Comparing the compensatory responses to floral damage by herbivores and heatwaves in the oilseed crop *Camelina sativa*

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Ecology and Evolutionary Biology

University of Toronto

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2019

Abstract

Tolerance is thought to evolve as a generalized response to different types of damage experienced in the plant’s lifetime. Flexible flowering schedules and the production of ‘spare’ late-produced flowers, which is widely observed, can be a very general mechanism used by plants to tolerate a variety of environmental insults. I have conducted two experiments to investigate how damage-induced shifts in flowering schedules in *Camelina sativa* contribute to tolerance of heatwaves and floral herbivory. We found that while heat damaged plants initiated compensatory responses through additional flower and branch production, reduced fertilization and increased seed abortion led to lower total yield than unheated plants. Floral herbivory did not result in compensatory growth, but rather a shift in resource allocation to remaining flowers, resulting in tolerance. *Camelina sativa* responds differently to heatwaves and herbivory because the extent of damage to plant tissues is greater during heat stress than floral herbivory.
Acknowledgments

I would first like to thank my supervisor Dr. Art Weis, for his guidance, enthusiasm for these projects, having confidence in me and supporting my goals. I learned so much thanks to Art, and I couldn't be more appreciative to have such a supportive supervisor. I wish to thank my committee members; Dr. James Thomson, for his valuable advice during committee meetings, and Dr. Tammy Sage, for sharing Camelina seeds, her expertise, and insightful suggestions to my experimental design that led to fruitful results. I would also like to thank Dr. Ben Gilbert and Dr. Njal Rollinson for serving on my defence committee.

Weis lab members have been supportive throughout my thesis project, especially Cameron So, Sydney Rotman and Sophia Fan. Thank you, Cameron and Sydney, for your encouragement and sense of humour, which made the day-to-day work more fun. I’m immensely grateful to Cameron for helping with both my experiments, his suggestions, statistics help, and knowledge about Camelina. Sophia has been a wonderful volunteer/work-study/ROP and friend, working incredibly hard on both my experiments, especially the silique dissections. I owe thanks to Michaela Fink and Natasha Dhamrait for helping with my herbivory experiment. This work would not have been possible without the help of many Weis lab volunteers. I am incredibly grateful to the especially dedicated volunteers Paula Pietraszkiewicz, Sarah Ravoth, Jacob Newfeld, Guadalupe Santos and Yiling Fu - thank you for sticking with me through both experiments and for your enthusiasm and hard work.

I would also like to thank Bill Cole, Andrew Petrie, Colin Bonner and Tom Gludovacz for their help setting up my experiments in the greenhouse and growth chambers. I’m grateful to Stephan Schneider - from fixing my car to finding me a pet cat, he was so helpful when I worked at KSR. I’m also grateful to my office mate Tia Harrison for her advice and kindness.

My family, especially my parents and grandparents, have been an incredible source of support during graduate school – I can’t thank you enough for your love and prayers. I’m thankful for my friends Alysha Johnson and Abbey, who have encouraged me throughout my degree. I’d like to give a special thank you to Cooper Papp, for always being there for me and encouraging me to reach my goals. Deo gratias.
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INTRODUCTION

Flower Damage: Extreme Heat and Floral Herbivory

In recent years, record breaking extreme heat events have increased globally and are attributed to anthropogenic climate warming (Jentsch et al., 2007; Coumou & Rahmstorf, 2012; Allen et al., 2019). Heatwaves are defined as extended periods of markedly hot temperatures relative to the average conditions for a region (Abeli et al., 2012). When mean temperature increases, the occurrence of minimum extremes declines and the frequency of maximum heat extremes intensifies, leading to novel environmental conditions (Jentsch et al., 2007). Heatwaves are becoming longer, more frequent and more intense (Meehl & Tebaldi, 2004). Extreme heat events abruptly push environments into conditions that stress plants, damage floral tissues and effect survival and reproductive success (Jentsch et al., 2007; Reyer et al., 2013; Zinta et al., 2014). Recent research suggests that climate variability, such as heatwaves, has a disproportionally greater effect on species when compared to increases in mean climate (Walther et al., 2003; Vasseur et al., 2014). Vasseur et al. (2014) found that incorporating changes in temperature variation into climate projection models results in substantial differences in the thermal performance of ectothermic invertebrates. Extreme events like drought and heavy rain can cause shifts in flowering length of equal magnitude to 10 years of gradual climate warming (Jentsch et al., 2009). Studying heatwaves in addition to the changes in mean temperature is necessary to determine the effect of climate change on species resilience and reproductive success.

Plant ecophysiological performance, as measured by net photosynthetic rate, CO₂ uptake rate and leaf water potential, generally decline during extreme climate events like heatwaves and droughts (Abeli et al., 2012). Flowering appears to be even more sensitive to heat extremes than vegetative growth (Abeli et al., 2012). Female and male reproductive organs within flowers fail at temperatures greater than 30°C (Sage et al., 2015). High temperatures reduce pollen production and viability, resulting in lower crop yield (Prasad & Djanaguiraman, 2014; Sage et al., 2015). High temperatures of 32°C have been noted to cause flower sterility and the development of small stamens, sepals and petals in Brassica napus (Polowick & Sawhney, 1988).

Decline in flower production due to heat stress leads to a corresponding decline in seed set. When summer field temperatures were greater than 29.5°C, Morrison and Stewart (2002) found reduced
flower production in several *Brassica* species, resulting in decreased seed yield through lower seed weight and number of seeds per pod. However, the degree of fitness reduction may depend on the timing of the heatwave in the plant’s flowering schedule. Optimal conditions in the first and second week of flowering are found to be crucial for seed set, and thus heatwaves that occur during this period are likely to negatively affect yield (Tayo & Morgan, 1979; Morrison & Stewart, 2002).

Herbivore damage is another stressor that reduces the reproductive success of plants. Despite advances in crop protection through pesticide use, the annual loss of crops due to herbivory is estimated to be between 25 – 40% worldwide (Oerke, 2006; Beddington, 2010; Fatouros *et al*., 2016). Brassicaceous oilseed crops host complex insect communities that attack roots, stems, leaves, buds, flowers, pods and seeds (Lamb, 1989). Floral herbivory is consumer damage to developing floral buds and parts of the flower, including the pistils, stamens, pollen, ovules, petals and sepals (McCall & Irwin, 2006). The primary herbivores of oilseed crops are aphids, Lygus bugs and pollen beetles, which feed directly on flowers and buds (Lamb, 1989). The Bertha armyworm and the brassica pod midge feed on seed pods (Lamb, 1989).

Although floral herbivores only feed on a small proportion of a plant’s total biomass, they can directly reduce fitness by damaging structures related to reproductive output, such as pollen, ovules, anthers and pistils (Krupnick & Weis, 1999; McCall & Irwin 2006; Soper Gordon & Adler, 2016). Oilseed crops can tolerate low levels of damage to flowers by retaining buds that would otherwise be aborted (McGregor, 1981; Tatchell, 1983; Lamb, 1989). However, intensive floral herbivory by the *Meligethes* pollen beetle results in substantial yield loss (Tulisalo & Wuori, 1986; Gagic *et al*., 2016). Floral herbivory can also damage tips of the main shoot and side branches, further reducing the plants ability to compensate (Lamb, 1989). Flower damage can indirectly reduce plant fitness when pollinator attraction and behaviour is altered. Pollinator visitation rates may decline when herbivory reduces floral appearance and display size, resulting in lowered pollen export and receipt (Krupnick & Weis, 1999; Carper *et al*., 2016).

Heatwaves and floral herbivory both disrupt plant mating by damaging open flowers, thus temporarily preventing successful pollen export and receipt. While flower-feeding insects damage flowers at only one stage of development, extended heat events will impact the whole inflorescence. Herbivore damage alters the source-sink resource relationship within a plant; by
removing current flowers, resources are freed to mature seed in later-opening flowers (Krupnick & Weis, 1999). Alternatively, extreme heat not only damages open flowers, it can cause seed abortion in immature fruits, disrupt development in buds and potentially damage inflorescence meristems. This damage to reproductive structures comes in addition to any disruption to photosynthesis systems.

