RESEARCH ARTICLE

Why do flowers wilt?

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ABSTRACT

- Resources salvaged when flowers wilt on a perennial plant could promote reproduction by, in preference order, the same flowers (Hypothesis 1), adjacent flowers on the same plant (Hypothesis 2), or during the next flowering season by the same plant (Hypothesis 3).
- We tested the above hypotheses for *Blandfordia grandiflora*, a perennial species, where some plants included flowers that were allowed to wilt, while equivalent flowers on other plants were prevented from wilting. The abilities of these plants to produce seed were determined by liberally pollinating all flowers. To test Hypotheses 1 and 2, seed set per flower and per plant were compared between plants with and without wilting flowers. To specifically test Hypothesis 3, reproduction was prevented in all flowers. For each experiment, flowering was monitored in the same plants during the next flowering season, thus also enabling Hypothesis 3 to be tested.
- The results were consistent with Hypothesis 3, but not with Hypotheses 1 and 2.
- Hence, we verified, for the first time, that plants may benefit from salvaging resources from wilting flowers and re-using these resources for subsequent reproduction. However, contrary to expectations, plants re-used these resources to promote reproduction during subsequent flowering, and not during current flowering by either the same flowers or other flowers on the same plant. The plants must have transferred resources from wilting flowers to underground corms and roots, which provided resources necessary for subsequent flowering. This is likely part of a general plant strategy to salvage resources invested in reproduction during one flowering season and reuse these resources during subsequent flowering.

INTRODUCTION

As flowers age, petals commonly wilt or senesce, with changes in colour, loss of turgor and transfer of constituents to other plant parts (Ma *et al.* 2018; Borghi & Fernie 2020). We refer to this process as flower wilting (e.g., Dong *et al.* 2016; Hobbhahn *et al.* 2017; Galetto *et al.* 2018). But why do plants do this?

Such flower wilting may be advantageous to a plant because it allows resources originally allocated to a flower to be resorbed and reused elsewhere, with consequent benefits in terms of plant reproduction (Jones 2013; Spigler 2017; Roddy *et al.* 2021), but this hypothesis has not been tested directly. Such resources may include the chemical constituents of various plant organs, e.g., floral nectar, pollen, seeds, or petals (Galetto *et al.* 2018; Borghi & Fernie 2020; Descamps *et al.* 2021) or plant photosynthate (Descamps *et al.* 2021) or chemical energy (Dong *et al.* 2016; Borghi & Fernie 2020). Experimental studies have, for example, demonstrated that the production and maintenance of flower petals can entail a cost to a plant which manifests as a trade-off between petal production and other aspects of plant reproduction (Andersson 1999, 2000, 2005, 2006). These studies demonstrate the potential benefit to a plant from resorbing and reusing resources in petals, as may occur during flower wilting. However, as far as we are aware, no study has yet directly demonstrated such benefits from flower wilting.

Indirect evidence of plants obtaining reproductive benefits through resource absorption as flowers senesce was described by Ashman (1992, 1994). Using the iteroparous, herbaceous plant *Sidalcea oregana* (Malvaceae), and working with 44 individual plants, Ashman examined the statistical relationship between investment in flowering in 1 year (i.e., 1989) and the magnitude of flowering effort in the next year (i.e., 1990). As measures of flowering in 1990 for each plant, which was the dependent variable in regression analysis, Ashman took the product of the number of inflorescences per plant and the diameter of a randomly chosen inflorescence (Ashman 1992, 1994). As independent variables for flowering in 1989, Ashman took total leaf area as the measure of plant size and two alternative sets of measures of reproductive investment for each plant (Ashman 1994). One set of measures of reproductive investment, referred to as 'static', consisted of the dry biomass content of three flower components (i.e., calyx, corolla complex, unpollinated ovaries) for four flowers that were removed per plant. The other set, referred to as 'dynamic', allowed for absorption of nutrients (i.e., nitrogen, phosphorus) from these flower components by reducing the dry biomass content by separately estimated percentages of these nutrients that were absorbed by plants during flower senescence (Ashman 1994). Ashman then found that the regression model based on the latter variables, which allowed for nutrient absorption and was labelled 'dynamic', explained a higher proportion of the

variance in flowering effort in the second year than the model based on the former variables, which did not include nutrient absorption and was labelled 'static' (Ashman 1994). Ashman thus concluded that 'dynamic estimates better reflect the true physiological cost of reproduction to plants in situ', and that 'nutrient resorption is important in determining realized reproductive costs' to a plant (Ashman 1994). In other words, plants must be able to 'recoup some of the nutrients invested in reproductive structures' and to use these resources to promote subsequent reproduction (Ashman 1994). However, direct evidence for this requires experimental manipulation of flower wilting and associated resource salvage, together with observations of subsequent plant reproduction.

