The Effect of Flower Maturity and Harvest Timing on Floral Extract from *Boronia megastigma* (Nees)

HAZEL S. MACTAVISH* and ROBERT C. MENARY

Department of Agricultural Science, University of Tasmania, G.P.O. Box 252-54 Hobart, Tasmania, Australia 7001

Development of floral organs during maturation of flower buds into fully open boronia flowers is described. The petals and functional anthers attain their maximum size prior to the non-functional anthers and the stigma. Organoleptic properties of the floral extract change with successive stages of bud development. The concentrations of extract and volatiles in the extract (% by f. wt) increase as buds mature, the extract concentration being highest in large buds and open flowers and the concentration of volatile compounds being highest in open flowers. The rate of flower and extract development was measured. Yields of flower material and floral extract per plant, and the concentration of total volatiles including β-ionone reach maximum levels when 70 % of flowers have reached anthesis. All measured factors decline after this point, except extract concentration (% of f. and d. wt) which is maintained up to 83 % open flowers.   © 1997 Annals of Botany Company

INTRODUCTION

Boronia megastigma Nees. (brown boronia, family Rutaceae) is an endemic shrub grown commercially in Tasmania for production of a highly valued floral extract. The strongly perfumed red-brown and golden boronia flowers are harvested in September. A yellow-brown waxy concrete is extracted from the flowers to yield between 0·3 and 0·7 % (by fresh flower weight) of extract, from which a viscous golden-coloured absolute may be prepared. Boronia extract has been fully described (Guenther, 1974; Davies and Menary, 1983; Weyerstahl *et al.*, 1994); β-ionone is the major volatile (12–30 % of total volatiles in the extract). Boronia extract has an odour that is 'powerful and characteristic; it recalls that of chopped spinach and blackcurrant buds, and after partial evaporation, like that of clove buds and infusion of tea' (Penfold and Phillips, 1927). Boronia absolute has an intense floral impact, its odour is reminiscent of cassis and violet. It has a natural fruity-green freshness entwined with the character of ripening hay and sweet tea; undertones of yellow freesias and raspberries emerge before finishing with a slightly spicy-herbaceous (cinnamon and tobacco leaf), woody dry-out (Guenther, 1974; Roberts, 1984; Weyerstahl *et al.*, 1994).

An axillary racemic flowering pattern occurs on boronia. A maximum of three flower buds initiate in autumn in each leaf axil on a current season's lateral. The most mature flowers usually occur in the axils of the third or fourth leaf below the apex and flower maturity decreases acropetally and basipetally from this point. Basal nodes initiate and mature earlier than apical nodes (Roberts, 1989; Roberts and Menary, 1994). After anthesis, flowers remain on the plant until abscission of the petals (about 6–8 d) and subsequent abscission of the remaining stalk (a further 2–7 d) unless fruit set occurs. Bussell, Considine and Spadek (1995) have described 12 developmental stages from very small flower buds through to seeds. Fruit set does not normally occur in Tasmania because the species of moth which is believed to pollinate boronia plants in their native Western Australia does not occur in Tasmania (MacTavish, 1995). Leggett (1979) found extract yield from boronia flowers was reduced at the beginning and end of the flowering period. At harvest, plants are completely harvested of all buds and flowers. Leggett's work was preliminary and did not consider the composition of the harvest in terms of the proportion of buds and flowers at each harvest. Quantitative information on the effects of harvest timing on the yield of floral material and extract obtained is therefore required.

MATERIALS AND METHODS

Plant material

Clonal plants of *Boronia megastigma* developed by the University of Tasmania were grown on commercial plantations in Tasmania over several growing seasons. Flowers were harvested by hand, separated into developmental stages as required, weighed and stored at −18 °C. The harvested material was divided into six developmental stages as described in Table 1.

Solvent

Technical grade petroleum ether (b.p. 40–60 °C) (pet. ether) was re-distilled prior to use.