Neither herbivory nor heatwaves are typically fatal, and so the plant has opportunity to regrow and replace the damaged parts. Recovery from floral damage can come in several forms. First, flowers produced at the end of the season typically fail to set fruit (Austen et al., 2015). When early flowers are damaged, resources can be diverted to mature these ‘spares’. Second, plants can increase late flower production by extending the flowering period and/or mobilizing lateral meristems to replace reproductive losses. Adjustments to the schedule of flower development and deployment would constitute mechanisms for tolerance to damage.

**Plant Tolerance to Damage**

Tolerance is the ability of a plant to maintain fitness by regrowth and reproduction despite suffering tissue damage (Stowe *et al.*, 2000). Tolerance was first studied by agricultural scientists who noticed that some plant varieties had a superior ability to regrow and reproduce after pest damage compared to others (Painter 1958). Due to the economic benefits of utilizing tolerant crops, research expanded to identify varieties that could withstand a wide array of abiotic and biotic insults.

Broadly considered, plant tolerance operates through several intrinsic mechanisms – adjustments in photosynthetic activity, mobilization of stored reserves, phenology changes, and through features of plant architecture (Tiffin, 2000). Increased photosynthetic rates in the remaining, undamaged tissues is a widely-observed response to damage (Tiffin, 2000). Zhu *et al.* (2014) found that when 30% of *Calligonum caput-medusae* sapling leaves were removed, photosynthetic rates in the remaining leaves increased, resulting in the growth of taller and thicker stems relative to control plants. Storage organs, such as taproots and tubers, can also contribute to tolerance; stored carbon can fuel aboveground regrowth and reproduction after damage (Strauss & Agrawal, 1999). Phenological response is also a mechanism of tolerance in plants that have a restricted growing season (Tiffin, 2000; Freeman *et al.*, 2003). Plants typically experience a delay in
growth, flowering and seed production following damage, and since it is essential that plants finish reproduction before the season ends, plant genotypes with a shorter delay can achieve greater tolerance (Tiffin, 2000). Passive mechanisms, including structural features that are already in place at the time of damage, are also important in providing tolerance in plants. For example, stem rigidity can enable a plant to remain upright after herbivore damage and high root-shoot ratios enhances nutrient uptake for regrowth following damage (Tiffin, 2000). In addition, the number and arrangement of meristems in a plant effects regrowth ability in the undamaged tissues (Marquis, 1996).

These tolerance mechanisms lead to compensatory growth – the increased growth rate and activation of dormant axillary meristems after damage occurs to dominant floral and vegetative meristems (Strauss & Agrawal, 1999). Compensatory growth changes the growth trajectories of plants, leading to replacement of some, all or excess tissues, referred to as undercompensation, full compensation and overcompensation, respectively (Tiffin, 2000). The occurrence and strength of compensation depends on a variety of factors, including timing of damage (Lennartson et al., 1998), type of damage (Strauss & Agrawal, 1999) and resource availability (Rosenthal and Kotanen, 1994). Lennartson et al., (1998) found overcompensation through increased axillary branch initiation and seed production in plants that were clipped earlier in summer compared to those damaged late, indicating that there may be a critical period for tolerance responses. In contrast, removing 50% of leaves in Barbarea vulgaris later in the growing season resulted in the largest increase in growth rate, although plants clipped at earlier time points were also able to regrow (Yoshizuka and Roach, 2011). Goldental-Cohen et al. (2018) found that the timing of flower removal and the position from which the flower was removed on olive inflorescences were both important determinants of the intensity of the damage response. When the flower buds were removed 3 weeks before anthesis, fruit set was increased by >50% (Goldental-Cohen et al., 2018). Removing the apical flower and flowers at lateral positions led to an increase in the number of developed fruits per inflorescence compared to the control (Goldental-Cohen et al., 2018). The type of damage also appears to evoke distinctive responses in the plant. Plant response to damage differs between simulated herbivory and natural herbivory (Agrawal, 1998). For example, while manual removal of cotton buds resulted in increased vegetative growth (Sadras, 1996), aphids feeding on phloem led to decreased branching (Rosenheim et al., 1997). Resources available in the environment and within the plant, which vary spatially and temporally, may also be critical to
plant tolerance responses. Within a plant, initiation and development of new meristems after damage is dependent on competition for resources between already developing vegetative tissues, flowers and seeds (Rosenthal & Kotanen, 1994).

**Tolerance in Light of Life History Strategy**

Fruit production and maturation is costly to plants because it consumes resources that could otherwise be put towards future growth and reproduction (Avila-Sakar *et al.*, 2001). However, when flowers are damaged and fail to produce fruit, resources are then freed, potentially allowing for enough regrowth and reproduction such that damage-induced losses are fully compensated (Avila-Sakar *et al.*, 2001; Bajcz & Dummond, 2017a).

We can consider two strategies plants may evolve to tolerate floral damage. The first strategy is similar to a bet-hedging strategy (Childs *et al.*, 2010; Simons, 2011). The plant produces a set number of flowers over a set deployment schedule. When floral damage occurs, plants use a fixed resource pool to make changes to post-damage yield investment, but do not produce additional buds through compensatory growth. The second strategy instead involves a flower deployment schedule that responds plastically to the environment. Plants use resources freed by flower damage to produce additional flowers through compensatory growth, resulting in an extended flowering schedule.

**Changes in Seed Investment in Response to Damage**

Bet-hedging is a type of evolutionary strategy that describes the optimal timing of and investment in life history events over the long term and under variable environmental conditions (Simons, 2011). Using a bet-hedging strategy does not yield the highest fitness in any one generation or environment, but rather maximizes the geometric mean fitness over the long term and results in a higher fitness across an array of different environments (Childs *et al.*, 2010; Simons, 2011). The geometric mean fitness is used to measure long term fitness of a genotype because it is multiplicative and therefore very sensitive to low fitness values in the years that environmental conditions are poor (Simons, 2011).

To further expand on this idea, we can imagine a plant species that has a developmental program that dictates that total flower output is determined during early growth, and these flowers are matured using a resource pool that reaches its limit at first flowering. Therefore, the number of
sources (resource pool) and sinks (flower buds) are fixed. In a constant environment, the optimal number of buds would match the plant’s expected resource pool. In variable environments, some flowers are destroyed by heatwaves and herbivore outbreaks. To maintain the highest geometric mean fitness in variable environments, the optimal number of flowers buds would be higher to account for potential losses. When there is no damage, the plant pays a cost to produce extra flower buds. However, when flowers are damaged, these extra buds are mobilized, improving the fitness lost to damage. This is a bet hedging-like strategy because the developmental program for initiating flower buds is fixed and set to maximize fitness across generations even if fitness is sub-maximal in some generations.

This strategy has been observed in plants that open flowers sequentially across the reproductive season and experience a decrease in seed set from first to last flowers (Stephenson, 1981; Thomson, 1989; Diggle, 1995; Kliber & Eckert, 2004). For example, Austen & Weis (2014) observed a decreasing probability of successful fruit set and siring seeds through time in *Brassica rapa*. Lovett Doust *et al.* (1986) observed no difference in the number of seed pods produced throughout *Arisaema triphyllum* plants, but upon more detailed analysis, found that the number of seeds decreased along the length of the branches from the base to the tip, with some distal flowers producing no seeds. The higher success of early flowers relative to those late in the season may be due to a structural advantage in early flowers or increased access to resource supplies early in the growing season (Stephenson, 1981; Diggle, 1995).

Despite consistent observations that late-produced flowers fail to both set seed and sire seeds on other plants, individuals continue to invest resources to produce ‘extra’ late flowers, which is seemingly maladaptive under benign conditions (Austen & Weis, 2014). However, when there is variability in environmental conditions, it may be risky to mainly invest in early flowers. Adjusting resource allocation toward the reproductive success of late-produced flowers may be more advantageous if a disturbance early in the season leads to the failure of early flowers. The extra flowers stand in reserve should the earlier-produced flowers be damaged. Several previous studies found that removing the first fruits produced by the plant resulted in an increase in seed set in the distal flowers produced after damage (Diggle, 1995; Avila-Sakar *et al.*, 2001; Bajcz & Drummond, 2017a). This demonstrates that in some species distal flowers have the potential to compensate for reproductive losses that occur to basal flowers earlier in the season.
**Plasticity in the Flower Deployment Schedule**

Bradshaw (1965) suggested that because crops experience variability in environmental conditions from year to year or even from one area of the field to another, adaptive plastic responses can help alleviate yield loss. Since plants cannot respond to damage using behavioural mechanisms and are restricted to the location that they germinate, shifting resource allocation toward compensatory growth after damage occurs can be adaptive (Bradshaw 1965).