To provide such direct evaluation of possible resorption and reuse of resources from wilting flowers, we hypothesized, for perennial plant species, that resources salvaged by a plant during a flowering season when its flowers wilt could be reused by the plant during that season for reproduction (e.g., seed set) by the same flowers (Hypothesis 1) or by adjacent flowers on the same plant (Hypothesis 2), or for reproduction during the next flowering season by the same plant (Hypothesis 3). Salvaged resources from wilted petals can be remobilized to the ovary of the same flower or other tissues outside this flower (Chapin & Jones 2007; Jones 2013; Broderick et al. 2014). Consequently, plants could reuse resources salvaged from wilted flowers for reproduction in those same flowers, other flowers on the same plant, subsequent reproduction later, or survival until later reproduction.

We further hypothesized that perennial plants would preferentially re-use resources salvaged from wilting flowers in reproduction by those same flowers, followed by reproduction at the same time in other flowers on the same plants, and followed by reproduction by the same plants during subsequent flowering. This seems likely as it should be easier for a plant to transfer and re-use resources close to wilted flowers, rather than from further away. Consistent with this, some studies have found that chemical constituents of wilted flowers have been transferred to adjacent ovaries, while observations of similar transfer to more distant locations have been rare (Rubio-Moraga et al. 2010; Shahri & Tahir 2011; Jones 2013). On the other hand, transfer of resources for reproduction between neighbouring flowers on the same plant is known to occur (e.g., Zimmerman & Pyke 1988c; Nepi & Stpiczynska 2007; Harder & Aizen 2010). Of course, re-use of resources from wilted flowers in one plant location does not exclude its occurrence elsewhere. We therefore aimed to test the following hypotheses, which apply to perennial plants and are not mutually exclusive.

HYPOTHESIS 1

Given the opportunity, plants will salvage and re-use resources from wilted flowers for reproduction by these same flowers through formation of fruits and seeds but may also re-use these resources for reproduction at the same time in other flowers on the same plant, or for subsequent flowering.

HYPOTHESIS 2

Given the opportunity, plants will salvage and re-use resources from wilted flowers for reproduction at the same time by other flowers on the same plant, if prevented from re-using resources salvaged from wilted flowers for reproduction in those same flowers. They may also re-use such resources during subsequent flowering.

HYPOTHESIS 3

If plants are prevented from re-using salvaged resources from wilted flowers for reproduction at the same time by both the same flowers and other flowers on the same plant, they will re-use these resources during subsequent flowering.

We aimed to evaluate Hypotheses 1 and 2 by comparing potential reproduction of plants on which flowers were artificially pollinated and allowed to wilt with plants on which flowers were similarly pollinated, but flowers or petals were removed before any wilting occurred. By artificially pollinating equivalent flowers on each plant with liberal quantities of outcross pollen, seed set per flower for these flowers and per plant should be determined by plant resource allocation, and not limited by pollen availability. We thus hypothesized that, for equivalent and pollinated flowers, average seed set per flower and per plant would be higher for plants with wilting flowers (i.e., W plants) than for plants with petals removed (i.e., R plants).

This experimental approach is similar to that previously taken to address potential cost to a plant to produce floral nectar (Pyke 1991; Ashman & Schoen 1997; Ornelas & Lara 2009) and yields information not otherwise available about plant trade-offs that arise through investment in floral resources.

We aimed to test Hypothesis 3 by comparing the frequencies of flowering during the next season after experimental treatment between plants with wilting flowers (i.e., W plants) and plants with petals removed (i.e., R plants).

We differentiated between the three hypotheses through experiments that allowed certain forms of resource salvage and reuse, while preventing others. We tested Hypothesis 2 by preventing resource transfer from wilting flowers to seed production by the same flowers while allowing resource transfer to adjacent flowers. We specifically tested Hypothesis 3 by preventing salvaged resources from wilting flowers being reused for seed production by any flowers on the same plant during the initial flowering season. Reuse of salvaged resources from one season for reproduction during the next season was always possible. For an annual plant species, Hypotheses 1 and 2 would apply, but not Hypothesis 3.

We carried out experiments to test these hypotheses using the perennial plant species *Blandfordia grandiflora* (Blandfordiaceae).

MATERIAL AND METHODS

Study species

Blandfordia grandiflora, commonly known as Christmas Bells, is well suited for our experimental design. Plants of this species consist of a basal leaf mass and underground root mass, all of which can be accommodated in a small flower pot (i.e., 15 cm diameter, 20 cm high) (Lamont et al. 1990; Johnson 1994; Ramsey et al. 1994). Most flowering occurs during November to February in our study area and individual plants flower no more than once during this flowering season (GHP, unpubl.

