Fruit and seed development in two *Hakea* species (Proteaceae)

P K Groom[1,2] & B B Lamont[1]

[1]Department of Environmental Biology, Curtin University of Technology, GPO Box U1987, Perth WA 6845
[2]Centre for Horticulture and Plant Sciences, University of Western Sydney, Locked Bag 1797,
Penrith South DC, NSW 1797
(present address for corresponding author)
✉ p.groom@uws.edu.au

Abstract

Hakea erinacea and *H. trifurcata* are related taxa that release their annual seed crop within a year of fruit maturity (weak serotiny). Both species produce similar-sized woody fruits (follicles) that protect two winged seeds. Fruit and seed development was completed 180 days (*H. erinacea*) and 215 days (*H. trifurcata*) after anthesis, with the fruits of both species reaching their maximum fresh mass during their 'green', non-woody state. This was associated with the commencement of wood formation in their fruit walls and the redistribution of certain nutrients (particularly phosphorus) from the fruit to developing seeds. Both *H. erinacea* and *H. trifurcata* retain chlorophyll in the walls of developing fruit, with *H. trifurcata* retaining chlorophyll in the mature fruit. This may have anti-herbivore properties whereby the developing green fruits are cryptically disguised within a background of similarly green leaves.

Introduction

Hakea erinacea Meissn. and *H. trifurcata* (Smith) R.Br. are related taxa (Barker *et al.* 1999) producing relatively small fruits (130–180 mg dry mass) compared with other species in the genus (Groom & Lamont 1996, 1997). Both species are weakly serotinous (minimal canopy seed storage) (Midgley *et al.* 1991), releasing most of their seeds annually from woody fruit (follicles) that open within a year of fruit maturing (Lamont *et al.* 1998, Groom *et al.* 2001). Each follicle possesses a longitudinal adaxial suture, which when ruptured exposes two valves each containing one compressed winged seed.

Hakea fruits remain green from anthesis to near maturity, except for those species that develop very large woody follicles (e.g. *H. platysperma*). Previous work on the seed and fruit development of the closely related genus *Grevillea* suggests that translocation of mineral nutrients from the fruit to seeds occurs as the fruit matures (Hocking 1981, 1982). Proteaceous seeds are packed with nutrients (Pate *et al.* 1986) and parent plants are efficient in redistributing essential nutrients from their fruits to their seeds and hence to their seedlings (Kuo *et al.* 1982, Lamont and Groom 2002).

An understanding of fruit and seed development will provide an insight into changes in nutrient content and biomass that occur as *Hakea* fruit develop woody walls. This paper records the development of fruits and seeds from anthesis to fruit maturity within two ecologically similar *Hakea* species (*H. erinacea* and *H. trifurcata*). The onset of wood formation (i.e. transition from the non-woody 'green' state) will be assessed in terms of changes in fresh and dry mass, partitioning of selected nutrients (N, P, K, Ca, Mg) between fruit and seeds, and chlorophyll content of the fruit wall.

Materials and Methods

Fruits were collected from a population (approximately 150 plants) of *H. erinacea* shrubs in the *Corymbia calophylla-Eucalyptus wandoo* woodlands of John Forrest National Park (31°37' S, 115°51' E) between August 1992 and February 1993. *H. trifurcata* fruits were collected from a stand (approximately 80 plants) in similar woodlands at Crystal Brook Reserve (31°59' S, 116°04' E), Kalamunda, between October 1992 and March 1993. By counting annual stem increments, plants in each population were estimated to be 15–20 years old. Fifty fruits per species were collected fortnightly beginning at the end of flowering (anthesis), with each collection representing a progressive stage of fruit development. Fruits were considered to be mature when there was no change in their dry mass and they contained fully developed seeds. For each collection, fruits (with seeds) had their fresh mass and dry mass (dried for 48 h at 60°C) recorded. After 90–100 days of development seeds were easily extracted from the developing fruit and weighed separately.