Using a plastic compensatory growth strategy assumes that plants have a developmental program dictating that successive buds can be promptly formed, so long as there are resources available to fuel this additional growth. Hence, new sinks (flowers) can be formed if there is a source (resources to be captured). There are never ‘extra’ flowers at the end of the season, because the optimal growth program is responsive to the environment and returns the maximal result in every generation. When there is no damage, each flower has the same chance of maturing seed and flowering ends when resources decline at the end of the growing season. Typically flower production increases over time, reaching one or more flower count peaks and declines as the growing season ends and as internal resources switch from investment in production of flowers to investment in seed maturation (Herrera, 1986; Wadgymar et al., 2014; Austen & Weis, 2015).

When some flowers are damaged, the resources that would have fueled seed development become available, and the increase in the resource poll triggers a profusion of replacement flowers, leading to short term fluctuations in flower and seed counts (Elzinga et al., 2007; Wadgymar et al., 2014; Austen et al., 2015). For example, flower removal over the span of 2 weeks led to a second peak in flower production that was not observed in control plants (Wells, 2001).

Plants that are adaptively plastic can detect and respond to factors affecting the display schedule, such as extreme heat or pests, and compensate for reproductive losses by initiating new flowers and vegetative tissues once the environmental insult has ended. For example, flower production decreases during extreme heat events and plants later compensate for lost reproductive success by increasing flowering after the heatwave has ended (Abeli et al., 2012). When the primary inflorescence of *Brassica napus* is damaged by heat, an increase in resource allocation toward production of lateral inflorescences has been observed (Young et al., 2004). Plants can also respond to heatwaves by altering the length of the flowering season. Decreases in flower production during stressful conditions may lead to accumulation of resources that can be used to
continue to flower later in the season (Cross et al., 2003). For example, flax that experiences 1-2 week heatwaves prolong the flowering period by 17 days (Cross et al., 2003). Prolonged flowering may be problematic if flowering extends into autumn, creating a risk for autumn frosts to destroy buds, flowers and developing seeds, further reducing reproductive success (Cross et al., 2003).

In practice, plants evolve a tolerance strategy is between these two extremes. The optimal strategy may depend on the type of damage, timing of damage, how resource acquisition rates change over the season, how quickly stored resources can be mobilized, and how quickly flower buds can be converted into fruits. The focus of my thesis is to investigate tolerance to heatwaves and herbivory in the oilseed crop Camelina sativa. I sought to determine if tolerance is conferred through phenotypic plasticity – by post damage increases in flower production, or through a bet hedging strategy that acts to reallocate resources to late-produced flowers, or both. To this end, I exposed plants to heatwaves – 5 day periods of elevated temperature, and herbivory – 3 day periods of 100% flower removal. The timing of damage could influence plant response, and so the damage treatment was applied either at the beginning, middle or toward the end of the flowering period. I found reduced fertilization in flowers opening during a heatwave. Plants overcompensated for these losses by increasing post-wave flower production. This led to high fruit production even in damaged plants. However, temperature induced seed abortion lowered seed yield per fruit. At the whole plant level, the plants’ response to the heatwave failed to fully compensate for damage. In contrast, floral herbivory did not result in increased post-damage flower or branch production. Instead, it appears that plants used a strategy in which resources were allocated toward seed production after damaged ended. All floral herbivory treatments produced yield equal to that of undamaged plants, resulting in full compensation.

METHODS

Study Organism

Camelina sativa (Brassicaceae), also known as false flax, is a fast-growing annual, native to the Mediterranean and central Asia (Guy et al., 2014). Plants reach harvest maturity 85-100 days from seedling emergence (Guy et al., 2014). Camelina flowers are small, yellow, self-pollinating, and mature into siliqual seed pods (Guy et al., 2014). Flower buds are first produced on the tip of
the main stem raceme, and as the plant ages, flowers are also produced on lower axillary racemes (Lamb, 1989). Nutrient efficient, it can grow in saline and low fertility soils, making it competitive in stressful environments (Budin et al., 1995).

First cultivated in Europe as an oilseed crop, *Camelina* is now gaining interest as a source of biofuel (Lu & Kang, 2008). It is easy and inexpensive to produce, has high seed oil concentration, can be integrated in existing cropping systems, and provides ecosystem services, such as pollinator food and habitat (Sindelar et al., 2017). Against these many favourable qualities, *Camelina* yield is substantially reduced at temperatures over 32°C (Carmo-Silva & Salvucci, 2012; Jewett, 2013).

I used recombinant inbred lines (RIL) of *C. sativa* that differ in branching patterns to determine the variability in plant response to damage. The RIL population (DSV Seeds, Lippstadt, Germany) was created by crossing two German flax cultivars, ‘Licalla’ and ‘Lindo’. The F1 generation produced by this cross was then selfed for 8 generations by single seed decent. I used fourteen genotypes; the two parent cultivars and the RILs 23, 158, 166, 206, 128, 9, 101, 162, 138, 36, 110 and 199. These genotypes were selected to decouple contributions of height and branching on the schedule of flower and inflorescence deployment.

**Heatwave Experiment**

*Plant Care*

Seeds were cold stratified at 4°C for 3 days prior to planting to increase germination success. Two seeds were planted per labelled 5.5 x 5.5 inch pot (Kordlock, SQL0550) in Pro-Mix BX Mycorrhizae growing medium. Pots were thinned to a single seedling after two weeks. Throughout the experiment plants were kept well-watered to minimize any effects associated with drought stress due to heatwaves. A general purpose fertilizer (20-20-20) with micronutrients was applied twice weekly. Plants were raised in 4 growth chambers (Econair) at the Plant Growth Facility for the University of Toronto, with 42 plants per chamber. Pots were kept evenly spaced to ensure equal light competition (Jewett, 2013). Light levels were measured using a photometer on the left and right side of the chamber twice per week, and adjustments were made to equalize chambers.
**Experimental Treatments**

Chambers were randomly assigned to one of four thermal treatments. Three of these simulated a heatwave during the early, middle, or late interval of the flowering schedule, while the fourth served as a control. Base thermal cycle was 24°C during the day and 18°C during the night. During heatwaves, I increased temperature to 38°C day / 28°C night for 5 days. The early heatwave treatment was imposed after greater than 50% of plants reached first anthesis. The peak heatwave was applied when most plants reached maximum flower production. The late heatwave was imposed as flower production declined. The control chamber remained at the base thermal cycle throughout.

I performed the experiment in two temporal blocks, with thermal treatment re-randomized among chambers for the second block to prevent confounding of temperature and chamber effects. I used six genotypes (Licalla, Lindo, 206, 158, 23, 166) to balance the trade-off between growth chamber space constraints and a suitable number of replicates per genotype. Each temporal block was comprised of 7 replicates each of the six genetic lines in each of four treatments (42 plants per chamber), for a total sample size of 336. The results from both temporal blocks showed similar patterns, so the datasets were combined.

My goal was to impose heatwaves at the equivalent points in the flowering schedule for all plants. In this way, earlier or later flowering lines would not ‘escape’ heat exposure. To synchronize flowering, I staggered sowing dates, with late-flowering types planted before the early-flowering types. In the first temporal block, I timed planting according to flowering times observed for the genetic lines in a previous greenhouse experiment. However, flowering times differed under growth chamber conditions, and first flowering occurred over a ~2-week span. As a result, genotypes were at somewhat different points in their flowering schedule when the treatment was applied. I corrected this in the second temporal block by using the date of first flower from plants grown in the growth chamber. Plants in the second block entered flowering within a 4-day span.