Fig. 1. Plants and flowers of *Blandfordia grandiflora*. (A) Area of natural flowering at Kara Plantation, near Port Macquarie, NSW, Australia; (B) Flowering plant; (C) Cutting flower petals near base; (D) Flowering stem with one flower having had almost all of each petal removed, but style remaining; (E) Some flowers as per D and one flower showing nectar production near base of style (arrow).

obs.). When flowering, plants usually have a single flower stalk, occasionally 2–3, that emerges vertically from the leaf mass with a height of up to about 1 m, on top of which are an average of 6–7 flowers arranged on a vertical raceme (Fig. 1A–E; Johnson 1994; Ramsey *et al.* 1994; Johnson & Burchett 1997). Flowers can thus be numbered sequentially from base to top, with the lowermost flower generally opening first followed by sequentially higher flowers. After the first flower has opened, no further flower buds are formed and so flower number is then fixed (GHP, unpubl. obs.).

Flowers are relatively large (ca. 6-cm long), generally bell-shaped, red in colour with yellow tips (Johnson 1994; Johnson & Burchett 1997; Ramsey *et al.* 1994), with stigma exerted or slightly inserted (Fig. 1A–D; GHP, unpubl. obs.), and with relatively thick and robust petals and, hence, these are easily manipulated (e.g., petal removal, Fig. 1C; stigma removal). Flowering under natural conditions peaks in December, hence its common name, and is stimulated by fire, with peak flowering 2–3 years post-fire (Johnson 1994; Griffith & Rutherford 2020). Flowering may also occur for plants cultivated in enclosures (Johnson & Burchett 1997). Flowers are largely self-incompatible and may produce moderate-sized fruits (i.e., up to 10–12 cm long; triangular in cross-section, with each side up to 10–12 mm long at widest point), each containing 50–100 seeds on average (GHP, unpubl. obs.). These fruits generally remain on the plant until they dry, split and shed their seeds (GHP, unpubl. obs.). Thus, seeds can be collected, counted and weighed if fruits are removed before dehiscence and allowed to continue maturing. This species is biologically similar to its congener *B. nobilis*, which has also been the subject of pollination-related experiments (Pyke *et al.* 1988; Zimmerman & Pyke 1988a, 1988b; Pyke 1991).

In *B. grandiflora* petals and sepals are combined in a single perianth with petal-shaped segments arranged in a roughly tubular corolla. Any removal of the perianth is therefore removal of both petal and sepal tissues. However, for simplicity, we refer to perianth segments as petals and to perianth removal as petal removal.

Study location and timing

This study was carried out on plants growing inside a large shade-house (i.e., about 100 × 30 m, enclosed top and sides with mesh-type material, no temperature control) on private property about 11 km north of Port Macquarie, lower north coast of NSW, Australia. This shade-house slightly reduced incident sunlight and rain, and functioned to protect the plants from damage by herbivores (mostly insects). Under natural conditions *B. grandiflora* is visited and pollinated by nectar-feeding honeyeaters (Meliphagidae) (Ramsay 1991; GHP, unpubl. obs.); the shade-house prevented such animal visitation. Inside this shade-house there were ca. 40,000 *B. grandiflora* plants, growing in 10 parallel beds, each about 1 m wide, all treated in the same manner.

Experiments were carried out on plants that flowered from late January to early February, and did not include any plants that flowered at other times. The plants in this shade-house were cultivated so that many were in flower between November and February each year, and these flowers were harvested for the cut-flower trade in December. This flower harvest had no impact on our research.

Experiments were carried out in three periods: 16 January to 1 February 2018, 16 to 31 January 2019, and 15 January to 5 February 2020, using similar but slightly different protocols (see below). Fruits were collected 6 weeks after the first two of these experiments were complete (i.e., 13–14 March 2018 and 12–14 March 2019). Seed formation was prevented for the 2020 experiments (see below), so no fruits were collected for these experiments. Whether plants reflowered during the season following experimental treatment was assessed for each year.

Control of flower wilting

Blandfordia grandiflora flowers change in appearance and orientation as they age. They are first detectable as small, green buds that point vertically upwards. They enlarge and become predominantly red and yellow, change orientation until they point downwards, and then begin to produce nectar (Fig. 1C). At this point the flower corollas open, anthers dehisce making pollen available, the style elongates with an expanding stigma at its tip, nectar production continues, and flowers begin to rise in vertical orientation. At about 7 days after opening, with flowers now pointing upwards, flower petal senescence and wilting commence, with petals beginning to change colour, becoming faded and losing colour until brown, and changing texture, becoming increasingly wrinkled, losing turgor, and eventually being shrivelled, with much less mass than just opened flowers. Fruit development may also occur at the same time, with the green style elongating and expanding. This phase occurs over a second period of about 7 days at the end of which the flowers have transformed into fully developed green fruits, pointing vertically upwards, with shrivelled, brown petals at their base. Flower age was assessed simply as the number of days since start of opening of the corolla.