For each collection, dried fruit and seed samples (including the wing) were ground and analysed for selected nutrients (N, P, K, Ca, Mg) by CSBP and Farmers Co., Perth based on 0.8 – 2 mg samples. N was determined by standard Kjeldahl digestion on bulked samples, whereas the remaining nutrients were determined from digestion in 9:1 nitric/perchloric acid. P was determined colorimetrically and cations (K, Ca, Mg) by atomic absorption spectrophotometry. Chlorophyll was extracted from five weighed samples per collection (5–10% w/v) of fruit material in *N,N*-dimethyl formamide and analysed spectrophotometrically using the extinction coefficients of Moran (1982).
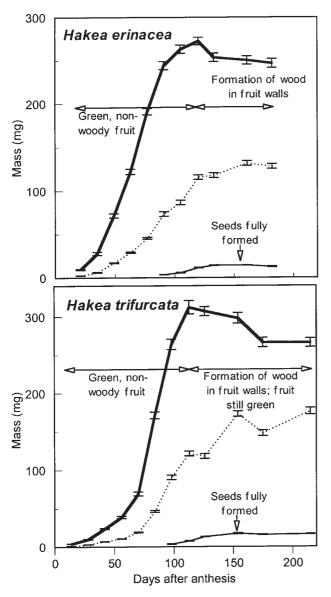
Figure 1. Fresh (thick solid line) and dry (dashed line) weight of fruit, and dry weight of seeds (thin solid line) during annual fruit development of *H. erinacea* and *H. trifurcata*. Data represent mean (± SE) from 50 fruits per species.

Results

Hakea erinacea fruits took 180 days to reach maturity, with wood formation in the fruit walls commencing 120 days after anthesis (Fig. 1). The similar sized *H. trifurcata* fruits took 215 days to reach maturity, with wood formation initiated after 110 days (Fig. 1). *H. trifurcata* seeds were slightly larger (16.4 ± 2.4 mg dry weight) than those of *H. erinacea* (12.5 ± 2.8 mg), although both species had similar final seed-to-fruit weight ratios (0.19; based on two seeds per fruit). Seeds were fully formed after 154 (*H. trifurcata*) and 161 (*H. erinacea*) days of development, 40 days after the commencement of fruit wood formation. Both species retained chlorophyll in their fruit walls during the non-woody ('green') phase of development. For *H. erinacea*, total fruit wall chlorophyll content stabilised around 0.24 mg g^{-1} between 40 to 133 days (Fig. 2), whereas *H. trifurcata* chlorophyll content

decreased with an increase in developing fruit size. Chlorophyll content of developing fruit was approximately one-third of leaves (0.6–0.9 mg g^{-1}). Chlorophyll was present in the walls of mature *H. trifurcata* fruits (0.09 mg g^{-1}), but not in the fruit walls of *H. erinacea*.

Of the five nutrients investigated, the total fruit nutrient content (in mg) for *H. erinacea* was near zero 21 days after anthesis (N: 0.039; P: 0.0004; K: 0.02; Ca: 0.032; Mg: 0.0009). These increased to a maximum 105 days (N: 1.18), 120 days (P: 0.017) and 160 days (K: 0.13, Ca: 0.06, Mg: 0.02) after anthesis (Fig. 3). For K, Ca and Mg, this was approximately 20 days before the onset of fruit wood production. Once fruit wood formation had commenced, the total nutrient content (in mg) of one *H. erinacea* seed (N: 1.18, P: 0.14, K: 0.11, Ca: 0.18, Mg: 0.05) was less than (N, P) or greater than (K, Ca, Mg) the amount present in a fully developed seed (Fig. 3). Calculating apparent translocation of each nutrient from the follicle as it
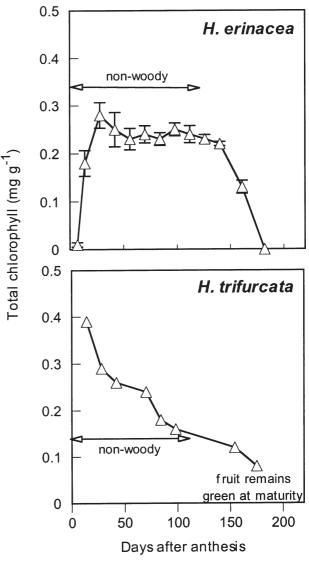