I monitored the success of flowers that opened before, during and after the heatwave. To identify these intervals, I tied a colour-coded string around the newest cluster of buds at the end of each flowering branch on the days the treatments were applied, and again when the base thermal cycle was restored (Plate 1). Flowers proximal to the first string were produced before the heatwave, those between the strings were produced during the heatwave, and those distal to the second string after. The control plants were also tied at the start and end of each heatwave treatment. After the
last treatment was applied, all plants were moved to the greenhouse to mature seeds. The greenhouse was set to ambient conditions and watered at lib.

Data Collection

To determine the effect of heatwaves on the schedule of flower deployment, I counted the number of open flowers on each plant every other day. To be considered an open, its petals must be unfurled, but it must not show signs of fertilization and fruit development (Plate 2). The two-day count interval assured the same flower was not counted twice. Heat damage to the meristem of racemes is expected to lead to the initiation of replacement flowering branches, and so I counted the number and position of branches bearing flowering racemes. Branches were categorized as primary, secondary, or tertiary, relative to their attachment to the main stem (Figure 1).

After plants matured, I counted the number of siliques on each branch, recording if they arose from flowers opened before, during, or after the heatwave. To gain detailed insights on reproductive success along individual branches, I collected siliques along the main stem, and from one middle and one lower primary branch. I collected siliques from the remaining primary branches together, then those from the secondary branches together. Thus, siliques could be categorized according to their branch’s position, their position along that branch and their exposure to the heatwave.

I quantified seed production as the aggregate seed mass per plant. Seeds were separated from siliques and weighed to the nearest 0.001g. Beyond the effect of heatwaves on total seed production, I explored impacts on seed quality. Specifically, I asked the following: Do heatwaves cause abortion of recently initiated seeds?; Do immature flower buds exposed to the heatwave later open and succeed: if flowers fail, is it due to failed fertilization or to embryo abortion? I examined individual siliques from two representative plants per genotype for each treatment, noting position of their branch on the plant (main stem, middle primary, lower primary) as well as their position along the inflorescence (see Figure 2). For each silique, I counted the number of unfertilized ovules, aborted seeds and mature seeds under a dissecting microscope (Plate 3). The mature seeds in each silique were weighed in aggregate. From this information, I sought to gain insights on survivorship through successive stages; the difference between the number of initial ovules and fertilized ovules represents failed fertilization, and the difference between the number of fertilized ovules and mature seeds represents seed abortion.
Herbivory Experiment

Plant Care

Seeds were sown as for the heatwave. Plants were raised in the Greenhouse Facility at the University of Toronto. The temperature ranged between 22-26°C during the day and 18-24°C at night. Plants were grown in Conetainer pots (Steuwe & Sons, Corvalis Oregon, USA) measuring 4 cm diameter and 21 cm height. Pots were organized into blocks (racks; see Plate 4), with a single replicate from each of the 14 genotypes per block.

Experimental Treatments

Each of the 60 treatment blocks was randomly assigned to one of four treatments. Three of these simulated floral herbivore damage during the early, middle, or late interval of the flowering schedule, while the fourth served as a control. During the treatment interval, 100% of the open flowers on the plant were removed each day over 3 successive days. As with the heatwave experiment, treatments were applied early in the flower schedules, at peak flowering and as flowering declined. After treatments were completed, plants remained in the greenhouse to mature seeds and were watered at lib. There were 4 treatments, 15 blocks per treatment, and 14 genotypes, for a total sample size of 840 plants.

Data Collection

Flowers were counted every other day, as for the heatwave experiment. After plants ceased flower production, I counted the number of siliques on each branch, recording if the flowers arose from flowers produced before, during or after herbivory. Silique counts were completed on approximately 50% of the plants. Once siliques matured, I collected the siliques produced before and after damage separately on every plant and quantified seed production as the aggregate seed mass per plant, weighed to the nearest 0.001g.

Statistical Analysis

Similar analyses were performed for both experiments. I used generalized linear mixed models (repeated measures) to determine if the pattern of flower production over time differed with the timing of the damage and with plant genotype. The purpose of this analysis was to determine if
treatment-induced changes in the plant flowering schedule could be characterized as under/overcompensation for damage. Since the treatment was applied at three different times, the pre- and post-treatment periods encompassed different time frames. This necessitated three separate analyses, each comparing the treatment to the control over the appropriate periods. Flower counts during the heat/herbivory event were not included in the analysis; flower production always decreased to 0.

The model included treatment, genotype, day and their interactions as fixed effects. The random effects were (1|Day) and (Day|Plant), as per conventional use in repeated measures analysis using GLMMs (Plonsky, 2015; Gadhave & Gange, 2016). Since the response variable is flower counts, a Poisson distribution with log link function was used in the model. This model structure was selected by contrasting it to alternative models with different random effects structures and then selecting the most efficient model using the likelihood ratio test. Differences between post-treatment flowering schedules of the control and treatment group would be indicated by a significant treatment*day interaction term. A significant treatment*genotype*day term would indicate that the response of flower deployment to the heatwaves varied among genotype.

I assessed the impact of damage on total seed production, total flower number and branching using general linear models, with treatment and genotype as fixed effects. When necessary, response data was log transformed as needed to satisfy the assumption of normality. Additional generalized linear models that included the interaction between treatment, genotype, and timing were used to determine how silique production as well as seed yield differed before, during and after the heatwave. Again, the different time frames for the early, peak, and late treatments necessitated three separate before/after analyses. If an ANOVA indicated significant terms, post hoc tests were then performed with alpha set to 0.05.

Additional analyses were performed for the heatwave experiment, using the data I collected for the number of unfertilized ovules, aborted seeds and mature seeds in siliques collected at different positions along branches. I used two-way ANOVAs to determine whether heatwaves cause ovule abortion pre- and post-damage, abortion of recently initiated seeds and failed fertilization of flowers open during the heatwave. I ran additional ANOVAs to determine if siliques produced after the heatwave can compensate for yield loss during the heatwave.
RESULTS

Heatwave Experiment

Heatwaves greatly impacted flowering and diminished seed yield in Camelina sativa. Plants reduced flower deployment during high temperature intervals, but afterwards they increased per day flower production, flowered for more days, or both. Increased flower production was in part achieved by increased branching after the heatwave treatment to replace damaged racemes. Despite increases in raceme, flower and silique production, seed yield of heat-damage plants was significantly lower than controls. The timing of heat damage did not affect yield loss. Undercompensation was primarily due to reduced fertilization of flowers and increased seed abortion in siliques, initiated prior to, and thus exposed to, heat events. Flowers produced following the heatwaves did not produce enough yield to make up for previous losses.

Flowering Schedule

Plants responded to heat damage by increasing per day flower production, flowering for more days, or both. In control plants, flower deployment increased to a peak 10-18 days after first anthesis. Thereafter they declined, and ceased flowering between days 22 and 28 (Figure 3). During heatwaves, the deployment of new flowers ceased, resulting in a decline in flower counts as the heatwave progressed. Following early, peak, and late heat treatments, there were ~ 3-day, 5-day and 8-day lags respectively, before flower deployment resumed. There was a surge in post-damage flowering that was greater than production by controls over the same time interval (Table 1, Figure 4). Heat-damaged plants also extended their flowering period relative to unheated controls (Table 2, Figure 5). The early and peak treatment groups flowered longer than controls (~1 week), and the late treatment groups flowered ~2 weeks longer. These differences were significant (p<0.05) in post hoc tests.

By counting the number of siliques produced by each plant we determined that aggregate flower production significantly differed between treatments (Table 2, Figure 6) and genotypes (Table 2, Figure 7). Heatwaves later in the flowering period resulted in greater total flower production than when heatwaves occur at the start or not at all (Figure 6). The damage*genotype interaction effect was not significant, indicating that in general, each genotype ranks similarly in flower production across all treatments (see Figure 7)
The repeated-measures GLMM models indicated that post damage flower deployment schedules differed between control and heat-damaged plants in all cases (Table 3). Thus, plants respond to damage by extending the flowering period and elevating post-damage flower production.