Manipulation of flowers so that wilting was prevented was therefore carried before flowers reached 7 days of age. Wilting was prevented for selected plants (i.e., R plants) in 2018 and 2020 by removing entire flowers at either age 6 days (Jan 2018 experiments) or when they were first open (i.e., age 1 day; Jan 2020 experiments). In 2019, wilting was restricted for selected plants (i.e., R plants) by removing almost all of each petal for flowers at age 6 days, leaving the style and the lowermost parts of petals intact (i.e., Jan 2019 experiments; Fig. 1C–E). On other plants (i.e., W plants), there was no removal of petals or flowers, and flowers were allowed to wilt naturally.

Nectar production

Nectar production occurs near the base of the style (Fig. 1E; GHP, pers. obs). Nectar then accumulates in non-senescent flowers at the base of the corolla, between the petals and style. With flower senescence, nectar production ceases and any nectar present is reabsorbed by the plant, presumably through the lower style, until none remains (GHP, unpubl. obs.). Consequently, some removal of nectar would have occurred when entire flowers were removed, either at age 6 days or age 1 day. To consider the magnitude and consequences of such nectar removal, we consequently sampled nectar from such flowers as we removed them. On the other hand, when almost all of each petal was removed but the style and proximal ends of petals left intact, or when flowers were not manipulated and were allowed to wilt, net nectar production would have been zero. In both circumstances, flowers absorb all nectar by the time wilting is completed (GHP, unpubl. obs.).

Resource limitation rather than pollen limitation of seed production

So that seed production by flowers was not limited by pollen receipt, and consequently determined by the plants' resource allocation, each open test flower on each plant was artificially pollinated on each experimental day using liberal quantities of pollen from a distant, unrelated plant. For this, 2–3 anthers that were dehiscing pollen were cut from a flower on the pollen source and rubbed across the stigma of the recipient flower. Because this procedure was carried out each day, individual flowers were generally pollinated multiple times over the course of the experiments. However, a few flowers never opened, and thus were not pollinated (i.e., zero pollinations) and were subsequently excluded from the analyses (see below).

Seed collection

About 6 weeks after fruits have fully developed, they senesce or mature and begin to split open until they drop their seeds on the ground. During this period, these fruits lose their green colour and become brown and faded; the three locules begin to separate, allowing seeds to fall out. Consequently, at 5–6 weeks after the end of the experimental treatments each year, fruits were collected and placed in separate open bowl-shaped trays until their seeds were counted and weighed. The fruits were kept in these trays until they had split and begun to lose seeds, which took a further 2–3 months. The trays retained seeds as the capsules split open, but seeds were also manually removed where splitting and seed fall was not quite complete.

Seed number and seed weight were determined per flower and per plant for each experimental plant group. Seed number and seed weight per plant were calculated by summing these values across all flowers on each plant. The following additional flower and plant variables were also recorded during the experiment: flower stem height (i.e., ground to point on stem of highest flower pedicel), flowers per stem, and flower position (i.e., flower number counting from lowermost flower).

Flowering in successive seasons

Some plants with tagged flowering stems from experiments in one season could be relocated in the following season, allowing us to determine which plants flowered again in the year after our experiments. Though dried out and faded brown, some flowering stems remained attached to plants a year after they had been included in our experiments. Plants that flowered in two successive seasons did so at about the same time each year, and so were surveyed for repeat flowering at close to the same time as experiments during the previous season. However, some flowering stems from the Jan 2018 and Jan 2019 experiments could not be located a year later, possibly because some tags had fallen off or stems were accidentally removed during routine garden maintenance, or stems were lying on the ground and could not be assigned to a particular plant. At the end of the Jan 2020 experiments, we therefore used metal stakes in the ground to indicate each experimental plant.

Experimental outcomes

For experimental plants, we determined seed set and seed weight for test flowers, seed set and seed weight per plant, and whether a plant flowered again in the next flowering season. This allowed us to test our hypotheses (see below).