TABLE 1. *Descriptions of developmental stages i–vi*

	Developmental stages					
Value	Very small bud Stage i	Small bud Stage ii	Medium-size bud Stage iii	Large bud Stage iv	Open flower Stage v	Stalk (no petals) Stage vi
Diameter (mm)	1–2	1·5–2·5	2–4	4–6	10	n/a
Weight of petal (% of bud weight)	10–15	20–25	25–30	35–50	50	n/a
Weight of bud as a % of stage v	10–15	25–30	35–50	65–80	100	50*
Diameter of functional anther (mm)	0·48	0·45	0·67	0·69	0·53	0·50
Diameter of non-functional anther (mm)	0·67	0·72	1·07	1·24	1·38	1·0
Diameter of stigma (mm)	1·44	1·49	2·21	2·62	3·71	3·0

* Developmental stage vi represents a flower from which the petals have abscised.

Extraction method

Thawed flowers (10–100 g) were covered with pet. ether and left at room temperature for 2 h. The solvent was then decanted and fresh solvent replaced on the flowers. After four 'washes' the solvent containing boronia concrete was dried down at reduced pressure by a rotary vacuum evaporator at 60 °C. Extracts of each developmental stage were made by homogenizing buds in pet. ether at the beginning of the first wash using an Omni mixer; this was necessary for extraction of stages i and ii.

Gas chromatography (GC)

A Hewlett Packard 5890 Series II GC equipped with a flame ionisation detector (FID), a split-injection system and a HP-1 cross linked methyl silicon gum column 30 m × 0·2 mm i.d., film thickness 0·33 microns, was used. (Carrier gas: N_2 @ 2 ml min^{-1}, head pressure 12 psi and split ratio of 1:50. Oven temperature programme: 50 °C for 1 min, then 10 °C min^{-1} to 250 °C. Injector temperature: 250 °C, detector temperature: 280 °C. Injection volume: 1 μL.)

Peaks were initially identified by GC/MS (Davies and Menary, 1983). Quantitative peak estimation was achieved by addition of octadecane as an internal standard, an FID response factor of one unit was used. Total volatiles were calculated as the fraction of the GC-analysable material eluting before n-heneicosane (Davies and Menary, 1983).

Organoleptic assessment

Organoleptic tests were performed in a 'double blind' test, in an aqueous medium at a dilution of 2×10^{-3}%. Solutions were ranked according to their floral, citrus and green bouquet, immediately and again 15 min later; solutions were tasted at the completion of their organoleptic test and ranked according to taste.

RESULTS

Development of buds of stage i into open flowers (stage v) incurred a gradual increase in the weight of the petals from 10 to 50 % of the total weight of the bud or flower (Table 1).
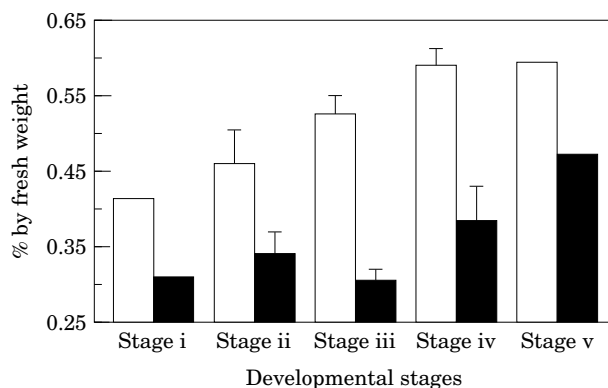

FIG. 1. Concentration of extract (% by f. wt, □) and concentration of total volatiles (% by f. wt × 10^{-3}, ■) in five developmental stages. Bars represent one s.d.

Maximum size of the functional anthers (0·69 mm diameter) occurred at stage iv, the release of pollen subsequently reduced the size of these organs. The diameter of the stigma increased at each developmental stage, especially between stages ii and iii and stages iv and v, during which it increased by more than 40 %. Senescence of stage v (open flowers) into stage vi (stalks from which petals have abscised) reduced the size of all organs.