Patterns of flower production reflected increased branching after heatwave treatment (Figure 8). The number of primary branches did not differ across treatments (Table 4). Heated plants produced significantly more secondary branches than unheated control plants (Table 4). In addition, plants heated late produced significantly more tertiary branches than the early-damaged, peak-damaged, and control plants (Table 4). This demonstrates that when racemes are damaged by heatwaves, plants respond plastically by producing new secondary branches. There were genetic differences in the number of primary, secondary, and tertiary branches produced, which reflects the underlying genetic differences between the recombinant inbred lines (Table 4). There was a significant interaction between treatment and genotype in secondary branch production, as genetic lines produced many more branches the later that heat treatments occurred (Table 4).

**Components of Yield**

Despite increased flower and silique production in plants exposed to heatwaves, seed yield declined compared to controls (Table 2, Figure 9). Post hoc tests did not reveal differences in seed yield among the three experimental groups. Genotypes differed in seed yield, with parental lines and RILs 206 and 158 producing significantly more yield than the low producing RILs 23 and 166 (Table 2, Figure 10). The interaction between treatment and genotype was not significant (Table 2).

Heat disrupted the development of young fruits and seeds. Pre-treatment seed yield was reduced in heat damaged plants (Tables 5, Figure 4). Fruit success declined over the flowering period in the control plants; their first-produced flowers set an average of ~11.8 seeds per silique, while late-produced flowers make only ~2.5 seeds. Early, peak and late heated plants made minimal flowers and virtually no seeds during the heatwave (Figure 4). Experimental treatments significantly rebounded in flower production once the heatwave ended, leading to increased yield after the heatwave compared to the control (Table 5, Figure 4).

Greater flower production and lower yield indicate higher flower failure rates in the experimental plants. I determined whether failures were due to lower fertilization success or increased seed abortion. Across all treatments, flowers initiated before the heatwave produced the same number
of initial ovules (Table 6, Figure 11). After heat treatments, early-, peak-, and late-heated flowers produced significantly fewer ovules than unheated flowers (Table 6, Figure 11). These post-treatment flowers were present as buds during the heatwave. Reduced ovule number after high temperature plays a role in the lack of compensatory ability of post-damage flowers, and therefore the overall reduced yield in experimental groups.

Recently opened flowers exposed to both early and peak heatwaves produced significantly less fertilized ovules compared to the control (Table 7, Figure 12a,b), indicating that reduced fertilization is an important factor in the failure of flowers during heatwaves. Reduced fertilization resulted in 10x fewer early and 4x fewer peak mature seeds than control flowers at the same position. Flowers exposed to the late heatwave contained a higher number of fertilized ovules compared to the control, but seed abortion resulted in < 1 seed produced on average while control plants produced ~ 2 seeds. (Figure 12c).

I found that peak and late heatwaves cause abortion of seeds in previously initiated siliques. Siliques exposed to the peak heatwave experienced significantly more seed abortion relative to the control (Table 8, Figure 13a). Control plants matured nearly all fertilized seeds (Figure 13a). Siliques exposed to the late heatwave also experienced significant seed abortion with only approximately 10% of fertilized ovules maturing to seed on average (Table 8, Figure 13b). There was no significant difference in mean number of mature seeds between late and control plants (Figure 13b).

Flowers produced after the heatwave were not successful enough to compensate for heat-induced losses (Figure 14). Immediately after the heatwave, there were less fertilized ovules and mature seeds in the early and peak treatments compared to the control (Table 9, Figure 14a,b). Siliques produced after the late treatment contained the same number of fertilized ovules and mature seeds as control plants (Figure 14c). These post-treatment flowers were present as buds during the heatwave. Siliques at the tips of branches produced less mature seeds compared to the control regardless of branch position on the plant (Table 9, Figure 15). Siliques produced at the tips of branches were not present as buds during the heatwave.
Herbivory Experiment

Simulated floral herbivory had less impact on *Camelina*’s flowering schedule and seed yield. Plants damaged at peak flowering produced fewer flowers overall than control plants, while those damaged early and late did not differ from the control. The 3-day flower removal treatments did not lead to the production of new racemes. Although floral herbivory treatments did not result in compensatory growth and flower production, yield did not differ between herbivory and control treatments. This indicates that *Camelina sativa* is tolerant to short-term floral herbivory.

**Flowering Schedule**

Following first flowering, control plants increased flower production until reaching a peak at 15-20 days, then declined during seed maturation (Figure 16). In the early-damage treatment, all genetic lines immediately resumed flowering after the three-day attack period. Several genetic lines failed to initiate a compensatory response when the herbivory treatment occurred later in the season (Figure 16). The length of the flowering schedule significantly differed between treatments (Figure 17). The early-removal treatment flowered for a shorter length of time than the control, peak and late treatments. The peak treatment flowered for a significantly longer period relative to the control (Figure 17).

By counting the number of siliques produced we determined the total number of flowers produced by plants in each treatment (Figure 18). Including the flowers removed during the 3-day herbivory treatments, the total aggregate number of flowers produced did not differ between flower removal treatments and the control (Table 10, Figure 18a). This indicates that herbivore-like damage was insufficient to alter the plants default architectural pattern of flower production. When the analysis excludes flowers removed by herbivory, there is a significant effect of treatment on flower production (Table 10, Figure 18b). The peak flower removal treatment produced significantly fewer flowers compared to the control treatment, as there were many flowers removed at maximum flower production. There was a significant difference in flower production between genetic lines (Table 10, Figure 19). The interaction effect between treatment and genetic line was not significant (Table 10), indicating that in general, each genotype ranks similarly in flower production across all treatments.
A repeated measures GLMM was used to model the flower deployment schedule and analyze differences between the control and treatment plants after herbivory. Flower deployment after early and peak herbivory differed significantly from the control through time (Table 11). Flower deployment after late herbivory did not differ from the control (Table 11), with similar, low levels of flower deployment until the end of the experiment.

Taken together, the results of the GLMM and ANOVA for total flowers produced indicates that although the day in which the flowers were deployed may differ from the control, the overall number of total flowers produced appears to be fixed and no additional flowers are produced.

Treatments did not significantly differ in total number of primary or secondary branches (Table 12, Figure 20). There was a significant difference in primary and secondary branch production across genotypes (Table 12), reflecting the inherent genetic differences in branch number between the RILs. The 3-day flower removal treatments did not lead to the production of new branches, potentially because the damage inflicted was not so intense that the production of new racemes was necessary to compensate for the reproductive loss. The damage*genotype interaction was not significant.

**Yield**

Yield did not significantly differ between treatments (Table 10, Figure 21), indicating that all plants were able to fully compensate for reproductive losses that occurred during the flower removal treatments. Genotypes differed significantly in yield, reflecting the underlying genetic differences between RILs (Table 10, Figure 22). The interaction effect was not significant (Table 10). RIL 206 consistently produced the highest seed yield across treatments, while RIL 166 was the lowest producing genotype. To determine the quality of the yield, the seeds collected will be used in future germination trials.

**DISCUSSION**

Plant tolerance to floral damage can be conferred through compensatory growth (increasing post-damage flower production), or through increasing the post-damage success rate for a set number of flowers. I found that *Camelina sativa* responds in either way, depending on the type of damage inflicted. Plants damaged by heat were not fully tolerant, despite initiating compensatory responses such as extending the flowering period and increasing the number of inflorescences.
Reproduction declined because extreme heat caused fertilization failure in flowers opening during the heatwave, as well as seed abortion in previously fertilized flowers. Flowers produced after the heatwave ended were not able to compensate for reproductive losses. This resulted in reduced yield in heated plants compared to the unheated controls. In contrast, *C. sativa* tolerated short periods of floral herbivory by increasing resource allocation to post-damage fruits. The improved success of the late-produced flowers compensated for flowers lost to herbivory. The compensatory response did not involve the initiation of additional flowering branches, nor an extension of the flowering period length. Although genotypes differed from each other in total flower production, branching number and yield, emphasizing the inherent genetic differences between recombinant inbred lines and parental lines, there were no detectable differences in their responses to damage of either type.

High temperatures can cause female and male floral organs to fail, leading to reduced crop yield (Zinn *et al.*, 2010; Sage *et al.*, 2015). I observed both lower fertilization and higher seed abortion in flowers that opened during the heatwave. Failed fertilization can be the result of reduced pollen production, germination and viability (Zinn *et al.*, 2010; Prasad & Djanaguiraman, 2014; Sage *et al.*, 2015) or ovule development and viability (Cross *et al.*, 2003). Chronic exposure to 35°C significantly reduced pollen tube growth, ovule number and embryo sac development in *Camelina sativa* (Lundsgaard-Nielsen, 2017); these factors likely play a significant role in reduced yield observed in my research with *Camelina* too.