Plant selection and treatments

Plants, chosen the day before each experiment (i.e., 15 Jan in 2018, 2019 and 2020), had a single flowering stem, no

malformed or visibly damaged flowers and no open flowers, but appearing had one or more flower buds likely to open the next day (i.e., large, brightly coloured, pointing downwards). These plants were chosen while walking alongside the beds in which they were growing and numbered successively as encountered. On each subsequent day for the next 2 weeks, each flower was examined and appropriate its experimental treatment applied. By the end of this period all, or almost all flowers had opened on each plant and been treated. However, because continuous flower opening within each plant occurred during this period, there was variability in the number of treatments per flower. For the Jan 2018 experiments, plants were selected if they had 5 or more total flowers, but no such restriction was imposed for the Jan 2019 and Jan 2020 experiments. Consequently, average number of flowers per plant was higher in 2018 than in the other two years (average number of flowers ± SE: 2018: 6.6 ± 0.3; 2019: 4.6 ± 0.2; 2020: 5.3 ± 0.2).

For all 3 years, there were two experimental groups of plants, one group with petal removal (i.e., Group R), the other group with petals intact throughout flower life span and thus able to wilt and senesce (i.e., Group W). To avoid spatial bias for treatment groups, assignment of treatment alternated as plants were numbered as encountered along the garden beds and incorporated into the experiments.

Experimental protocols

Experiments were designed to compare plants on which flowers wilted naturally with plants where some or all flowers were manipulated such that they had minimal or no wilting. However, as described below, this was achieved differently for the three experimental years.

Hypotheses 1 and 3 (2019 experiments)
For one group of plants (i.e., R plants), petals were removed from all flowers when they reached age 6 day, thus preventing petal wilting. As flowers reached this age, each petal was cut at about 5 mm from its base (Fig. 1C) and removed, thus preventing possible resource salvage from these petals. Furthermore, nectar production by flowers should not have been affected by this treatment as nectar accumulation is restricted to the lowermost part of the corolla, below where petals were cut, and all nectar is ultimately absorbed by each flower resulting in zero net nectar production.

On other plants (i.e., W plants), there was no manipulation of flower petals, and thus normal petal wilting could occur. Net nectar production by flowers on these plants should also have been zero, as all nectar is ultimately absorbed by each flower.

For both groups of plants, all flowers on each experimental plant were considered test flowers and so were artificially pollinated each day when open and collected as fruit 5–6 weeks after completion of experimental treatments. Consequently, individual flowers were typically pollinated multiple times.

Hypothesis 1 predicts that seed set and seed weight per flower and per plant would be greater for plants with flowers allowed to wilt (i.e., W plants) than for plants where flower petals were removed and therefore wilting prevented (i.e., R plants). It was also hypothesized (Hypothesis 3) that the W plants would be more likely to flower again during the next season than the R plants.

Hypotheses 2 and 3 (2018 experiments)
In this case, reuse of resources from flower wilting by the same flowers was prevented (see below). The flowers on each plant were divided into lower and upper stem halves, with the flowers in the lower half having styles cut when they opened and either no further treatment (i.e., W plants with wilting flowers) or flowers removed at age 6 days (i.e., R plants with flowers removed). Thus, these flowers were prevented from forming fruits and seeds and were either removed or allowed to wilt. Transfer of salvaged resources from flower wilting to seeds of the same flowers was therefore prevented. The lower half included n/2 flowers if the number n of flowers per stem was even and included (n + 1)/2 flowers if n was odd.

The flowers in the upper half were designated test flowers, artificially pollinated each day when they were open, and collected as fruits after another 5–6 weeks. Transfer of salvaged resources from wilted flowers to seeds of adjacent test flowers was therefore possible. Therefore, similarly to Hypothesis 1, it was hypothesized that seed set and seed weight per flower for test flowers and per plant would be greater for plants with flowers allowed to wilt (i.e., W plants) than for plants where flowers were removed and thus prevented from wilting (i.e., R plants). However, in this case, test flowers were adjacent to flowers that wilted and not the same flowers. It was also hypothesized (Hypothesis 3) that W plants would be more likely to flower again during the following season than R plants.

Hypothesis 3: (All experiments)
Reuse of salvaged resources from flower wilting for seed production by the same plants during the initial season was prevented (see below). There were two groups of plants differing only in whether all flowers were allowed to wilt (i.e., W plants) or were prevented from doing so (i.e., R plants), with no difference between these two groups in terms of seed production, which was prevented for both groups. For R plants, removing each flower when first open (i.e., age day 1) prevented these flowers wilting, and also prevented seed production by these flowers. For W plants, when each flower was first open (i.e., age day 1), removing the stigma and style tip prevented seed production for these flowers. As neither W nor R plants could set seed, any effect of salvaging resources from wilted flowers would be delayed until a subsequent reproductive episode, so there was no collection of fruits for this 2020 experiment. However, whether plants from this experiment re-flowered during the next flowering season was determined by inspecting them at about the same time in 2021 (i.e., 14 Jan 2021). Similarly, whether plants from the 2018 and 2019 experiments re-flowered during the next flowering seasons was determined by inspecting them at about the same times in 2019 and 2020 respectively (i.e., 15–16 Jan 2019, 19 Jan 2020).