The yield and composition of extract was assessed in flower buds at five developmental stages (i–v), (Fig. 1). There was a linear increase in the concentration of extract (% by f. wt) between buds of stages i and iv, from 0·42 to 0·6 %. There was no significant increase in extract concentration between stage iv and v (open flowers). The concentration of volatiles was variable, and was highest in stages iv and v (0·48 % × 10^{-3} by f. wt). Six individual volatiles were studied including α-pinene, β-ionone, dodecyl acetate, methyl jasmonate and methyl epijasmonate and (Z)-heptadec-8-ene. These compounds were selected because they represent a range of volatilities; α-pinene and (Z)-heptadec-8-ene do not contribute to the floral bouquet, however the former is highly volatile and typical of vegetative material, and the latter is a major component of the floral extract. β-ionone, dodecyl acetate and (Z)-heptadec-8-ene occurred in extract from all five devel-
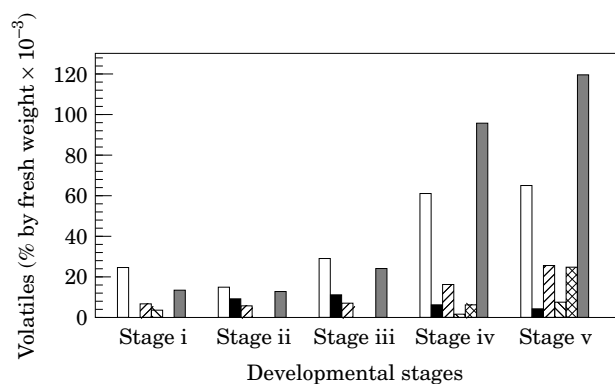
Fig. 2. Concentration of six different volatiles (% by fresh flower weight $\times 10^{-3}$) in five developmental stages. β-ionone (□); α-pinene (■); dodecyl acetate (▨); methyl jasmonate (▧); methyl epijasmonate (▨); (Z)-heptadec-8-ene (▨).

Fig. 3. Percentage of each developmental stage at harvest during the flowering period. Stage i (△); stage ii (□); stage iii (○); stage iv (▲); stage v (■); stage vi (●).

Fig. 4. Concentration of extract as a % of f. wt (--□--) and d. wt (—◆—) throughout flowering. Bars represent s.e.

opmental stages (Fig. 2). The concentrations of all three compounds increased rapidly after stage iii wherein they occurred at 20, 5 and $20\% \times 10^{-3}$ (by f. wt), respectively, reaching maximum levels in extract from stage v at 65, 25 and $120\% \times 10^{-3}$ (by f. wt), respectively. Methyl epijasmonate occurred only in extract from stages iv and v; highest concentrations were found in extract from stage v ($30\% \times 10^{-3}$ by f. wt). Methyl jasmonate occurred in extract from stages i, iv and v, reaching maximum levels in extract from stage v (approx. $10\% \times 10^{-3}$ by f. wt).

The dominance of 'non-floral' volatiles in extract from stage i may be perceived organoleptically; the extract was green, astringent and citrus. α-pinene reached a maximum level of approx $10\% \times 10^{-3}$ (by f. wt) in extract from stage ii and subsequently declined. As the concentration of floral volatiles increases, floral notes became evident in organoleptic assessment of extracts. Fruity fragrances were evident in extract from stage iii buds, becoming well balanced and combining with floral and jasmine notes in extract from stage iv (large buds). The characteristic woody notes and hints of jasmine and rose become evident in extract from open flowers, the developmental stage with generally the highest concentration of floral volatiles examined.

The percentage by number of each developmental stage throughout the flowering period was assessed on a single plant, typical of that clonal stand (Fig. 3). During the first 22 d of sampling there were virtually no open flowers (stage v) observed. Most of the buds were stages i, ii and iii prior to this time. Stage iv buds were present until 65 d after the first sample, although they never comprised more than 25% of the crop. After 22 d of sampling the percentage of stage v flowers increased exponentially and the percentage of stage iii declined rapidly from a maximum comprising 70% of the crop. The percentage of open flowers reached a maximum of 90%, 55 d after the first sampling, after which point the percentage of stage vi (senescent flowers from which the petals have abscised) increased rapidly.