I found higher seed abortion in siliques that were maturing at the time of the heatwave compared to control plants. Yield reductions in response to heat stress has been studied in other plant species as well. For example, Cross *et al.* (2003) found seed abortion in flax flowers fertilized 2 days before the start of heat stress, leading to decreasing seed weight as the period of heat stress increased. Similar trends of reduced yield were also found in canola (Young *et al.*, 2004), bean (Gross & Kigel, 1994; Prasad *et al.*, 2002) and maize (Ruiz-Vera *et al.*, 2015).

I found post-treatment flowers contained fewer unfertilized ovules than those deployed pre-treatment, whereas on control, flowers produced during the corresponding time periods had equal numbers of ovules. A possible explanation for this observation may be that the initiation of additional secondary and tertiary branches skewed source-sink relationships, resulting in lower per flower resource allocation during the post-damage period (Diggle, 1995; Kliber & Eckert, 2004).
When heatwaves damage open flowers, resources become available to instead produce new branches with more resource-sinking flowers. Heatwaves also damage buds, inflorescence meristems and leaf tissue. We noticed damage to buds and meristems (Plate 5), reducing the potential for future reproduction, unless new sinks are made. Heat stress causes reduced net photosynthetic rate, CO₂ uptake rate and leaf water potential in leaves (Abeli et al., 2012), leading to diminished sources of photosynthate, impeding the production of additional resource sinks. Taken together, heat causes source-sink relationships to become unbalanced. Not only does heat diminish success of previously produced fruit and open flowers, but heat-stressed leaves further reduce the plant’s ability to acquire enough resources to adequately compensate during the recovery period.

I observed greater compensatory growth when plants were heated later in the flowering season because heat damages much of the previously created reproductive sinks. Plants heated later produced the highest number of flowers and branches and flowered for the longest time. Damage to siliques and branch meristems requires the initiation of entirely new branches and the production of a substantial number of new flowers. Heat damage resulted in a delay in reproduction as the plant acquires the necessary photosynthetic resources for regrowth. Damage at the end of the flowering period can be devastating for plants if they cannot respond before the growing season ends, reducing the opportunity for late-damaged plants to reproduce. Conversely, early heatwaves do not lead to as strong of a compensatory growth response as late season heatwaves. Though early flowers and tissues are damaged, matured siliques are not yet present. Damage to unpollinated flowers has a lower fitness cost compared to pollinated flowers and siliques; seeds are more valuable than unfertilized ovules in a new flower (McCall & Irwin, 2006). After early season heat damage, there is still time remaining to accumulate resources, recover and continue reproduction before the growing season ends.

Fruit production and maturation can be costly to plants because it uses resources that could otherwise be put towards future growth and reproduction (Avila-Sakar et al., 2001). However, when flowers are removed and therefore fail to produce fruit, resources are then freed, potentially allowing for enough regrowth and reproduction such that damage-induced losses are fully compensated (Avila-Sakar et al., 2001; Bajcz & Dummond, 2017a). Although we did not observe compensatory growth, we found no difference in seed weight across floral herbivory treatments, indicating full compensation through adjustment in resource allocation to subsequent seed set.
Bajcz and Dummond (2017b) found flower removal in lowbush blueberry led to greater seed yield and vegetative mass than plants with natural reproductive levels. Previous studies have found that reproductive traits like fruit set (Trueman & Turnbull, 1994), fruit weight (Daugaard, 1999), rate of fruit development (Maust et al., 1999), flower production rate (Hartemink et al., 2004), seed set (Gómez & Fuentes, 2001), and seed quality (Dong et al., 2005) increased following flower removal (Bajcz & Dummond, 2017a).

The use of a bet-hedging-like strategy was most apparent in plants damaged at maximum flowering. We found that the peak treatment produced significantly less total flowers compared to the undamaged control. Perhaps this is because there were many more flowers removed during maximum flower production compared to earlier or later in the flowering period. Despite the deficit in flower number, plants in the peak treatment still fully compensated in yield, indicating a strategy similar to bet-hedging was used. When resource sinks (flowers) are removed during maximum flower production, resources are then freed to invest in maturing fruit on subsequently produced flowers. Plants in the early and late treatments produced enough flowers to approximately equal flower production in the control treatment. At the start and end of the flowering period, few flowers are produced per day and the loss of these flowers may not have been great enough to result in a significant difference in aggregate flower production. In addition, the loss of less successful late-produced flowers may not have been a substantial reproductive loss. Therefore, the production of extra flowers may not have been necessary for compensation to occur, so early- and late-damaged plants instead invested in fruit maturation and deployed flowers at the same rate as control plants.

It is possible that floral herbivory over a three-day period was not long enough to sufficiently impact yield and incite compensatory growth responses. Flowers are a relatively inexpensive reproductive sink compared to seed bearing fruits (McCall & Irwin, 2006), lasting only for approximately <2 days in Camelina. Even when flowers are damaged, the potential for future reproduction is high because there are many buds available for deployment after damage, as well as lateral meristems to produce new inflorescences. Removing fruits, however, comes at a greater reproductive cost to the plant as more time and resources have been invested in fruit maturation and seed production, potentially leading to a more intense compensatory response. Avila-Sakar et al. (2001) found removal of immature Cucurbita pepo fruits freed resources, leading to faster growth and flower production. In addition, root, stem and leaf herbivory can reduce resource
acquisition and photosynthetic capabilities, altering source-sink relationships and the capacity to compensate (Lamb, 1989).

*Camelina* responds differently to heatwaves and herbivory because the extent of damage to plant tissues is greater during heat stress than floral herbivory. Heat events reduce yield by damaging previously produced fruit, open flowers at the time of the heatwave, flower buds, and photosynthetic tissues, necessitating the regrowth to ensure some reproduction. Alternatively, floral herbivory only damages the open flowers removed during the three-day damage period, while no damage was done to other plant tissues; the overall damage inflicted on the herbivorized plant was less intense than heatwaves. As a result, plants that experienced floral herbivory fully compensated, while heat-damaged plants undercompensated.

Another possible reason why *Camelina* tolerated simulated floral herbivory but not heat damage is the frequency that the two damage types strike in the historical environment, shaping the evolution of plant developmental programs. Members of the Brassicaceae family are frequently damaged by *Meligethes* pollen beetles and other floral herbivores (Lamb, 1989). A flower deployment schedule that ‘hedges the bet’ on insect attack – by wasting resources on late-deployed flowers when un-attacked so that resources are available when attacked – could reasonably increase the long-term geometric mean fitness. In an environment with frequent herbivore attacks, selection could favour a plant developmental program that adjusts source-sink relationships such that total flowers deployed becomes set as the plant reaches maturity, but flower maturation is conditional. When flower maturation is conditional, plants can selectively mature undamaged ‘extra’ fruit when herbivory occurs earlier in the flower deployment schedule. The high failure rate of late-season flowers seen in plants more generally (Austen *et al.*, 2015) could reflect a bet-hedging strategy not only against herbivory, but also fertilization failures. These ecological causes of flower failure occur frequently enough that the “insurance policy” provided by the extra flowers pays for itself over the long term.

In contrast, extreme heat events have been infrequent in recent geological history (Allen *et al.*, 2019). A bet-hedging strategy that optimizes between normal conditions and heatwaves might be possible, but perhaps at the expense of growth and reproduction under typical conditions. The cost of the ‘insurance policy’ could be so high, while the infrequent risk of heatwaves so low, that bet-hedging cannot invade the prevailing strategy. *Camelina* has evolved under both natural and artificial selection to maximize yield in its historic environments, requiring a developmental
program that balances source-sink interactions under the ordinary range of conditions. When the plant is exposed to extraordinary conditions like heatwaves, it appears the source-sink relationships become unbalanced; both sources and sinks are diminished for a period following the heat event. A pathological result – production of more flowers than are matured into siliques – may follow from such an imbalance.