For experiments in all 3 years, it was hypothesized that W plants would be more likely to re-flower during the next season than R plants.

Other flower and plant variables

Other flower and plant variables could affect the results through associated patterns of resource availability and provision. These variables include height of flowering stem, number

Table 1. Results of a general linear mixed model for seed set and seed weight (mg) per flower (for each plant) vs treatment, flower position, stem height, number of flowers per stem (Num Fls), number of pollinations per flower (Num pollinations), and plant ID nested within treatment, with constant included (n = 228; 18 flowers with zero pollinations excluded).

effect	seed set per flower				seed weight per flower			
	coefficient	*F*-ratio	df	*P*-value (* = sig)	coefficient	*F*-ratio	df	*P*-value (* = sig)
Constant	3.89	0.04	1	0.84	−573.4	1.01	1	0.32
Treatment (R = 0; W = 1)	0.76	0.33	1	0.57	38.3	1.11	1	0.29
Flower position	−5.29	38.30	1	<0.001*	−107.5	24.10	1	<0.001*
Stem height	0.63	1.09	1	0.30	26.6	5.37	1	0.02*
Num. Flowers	0.50	0.03	1	0.86	Omitted			
Num. pollinations	1.85	1.13	1	0.29	92.5	4.27	1	0.04
Plant ID (within treatment)		2.38	49	<0.001*		2.93	50	<0.001*

Number of flowers per stem was omitted as a variable from analysis for seed weight because it was highly correlated with flower position and failed the tolerance test. The distributions of model residuals, for both seed set and seed weight, were not significantly different from normal (seed set: K-S test statistic = 0.04, P = 0.49; seed weight: K-S test statistic = 0.05, P = 0.24). Adjusted P threshold for significance is 0.02 (i.e., 0.15/7). Results that are significant (i.e., sig) are marked with *.

of flowers per stem, flower position on stem, and plant ID. Consequently, these were considered as random factors.

Statistical analyses

Whether experimental treatment affected seed set or seed weight per flower was evaluated with a General Linear Mixed Model where the dependent variable was seed number or seed weight per flower and independent variables were plant, nested within treatment group, and the additional plant and flower covariates (i.e., flower stem height, number of flowers on stem, number of pollinations, flower position). Whether experimental treatment affected seed set or seed weight per plant was similarly evaluated, but without flower position as a variable. In all cases, normality of residuals was confirmed with a Kolmogorov–Smirnov (Lilliefors) test.

Whether plant treatment affected likelihood of re-flowering during the next flowering season was evaluated using Pearson Chi-square analysis for each study year and a stepwise General Linear Mixed Model for all years combined. In the latter case, the dependent variable was whether a plant flowered a year after experimental treatment (No = 0; Yes = 1) and the independent categorical variables were treatment (R and W), study year and treatment x study year interaction. Although treatments differed slightly between years, we carried out this analysis with years combined because, for each year, we had similar plants with wilting flowers and plants for which equivalent flowers were prevented from wilting and determined whether these plants re-flowered a year later. These analyses were carried out using the statistical software *SYSTAT* (Wilkinson 1990).

Our analyses involved multiple tests and so we adopted adjusted threshold probabilities (P) for significance (Wright 1992; Chandler 1995). Here, tests of Hypotheses 1 and 2, based on seed set or seed weight per flower or per plant constituted eight 'families' of tests. Tests of Hypothesis 3 constituted another 'family' of tests. For each of these families of tests, we adopted an overall 'experiment-wise' threshold P-value of 0.15, to which we applied a Bonferroni correction by dividing this value by the number of tests involved, to yield the final P threshold value for significance (Wright 1992;

Chandler 1995). This approach contrasts with the commonly adopted application of a Bonferroni correction to an experiment-wise P-value of 0.05, which results in an unacceptably high rate of Type II errors (Perneger 1998; Nakagawa 2004). Further details are presented in the Results.

RESULTS

Hypothesis 1: Re-use of salvaged resources from wilted flowers by the same flowers (2019 experiments)

There was no evidence that salvaged resources from wilted flowers are re-used to support reproduction by the same flowers. Contrary to this hypothesis, there were no significant differences between W and R plants in terms of seed number and seed weight per flower, and seed number and seed weight, summed across flowers, per plant (Table 1).

Both seed set and seed weight per flower decreased significantly with increasing flower position and increased with increasing flowering stem height (Table 1), and seed weight per plant increased with increasing height of the flowering stem (Table 2). The effect of Plant ID was also significant, but the numbers of flowers per stem and pollinations had no significant effect on any of the variables (Tables 1 and 2).