Plants were harvested of all buds and flowers present at 7 d intervals during the flowering period and the percentage of open flowers in the sample was calculated. At later stages of flowering ($> 85\%$), it is difficult to assess the exact proportion of open flowers because of petal fall from over-
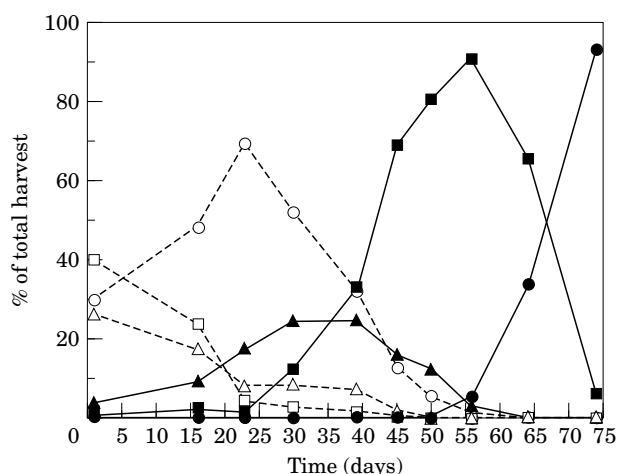
mature flowers. The concentration of extract (% by f. wt) in the material harvested throughout flowering, including flowers and any buds present, increased from 0·36 to 0·48% between 10 and 37% open flowers (Fig. 4). There was no further significant increase in extract concentration on a fresh weight basis between 37 and 80% open flowers (Fig. 4). The concentration of extract (% by d. wt) increased throughout flowering in a similar way to the fresh weight percentage (Fig. 4). However, there were greater increases in extract concentration by dry weight in later stages of flowering, due to the decline in the dry weight of harvested material during the flowering period from 32% d. wt at 10% open flowers to 25% d. wt at 83% open flowers (data not shown).
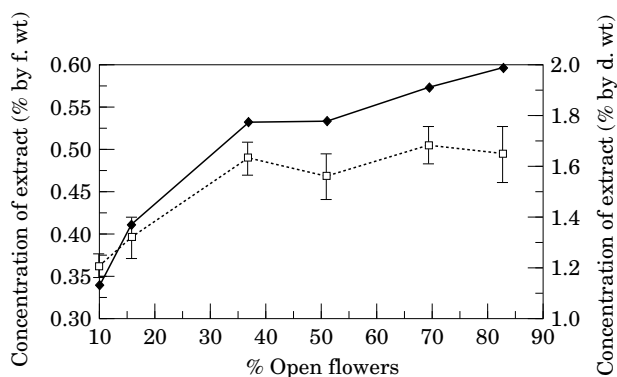
As the percentage of open flowers increased, the yield of floral material per plant increased until 70% of flowers were open and then rapidly declined (Fig. 5). Yield of extract (mg per plant), calculated using the yield of floral material per plant and the concentration of extract (% by f. wt) in the whole sample (buds and flowers) at each harvest date, increased rapidly between 17 and 53% open flowers, reached a maximum between 53 and 69% and then rapidly declined (Fig. 5). The harvest was separated into open flowers and
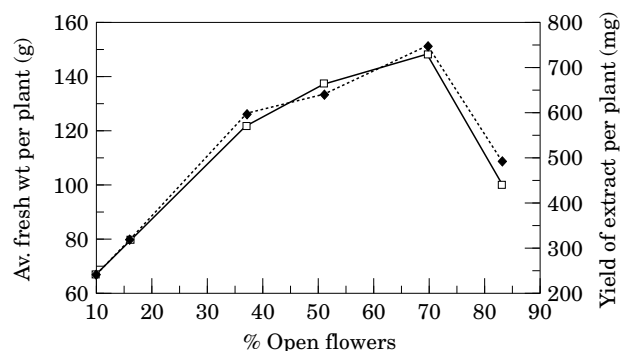
FIG. 5. Concentration of flower and bud material (—□—) (g) and extract (---◆---) (mg) per plant throughout flowering.
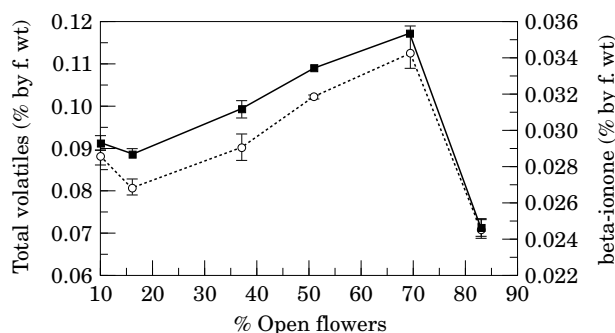

FIG. 6. Concentration of β-ionone (--○--) and total volatiles (—■—) in flowers throughout flowering (% by f. wt). Bars represent two s.d.

buds (stages ii, iii and iv) and extractions made from each sample throughout the flowering period. This enabled the contribution of open flowers and buds to the extract from the total harvested sample to be calculated. The contribution made to the total extract by open flowers increased throughout flowering, most rapidly between 38 and 50 % open flowers (data not shown). The concentration of extract in open flowers early in the flowering season was greater than that later in the season (17 % of flowers contribute 32 % of extract; 83 % of flowers contributed 80 % of extract).