In this experiment, we defined tolerance as damaged and control plants producing equal aggregate seed weight. However, this measure can overestimate true yield because it gives no indication of seed quality, measured by viability, germination, seedling survivorship (Stowe et al., 2000). We observed reduced appearance, smaller size and malformed shape of some heated seeds (Plate 6). Decreased germination rate, seed mass, seed vigor and weight per seed have been observed in plants exposed to high temperature (Joshi et al., 2016; Rashid et al., 2018; Sita et al., 2018). Herbivory can also effect seed quality, harvestability and oil content. Meligethes pollen beetle damage can delay maturation and cause the crop to mature unevenly (Lamb, 1989). As a result, the growing season may be extended, risking frost damage, and yield may be lost as mature pods drop seed before harvest (Lamb, 1989). Since oil makes up ~40% of seed weight in oilseed crops (Lamb, 1989) and is commercially important, determining seed quality is an important next step in the completion of this experiment. We plan to do this by weighing individual seeds and performing germination trials.

As climate extremes become longer, more frequent and more intense (Meehl & Tebaldi, 2004), agronomists will be challenged to breed lines that can maintain yield. The fact that the genotypes used in this study showed no yield difference in response to heat damage does not bode well for plant breeding. There does not seem to be genetic variation in the direction one would wish to select to maintain yield during high temperatures. Future research should investigate heat tolerance in additional Camelina cultivars and their RILs to determine if there is genetic variation to maintain yield when heat stressed.
Tables

Heatwave Experiment

**Table 1** – Effects of heat damage and genotype on silique production during the Pre- and Post-damage periods. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Pre- and Post-damage periods also differed. This necessitated three different GLMM analyses, each comparing one of the damage treatments to a single control group.

<table>
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<th>Damaged Early</th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
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<td><strong>F-value</strong></td>
<td><strong>F-value</strong></td>
<td><strong>F-value</strong></td>
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<tr>
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<td>112.1***</td>
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<td><strong>Error DF</strong></td>
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*P<0.05; **P<0.01; ***P<0.001
Table 2 – Effects of heat damage and genotype on the length of the flowering period, total flower production and yield.

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<th>Length of Flowering Period</th>
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<th>Yield</th>
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*P<0.05; **P<0.01; ***P<0.001
Table 3 – Effects of heat damage, genotype, and date in the flowering period on flower deployment after the heat treatment. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of Post-damage periods differed. This necessitated three different GLMM analyses, each comparing one of the damage treatments to a single control group.

\[
glmer(\text{Flowers} \sim \text{Damage*Genotype*Day} + (1|\text{Day}) + (\text{Day}|\text{Plant}), \text{family}=\text{poisson(link=\"log\")})
\]

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th></th>
<th>Damaged at Peak</th>
<th></th>
<th>Damaged Late</th>
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<td>739.3***</td>
<td>10</td>
<td>22.6*</td>
</tr>
<tr>
<td>Date:Genotype</td>
<td>60</td>
<td>137.5***</td>
<td>64</td>
<td>281.9***</td>
<td>61</td>
<td>158***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>5</td>
<td>20.2**</td>
<td>24</td>
<td>124***</td>
<td>6</td>
<td>17.8**</td>
</tr>
<tr>
<td>Damage:Date:Genotype</td>
<td>60</td>
<td>179.5***</td>
<td>45</td>
<td>75.8**</td>
<td>45</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
**Table 4**– Effects of heat damage and genotype on the number of primary, secondary and tertiary branches produced.

<table>
<thead>
<tr>
<th></th>
<th>Primary Branch</th>
<th>Secondary Branch</th>
<th>Tertiary Branch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
<td>DF</td>
</tr>
<tr>
<td>Damage</td>
<td>3</td>
<td>1.3</td>
<td>3</td>
</tr>
<tr>
<td>Genotype</td>
<td>5</td>
<td>15.1***</td>
<td>5</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>15</td>
<td>1.2</td>
<td>15</td>
</tr>
<tr>
<td>Residuals</td>
<td>312</td>
<td>296</td>
<td>136</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table 5—Effects of heat damage and genotype on seed yield during the Pre- and Post-damage periods. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Pre- and Post-damage periods also differed. This necessitated three different GLMM analyses, each comparing one of the damage treatments to a single control group.

\[
\text{lmer(Yield ~ Damage*Genotype*Pre/Post + (1|Temporal\_Block))}
\]

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>1</td>
<td>26.5***</td>
<td>32.5***</td>
</tr>
<tr>
<td>Genotype</td>
<td>5</td>
<td>9.1***</td>
<td>9.3***</td>
</tr>
<tr>
<td>Pre/Post-Damage</td>
<td>2</td>
<td>177.1***</td>
<td>60.1***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>5</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Damage:Pre/Post</td>
<td>2</td>
<td>128.7***</td>
<td>288.8***</td>
</tr>
<tr>
<td>Genotype:Pre/Post</td>
<td>10</td>
<td>18.4***</td>
<td>6.3***</td>
</tr>
<tr>
<td>Damage:Pre/Post:Genotype</td>
<td>10</td>
<td>2.8**</td>
<td>6.1***</td>
</tr>
</tbody>
</table>

Error DF 396 395 467

*P<0.05; **P<0.01; ***P<0.001
Tables 6 – Effects of heat damage and position along the branch on the number of initial ovules produced Pre- and Post-damage periods. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Pre- and Post-damage periods also differed. This necessitated three different two-way ANOVA analyses, each comparing one of the damage treatments to a single control group.

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
<td>DF</td>
</tr>
<tr>
<td>Damage</td>
<td>1</td>
<td>32.6***</td>
<td>1</td>
</tr>
<tr>
<td>Pre/Post</td>
<td>1</td>
<td>12.1***</td>
<td>1</td>
</tr>
<tr>
<td>Damage:Pre/Post</td>
<td>1</td>
<td>6.6*</td>
<td>1</td>
</tr>
<tr>
<td>Residuals</td>
<td>144</td>
<td>185</td>
<td>159</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table 7 – Effects of heat damage and silique component on the number of initial ovules, fertilized ovules and mature seeds produced in flowers that opened during the heatwave. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Pre-damage periods also differed. This necessitated three different two-way ANOVA analyses, each comparing one of the damage treatments to a single control group.

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>$F$-value</td>
<td>DF</td>
</tr>
<tr>
<td>Damage</td>
<td>1</td>
<td>86.1***</td>
<td>1</td>
</tr>
<tr>
<td>Silique Component</td>
<td>2</td>
<td>114.1***</td>
<td>2</td>
</tr>
<tr>
<td>Damage:Silique</td>
<td>2</td>
<td>19.6***</td>
<td>2</td>
</tr>
<tr>
<td>Component</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residuals</td>
<td>45</td>
<td></td>
<td>48</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table 8 – Effects of heat damage and silique component on the number of initial ovules, fertilized ovules and mature seeds produced in siliques maturing during heatwaves. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Pre-damage periods also differed. This necessitated three different two-way ANOVA analyses, each comparing one of the damage treatments to a single control group.

<table>
<thead>
<tr>
<th></th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
</tr>
<tr>
<td>Damage</td>
<td>1</td>
<td>15.6***</td>
</tr>
<tr>
<td>Silique Component</td>
<td>2</td>
<td>188.4***</td>
</tr>
<tr>
<td>Damage:Silique Component</td>
<td>2</td>
<td>5.9**</td>
</tr>
<tr>
<td>Residuals</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table 9 – Effects of heat damage and silique component on the number of initial ovules, fertilized ovules and mature seeds produced in flowers that opened after heatwaves. Heat damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of the Post-damage periods also differed. This necessitated four different two-way ANOVA analyses, each comparing one of the damage treatments to a single control group.

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th>Damaged at Peak</th>
<th>Damaged Late</th>
<th>Tip of Branch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
<td>DF</td>
<td>F-value</td>
</tr>
<tr>
<td>Damage</td>
<td>1</td>
<td>308.6***</td>
<td>1</td>
<td>259.7***</td>
</tr>
<tr>
<td>Silique Component</td>
<td>2</td>
<td>200.3***</td>
<td>2</td>
<td>288.0***</td>
</tr>
<tr>
<td>Damage:Silique Component</td>
<td>2</td>
<td>24.1***</td>
<td>2</td>
<td>10.9***</td>
</tr>
<tr>
<td>Residuals</td>
<td>192</td>
<td>183</td>
<td>81</td>
<td>1362</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Herbivory Experiment

Table 10 – Effects of herbivore damage and genotype on the length of the flowering period, total flower production and yield.