Hypothesis 2: Re-use of salvaged resources from wilted flowers by other flowers on the same plants (2018 experiments)

Similarly, there was no evidence that salvaged resources from wilted flowers are re-used to support reproduction by other flowers on the same plant. Contrary to this hypothesis, there were no significant differences between W and R plants in terms of seed number and seed weight per flower, and average seed number and average seed weight, across flowers, per plant (Table 3). Both seed set and seed weight per plant increased with number of flowers per stem, but neither seed set nor seed weight per flower was significantly affected by this variable (Tables 3 and 4). The effect of Plant ID was significant, but stem height and number of pollinations had no significant effect on any variables (Tables 3 and 4).

Table 2. Results of a general linear mixed model for seed set and seed weight (mg) per plant vs treatment, stem height and number of flowers per stem, and number of pollinations per plant, with constant included (n = 54; one plant with zero pollinations omitted).

	seed set per plant				seed weight per plant			
effect	coefficient	F-ratio	df	P-value (* = sig)	coefficient	F-ratio.	df	P-value (* = sig)
Constant	−59.29	2.25	1	0.14	−1,440.6	1.69	1	0.20
Treatment (R = 0; W = 1)	7.54	0.77	1	0.39	190.2	0.62	1	0.44
Stem height	1.83	4.39	1	0.04	48.8	3.96	1	0.05
Num. Flowers	14.31	2.99	1	0.09	319.8	1.90	1	0.18
Num. pollinations	1.53	0.62	1	0.43	40.5	0.55	1	0.46

For both seed set and seed weight per plant, the distribution of residuals was not significantly different from normal (seed set: K-S test statistic = 0.08, P = 0.59; seed weight: K-S statistic = 0.06, P = 0.94). Adjusted P threshold for significance is 0.03 (i.e., 0.15/5). None of the results is significant.

Table 3. Results of a general linear mixed model for seed set and seed weight (mg) per flower (for each plant) vs treatment, flower position, stem height (cm), number of flowers per stem, number of pollinations per flower, and plant nested within treatment, with constant included (n = 109; 4 flowers with zero pollinations excluded).

	seed set per flower				seed weight per flower			
effect	coefficient	F-ratio	df	P-value (* = sig)	coefficient	F-ratio	df	P-value (* = sig)
Constant	22.20	0.12	1	0.73	77.75	0.21	1	0.65
Treatment (R = 0, W = 1)	−1.20	0.07	1	0.79	7.83	0.07	1	0.80
Flower position	−6.85	3.97	1	0.05	−17.61	5.10	1	0.03
Stem Height	0.06	0.01	1	0.93	2.03	0.03	1	0.87
Num. Flowers	11.28	1.32	1	0.26	5.98	0.01	1	0.94
Num. pollinations	1.52	0.36	1	0.55	3.89	0.46	1	0.50
Plant ID (within Treatment)		4.17	33	<0.001*		10.27	33	<0.001*

The distributions of model residuals, for both seed set and seed weight per flower, were not significantly different from normal (seed set: K-S test statistic = 0.07, P = 0.14; seed weight: K-S test statistic =0.09, P = 0.03). Adjusted P threshold for significance is 0.02 (i.e., 0.15/7). Results that are significant (i.e., sig) are marked with *.

Table 4. Results of a general linear mixed model for seed set and seed weight (mg) per plant vs treatment, stem height, number of flowers per stem, and number of pollinations per plant, with constant included (n = 38).

	seed set per plant				seed weight (mg) per plant			
effect	coefficient	df	F-ratio	P-value (* = sig)	coefficient	df	F-ratio	P-value (* = sig)
Constant	−166.67	1	7.34	0.011	−477.1	1	5.79	0.02*
Treatment (R = 0, W = 1)	−0.94	1	0.002	0.97	15.8	1	0.20	0.66
Stem height	1.08	1	1.16	0.29	4.07	1	1.59	0.22
Num. Flowers	43.56	1	33.71	<0.001*	89.7	1	13.84	0.001*
Num. pollinations	1.81	1	0.72	0.40	6.6	1	0.92	0.34

No plants had zero pollinations. For both seed set and seed weight per plant, the distribution of residuals was not significantly different from normal (seed set: K-S test statistic = 0.09, P = 0.57; seed weight: K-S statistic =0.10, P = 0.42). Adjusted P threshold for significance is 0.03 (i.e., 0.15/5). Results that are significant (i.e., sig) are marked with *.