Figure 2. Total chlorophyll content of the fruit wall during annual fruit development of *H. erinacea* and *H. trifurcata*. Arrowed-line represents period during fruit development when fruit were fleshy and non-woody. Data represent mean (± SE) from 5 fruits per species.
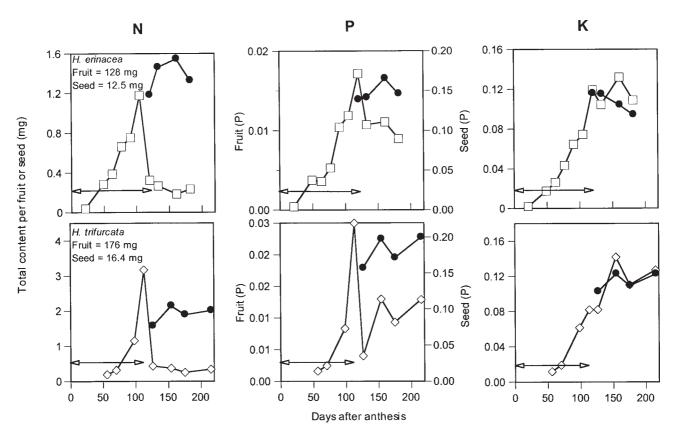
Figure 3. Total nutrient content of N, P and K in the fruit (open symbol) and seed (filled symbol) of *H. erinacea* (top row) and *H. trifurcata* (bottom row). Ca and Mg show similar trends as K. For P, fruit and seed data are on separate axes. Arrowed-line represents period during fruit development when fruit were fleshy and non-woody. Data calculated from the average fruit dry mass and the dry mass of one seed.

matured (after Hocking 1982) showed that N and P were redistributed with the greatest efficiency (80% and 48% respectively) and K, Ca and Mg with much less efficiency (0.5–19%). A decrease in total fruit nutrient content was associated with the onset of wood formation in the fruit walls of *H. erinacea* (Fig. 3).

The total nutrient content (in mg) in the fruit of *H. trifurcata* reached a maximum 110 days after anthesis (Fig. 3) and was synchronised with the beginning of fruit wood formation. The total fruit nutrient content (in mg) for *H. trifurcata* reached its maximum (N: 3.17; P: 0.025; Ca: 0.56; Mg: 0.24) 115 days after anthesis (Fig. 3). Potassium peaked at 0.14 mg 150 days after anthesis. At the commencement of fruit wood formation the total nutrient content of one *H. trifurcata* seed was less than (N, P, Mg) or greater than (K, Ca) the amount present in a fully developed seed (Fig. 3). Redistribution efficiency of nutrients translocated from maturing follicles to seeds in *H. trifurcata* was low for K and Ca (10–15%), moderate for N and Mg (24–32%) and high for P (63%).

Discussion

Hakea erinacea and *H. trifurcata* have similar fruit and seed size, and produce mature fruits and seeds within similar developmental time-frames. The fruits of both species reached their maximum fresh mass during their 'green', non-woody state, occurring with the commencement of wood formation in their fruit walls.

Developing fruits are an important store of essential nutrients (*e.g.*, N, P) (*cf* Hocking 1981, 1982) that were translocated to the two winged seeds at the onset of fruit wood formation. Within the Proteaceae, woody fruit protect the seeds from external damage by heat, desiccation and granivores, as well as providing an economical method of redistributing certain nutrients to the developing seeds (Stock *et al.* 1991, Lamont & Groom 1998).

The timing of fruit wood formation in *Hakea* follicles may be related to fruit size, which in turn is a function of the ability of the species to store seeds within their canopy (serotiny). Weakly serotinous *Hakea* species typically possess smaller, thinner walled follicles with less dense wood than more serotinous species (Groom & Lamont 1997). By allocating a relatively small amount of carbon (130–180 mg dry weight) into their thin woody fruit walls, the weakly serotinous *H. erinacea* and *H. trifurcata* provide limited seed protection over a relatively short period of time, without affecting the efficiency with which certain nutrients are redistributed to the developing seeds. For fire-prone plant species, weak serotiny has evolved because costs (carbon invested in protection) outweigh the benefits (maximising recruitment) (Midgley 2000). Differences between maturation time and degree of serotiny is supported by Hocking (1982) who showed that the non-serotinous, leathery follicles of *Grevillea wilsonii* matured within 9 weeks of anthesis, compared with the 25 weeks of the serotinous, woody fruit of the co-occurring *Hakea undulata.*

Both *H. erinacea* and *H. trifurcata* retain chlorophyll in the walls of developing fruits, with only *H. trifurcata* retaining chlorophyll in the mature fruit. This may enable the developing fruit to provide its own supply of photosynthates, although it is more likely that leaves are a major source of carbon for fruit and seed growth. Green developing fruits may have anti-herbivore properties, akin to the cryptic mimicry experienced by the mature fruit of *Hakea trifurcata* (Groom *et al.* 1994), and other weakly serotinous species (e.g. *H. prostrata*), whereby the developing green fruit are disguised within a background of similarly green leaves.

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