The concentration of total volatiles and β-ionone in flowers and buds declined between 10 and 16 % open flowers, then increased to maximum levels when approx. 73 % of flowers were open (Fig. 6). The concentrations of total volatiles and β-ionone subsequently decline rapidly to reduced levels compared to those at 10 % open flowers.

DISCUSSION

The developmental stages of boronia flowers have been described and their contribution to extract yield and composition assessed. The composition of the harvest throughout flowering in terms of the percentage of each developmental stage present has been studied for its effect on the yield of flower material and extract per plant.

Maximum concentration of extract occurs in stage iv buds; maximum concentration of volatile compounds occurs in stage v (open flowers). The organoleptic properties of extracts develop as buds mature, reaching full palate at stage v. The maximum yield of flower material (and extract) per plant occurs at around 70 % open flowers; the remainder of the harvest at this time comprises stages iii and iv (15 % each). Later in the flowering period, stage iii buds develop into stage iv buds which contain the highest concentration of extract, at a faster rate than stage iv buds reach anthesis and become stage v flowers; existing stage v flowers are ageing, and increasingly more senescent flowers (stage vi) occur (Fig. 4). The depletion in flower and extract yield per plant after 70 % open flowers is initially brought about by the reduction in fresh (and dry) flower mass in ageing flowers prior to visible signs of senescence such as abscission of petals (conversion of stage v into stage vi). After 80–85 % of flowers have opened, rapid senescence of stage v flowers into stage vi senescent flowers from which the petals have abscised brings about the continuing trend of declining yields of flowers and extract per plant.

The dry weight of harvested material as a percentage of fresh weight declines during the flowering period. This may occur because open flowers which comprise increasingly more of the harvest throughout flowering contain more moisture than stages i–iv. It may also be due to catabolism of proteins, sugars and carbohydrates and the subsequent removal of catabolized products from stage v flowers prior to senescence (stage vi) and subsequent abscission of the unpollinated flower.

The concentration of volatile compounds continues to increase between stages iv and v indicating that synthesis of volatile compounds occurs later than non-volatile components of the extract, such as waxes, pigments and fatty acids (Davies and Menary, 1983). The concentration of volatile compounds declines after 70 % of flowers have opened. However, the concentration of extract (% of f. and d. wt) does not decline until after 83 % of flowers have opened, indicating that catabolism of non-volatile components of the extract occurs later than catabolism of volatile compounds.

The volatile compounds potentially active in attraction of pollinators to boronia flowers such as β-ionone, dodecyl acetate, methyl jasmonate and methyl epijasmonate, are thus short-lived products, of which the catabolism and removal from the flower or conversion into other, possibly volatile compounds precedes abscission of unpollinated flowers from the plant. It is evident that from an ecological perspective, boronia flowers produce volatile compounds to attract pollinators both to the individual flower (volatiles are maximized just after anthesis) and to other flowers which may open later (volatiles are not depleted immediately after anthesis). Pollination may stimulate changes in the metabolic regulation of production of volatile compounds. Volatiles are also present in the developing fruit and synthesis of volatiles such as limonene may continue during fruit maturation (Bussell *et al.*, 1995).

Petals and functional anthers have a greater relative increase in size between bud stages i and iii than do the non-functional anthers and stigma. The latter two organs have a

relatively large increase in size between stages iv and v, whereas petal size remains the same between these stages, and functional anthers actually decrease in size (due to pollen production). Boronia flowers appear to be protandrous: the functional anthers produce pollen and senesce prior to maximum expansion of the stigma. Bussell *et al.* (1995) note that the stigma is the last organ to senesce and abscise. Bussell *et al.* (1995) also report that petals do not abscise until pollination has occurred, however we describe it as a normal phenomenon associated with flower senescence, unrelated to pollination.

From a commercial perspective, it is desirable to maximize yields of flower and extract from a crop, and this may be achieved by harvesting the majority of the crop when approximately 70 % of flowers have reached anthesis. Further work is required to determine the optimum harvest time for each clone grown commercially.

ACKNOWLEDGEMENTS

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