Hypothesis 3: Re-use of salvaged resources from wilted flowers for reproduction by plants in next flowering season

In contrast to Hypotheses 1 and 2, tests of Hypothesis 3 revealed that salvaged resources from wilted flowers are re-used to support reproduction during the next flowering season. Here there were three tests, so the adjusted P threshold for significance is 0.05. In all 3 years, reflowering during the season following experimental manipulation was more likely for W than R plants. This was significant for experimental manipulations in 2018 (Reflowered: R treatment: 3 plants, W treatment: 8 plants; No reflowering: R: 12 plants, W 8 plants; Pearson χ^2 = 3.044, df = 1, P [one-tail] = 0.04) and 2020 (Reflowered: R treatment – 33 plants, W – 38 plants; No reflowering: R – 94 plants, W – 62 plants; Pearson χ^2 = 3.758, df = 1, P [one-tail] = 0.03). However, this was not significant in 2019 (Reflowered: R – 5, W – 9; No reflowering: R – 15, W – 10; Pearson χ^2 = 2.119, df = 1, P [one-tail] = 0.07). For all years combined, reflowering during the season following experimental manipulation was significantly more likely for W than R plants (F = 8.18; P [one-tail] = 0.003), with no significant effect for either study year (F = 0.16; P = 0.86) or the treatment x study year interaction (F = 0.63; P = 0.53).

Table 5. Comparing average weights of nectar sugar removed and seeds developed.

experiment/protocol	nectar sugar removed per flower (mg)	seed weight per flower (mg)
2018/age 6-days flowers removed	10.20 ± 1.25 (n = 58)	151 ± 7 (n = 114)
2019/age 6-days petals removed	Assumed zero	676 ± 39 (n = 228)
2020/age 1-day flowers removed	5.46 ± 0.24 (N = 223)	No seed production

Weights of nectar sugar removed per removed flower and seed weight per flower

The average weight of nectar sugar removed per removed flower was small relative to the average seed weight per flower. The average weight of sugar removed per flower was larger for flowers removed when 5 days old than for flowers removed when 1 day old (Table 5), and both were <7% of seed weight per flower (Table 5).

DISCUSSION

We obtained the first direct evidence that plants re-use salvaged resources from flower wilting to support reproduction. However, contrary to expectation, our study plants (*B. grandiflora*) did not preferentially re-use such resources for reproduction by either the same flowers (Hypothesized 1st preference) or other flowers on the same plants (Hypothesized 2nd preference). Instead, they re-used these resources for reproduction during the next flowering season (Hypothesized 3rd preference).

The following observations suggest that this pattern of re-use of resources from wilted flowers may be part of an overall strategy to salvage and re-use resources invested in reproduction, other than the current crop of seeds, in subsequent flowering. In *B. grandiflora*, mature seeds are released from wilted flowers and fall to the ground, while the remaining flowering stem also wilts, leaving a faded and dried-out skeleton. Hence, these plants must have mechanisms and pathways for transfer of resources from flowering stem and attached flowers to underground storage in corms and roots. These underground resources could then be used for production of new flowering stems during subsequent flowering.

That the average weight of nectar sugar removed when a flower was removed was small relative to average seed weight per flower suggests that such nectar removal is unlikely to have significantly affected the results of our experiments. Furthermore, nectar sugar removal did not occur for the experiment carried out in Jan 2019 and so was not relevant for that experiment. Removal of other nectar constituents besides sugars is also likely to be unimportant as non-sugar components of nectar generally make up a very small proportion of the dry mass of nectar (Pyke & Ren 2023).

That the number of pollinations had no significant effect on either seed set or seed weight for both individual flowers and whole plants confirms our assumption that our artificial pollination resulted in seed production being determined by allocated plant resources and not being pollen limited. That seed number and seed weight were sometimes significantly affected by flowering stem height, number of flowers per stem and flower position indicates that plants may differentially allocate resources to seed production, depending on these variables. This warrants further investigation.

Our results point to the areas for further research: salvage and reuse of resources from wilting flowers in additional plant species, particularly annual species; determining the chemical nature of these resources and mechanisms by which they are transformed and transported; tracking the nature and movement of resources from wilting flowers to other plant parts, possibly using labelling techniques; quantitative assessment in terms of plant fitness of the costs and benefits arising from allocation of resources to petals, nectar and other floral parts and subsequent salvage and re-use of these resources. There remains much to do.

CONCLUSION

We provide the first direct demonstration that plants can salvage resources from wilting flowers and reuse these resources to promote subsequent reproduction, as part of a strategy of salvaging resources allocated to reproduction during one flowering season and re-using these resources for subsequent flowering, but further research is required to understand this phenomenon.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

GHP initiated the project, carried out the lab work and led the writing. GHP and JRMK carried out the fieldwork. All authors discussed the project as it evolved, contributed to the writing, and agreed on the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data have been deposited in Dryad.

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