by a flower is related to the volume of the nectary parenchyma. Although no precise measurements were performed here, microscopic observations of the nectary parenchyma suggested that there is no difference between the two morphs. Similarly, no difference in sugar supply by phloem sap to inflorescences of the two morphs was detected. However, as phloem sap at the tip of the peduncle of an inflorescence brought sugars not only for nectar but also for morphogenesis and development of the multiple reproductive structures that coexist in a raceme, more precise measurements, on a flower scale, would be required to invalidate the hypothesis of a higher sugar supply to thrum flowers.

Factors affecting nectar secretion

Nectar production of flowers was positively correlated with the number of newly open flowers per plant, which fluctuated with plant age. The amount of nectar produced per flower and per plant was highest during the flowering peak, which occurred 1 month after the first anthesis. According to Alekseyeva and Bureyko (2000), the flowering peak in the field corresponds to the period when the highest bee visitation rate is observed. Inflorescences at the top of the main stem, which are a priori more accessible for pollinators than lower inflorescences owing to a lack of leaf cover, had the greatest nectar production per flower.

Within a floral morph, nectar volume per flower varied among individuals. Although Pleasants and Chaplin (1983) found a positive relationship between nectar production and root weight in *Asclepias quadrifolia* and a Russian study, quoted by Namai (1990), reported a correlation ($r^2 = 0{\cdot}62$) between nectar production and leaf area in buckwheat, no relationship between nectar production per plant and root, axis, inflorescence and lamina weights was found in the present study, using a homogeneous population growing under controlled conditions. Thus, under the conditions used here, a difference in organ biomass was not the cause of the variation inm nectar production by plants between individuals from the same morph.

As in many angiosperms (van Doorn and van Meeteren, 2003), transferring buckwheat plants to dark suppressed flower anthesis. Nectar secretion was also drastically reduced in the few flowers that opened following transfer to darkness. Interestingly, darkening the inflorescence only inhibited flower opening but did not prevent nectar accumulation in the few flowers that succeeded in reaching anthesis. These findings demonstrated a direct action of light on the reproductive structures that mediates flower opening and also indicate that nectar secretion is primarily dependent on flower opening. As leaf excision of plants that are otherwise kept in the light reduces both anthesis and nectar accumulation in the flowers that opened, a role of leaves in these processes was also evident. However, photosynthetic activity, which supplies reproductive structures with sugars that are a source of energy necessary for flower opening and for the constituents of nectar, is most probably implicated. If photosynthesis is actually the contributing factor, the observations that, after defoliation, anthesis of a high proportion of flowers is maintained and that nectar accumulation still occurs, although at a lower rate and with a reduced sugar concentration, suggest that tissues, other than leaves (such as flower receptacles,

inflorescence peduncles, cyme bracts and main stem which all possess chloroplasts), provide the inflorescences with assimilates. According to Pacini and Nepi (2007), the plant parts often involved in the production of nectar sugars by photosynthesis are the flower pedicel, the calyx, the ovary and the adjacent leaves. In other respects, storage organs might also contribute to nectar production. Búrquez and Corbet (1998) demonstrated with defoliation experiments conducted on *Impatiens glandulifera* that only a fraction of the day's nectar secretion depends on the day's photosynthesis, while another fraction must be mobilized from stored assimilates in storage organs.

The light-dependent nectar quantity produced by flowers has also been reported for *Thymus capitus* (Petanidou and Smets, 1996) and is corroborated, in the case of buckwheat, by the observation that nectar production by flowers of excised inflorescences significantly increased by increasing irradiance at the reproductive structure level ['low' irradiance (100 ± 10 μmol m^{-2} s^{-1}): 0·08 μL per flower vs. 'high' irradiance (200 ± 10 μmol m^{-2} s^{-1}): 0·13 μL per $flower^{-1}$; Cawoy (2007)]. With regard to the impact of leaf excision on nectar production, it appears that there is no uniform response among species. A drastic defoliation did not decrease nectar accumulation in *Asclepias syriaca* or *Brassica napus* (Southwick, 1984; Cresswell *et al.*, 2001), while a limited defoliation was reported either to decrease or to increase nectar accumulation depending on species. A decrease was recorded for *Penstemon confertus*, *Hedysarum alpinum* and *Epilobium angustifolium* while an increase was found in *Hedysarum alpinum* and *Oxytropis monticola* (Cartar, 2004).

In conclusion, the present study establishes that nectar secretion in buckwheat is strongly influenced by floral morph type, plant age, inflorescence position on the stem and light. It demonstrated that light has a dual role. First, light acts directly on the reproductive structures, triggering flower opening that is required for the initiation of nectar secretion. Second, light operates probably by activating photosynthesis, which also stimulates flower opening and regulates nectar accumulation in flowers that reach anthesis. To improve nectar production in the field, further studies under controlled conditions investigating the impact of other factors such as soil fertility, air moisture, soil water content, temperature and fruit set could be useful (Pacini and Nepi, 2007). Field experiments aiming to investigate the likely links between nectar secretion, insect attractiveness and seed set and to compare nectar production of different cultivars would be also of interest.

ACKNOWLEDGEMENTS

This work was supported by the Fonds National de la Recherche Scientifique (FNRS) of Belgium (Fonds de la Recherche Fondamentale Collective contract 2·4562·99) and by the Université catholique de Louvain (Fonds Spéciaux de Recherche). A-L.J. is a research associate of the FNRS. This is publication number BCR126 of the Biodiversity Research Centre, Université catholique de Louvain, Louvain-la-Neuve, Belgium.

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