<table>
<thead>
<tr>
<th></th>
<th>Length of Flowering Period</th>
<th>Lifetime Flower Production ∨</th>
<th>Total Flower Production •</th>
<th>Yield (seed weight(g))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
<td>DF</td>
<td>F-value</td>
</tr>
<tr>
<td>Damage</td>
<td>3</td>
<td>11.3***</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>Genotype</td>
<td>13</td>
<td>6.9***</td>
<td>13</td>
<td>16.9***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>39</td>
<td>0.9</td>
<td>39</td>
<td>0.8</td>
</tr>
<tr>
<td>Residuals</td>
<td>781</td>
<td>607</td>
<td>607</td>
<td>607</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001

∨ including flowers removed during herbivory treatments

• excluding flowers removed during herbivory treatments
Table 11 – Effects of herbivore damage, genotype, and date in the flowering period on flower deployment after damage. Herbivore damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of Post-damage periods differed. This necessitated three different GLMM analyses, each comparing one of the damage treatments to a single control group.

\[
glmer(\text{Flowers} \sim \text{Damage*Genotype*Day} + (1|\text{Day}) + (\text{Day}|\text{Plant}), \text{family=poisson(link="log")))
\]

<table>
<thead>
<tr>
<th></th>
<th>Damaged Early</th>
<th>Damaged Peak</th>
<th>Damaged Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>17 167.3***</td>
<td>5 16.5**</td>
<td>13 52.2***</td>
</tr>
<tr>
<td>Damage</td>
<td>5 16.6**</td>
<td>1 2.6</td>
<td>11 20.9*</td>
</tr>
<tr>
<td>Genotype</td>
<td>12 67.2***</td>
<td>5 42.5***</td>
<td>19 64.1***</td>
</tr>
<tr>
<td>Date:Damage</td>
<td>10 32.1***</td>
<td>5 13.7*</td>
<td>5 5.4</td>
</tr>
<tr>
<td>Date:Genotype</td>
<td>42 154.3***</td>
<td>25 74.2***</td>
<td>13 46.9***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>7 7.8</td>
<td>5 12.2*</td>
<td>8 19.2*</td>
</tr>
<tr>
<td>Damage:Date:Genotype</td>
<td>33 81.6***</td>
<td>19 50.6***</td>
<td>8 2.9</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table 12 – Effects of herbivore damage and genotype on the number of primary, secondary and tertiary branches produced.

<table>
<thead>
<tr>
<th></th>
<th>Primary Branches</th>
<th></th>
<th>Secondary Branches</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>F-value</td>
<td>DF</td>
<td>F-value</td>
</tr>
<tr>
<td>Damage</td>
<td>3</td>
<td>0.8</td>
<td>3</td>
<td>1.4</td>
</tr>
<tr>
<td>Genotype</td>
<td>13</td>
<td>10.6***</td>
<td>13</td>
<td>2.5**</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>39</td>
<td>0.8</td>
<td>39</td>
<td>0.9</td>
</tr>
<tr>
<td>Residuals</td>
<td>607</td>
<td></td>
<td>227</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Plate 1 – Colour-coated string tied around the newest cluster of buds to mark the start of the heatwave treatment.
Plate 2 – Examples of A) open flowers and B) closed flowers. Open flowers were counted because they are still receptive to fertilization. Closed flowers show signs of fertilization, such as the early stage of silique formation.
Plate 3 – Silique dissection in the heatwave experiment. The ovules, aborted seeds and successful mature seeds are labeled. The number of funiculi indicate the total number of ovules produced.

Unfertilized ovules are smallest and white, often still attached to a funiculus. Small orange structures are fertilized ovules that did not progress through seed maturation. Mature seeds are largest and orange-brown.
Plate 4 – Randomized layout of herbivory blocks (racks). Each labelled plant number represents one of the 14 genetic lines. Position of the genetic line was randomized in each block. All plants in each block belong to the same treatment.
**Plate 5** – Aborted buds during heat stress. A) Buds damaged by high temperature appear pale yellow and dry (red arrow), and eventually fall off the plant leaving the pistil and pedicel behind (orange arrow). B) Image depicting aborted buds and post treatment flowering. The aborted buds can represent the lag experienced after heatwaves as plants produce undamaged buds.
Plate 6 – Variation in the shape and size of seeds collected from heat stressed plants.
Figure 1 – Branch classification. Branches were classified as primary, secondary and tertiary, as indicated in the diagram. The main stem also produces flowers at the tip.
Heatwave Experiment

**Figure 2** – Collected silique positioning along branches. The illustrated strings tied around each branch indicate the start and end of each heatwave. The numbers above each branch indicate the position that individual siliques were collected, with 1 – base of the branch, 2 - start of the early heatwave, 3 - end of the early heatwave, 4 – start of the peak heatwave, 5 – end of the peak heatwave, 6 – start of the late heatwave, 7 – end of the late heatwave and 8 – tip of the branch.
Figure 3 – Average flower deployment through time for each treatment. The vertical rectangular blocks on each graph indicate the period on the x-axis when heatwaves occurred. The coloured lines indicate the mean flower production per day for each genetic line used, with colours corresponding to the genetic lines indicated on legend at the bottom of the figure.
Figure 4 – Average number of flowers (A, C, E) and yield (B, D, F) produced in the experimental and control treatments before, during and after the heatwave. The treatment is denoted by the colour of the bars, corresponding to the legend to the right of each figure. The error bars represent the standard error.
Figure 5 – Average length of the flowering season for each treatment. The treatment is denoted by the colour of the boxplots, corresponding to the labels on the x-axis.
Figure 6 – Average aggregate number of flowers produced per plant in each treatment. Treatment is indicated on the x-axis. The error bars represent the standard error.
**Figure 7** – Average aggregate number of total flowers produced per plant in each treatment and genotype. Treatment is indicated on the x-axis and is represented by a differently shaded background colour. The genotypes correspond to the legend on the right side of the figure. The error bars represent the standard error.
Figure 8 – Average number of primary, secondary, and tertiary branches produced by plants in each treatment. The treatment corresponds with the legend to the right. The error bars represent the standard error.
**Figure 9** – Average aggregate seed yield produced per plant in each treatment. Yield is measured in aggregate seed weight (g). Treatment is indicated on the x-axis. The error bars represent the standard error.
Figure 10 – Average aggregate seed yield per plant in each treatment and genotype. Yield is measured in aggregate seed weight (g). Treatment is indicated on the x-axis and is represented by a differently shaded background colour. The genotypes correspond to the legend on the right side of the figure. The error bars represent the standard error.
Figure 11 – Average number of initial ovules produced before and after the A) early, B) peak, and C) late heated treatments. The error bars represent the standard error.
Figure 12 – Average number of initial ovules, fertilized ovules and mature seeds in flowers that opened immediately before the A) early, B) peak and C) late heatwaves. The decline between initial ovules and fertilized ovules indicates failed fertilization. The decline between fertilized ovules and mature seeds indicates seed abortion. The error bars represent the standard error.
Figure 13 – Average number of initial ovules, fertilized ovules and mature seeds in siliques maturing during the A) peak and B) late heated treatments. In the early-damaged treatment, there were no maturing siliques at this stage to collect. The decline between initial ovules and fertilized ovules indicates failed fertilization. The decline between fertilized ovules and mature seeds indicates seed abortion. The error bars represent the standard error.
Figure 14 – Average number of initial ovules, fertilized ovules and mature seeds in flowers produced immediately after the A) early B) peak and C) late heated treatments. The decline between initial ovules and fertilized ovules indicates failed fertilization. The decline between fertilized ovules and mature seeds indicates seed abortion. The error bars represent the standard error.
Figure 15 - Average number of initial ovules, fertilized ovules and mature seeds in flowers produced at the tips of racemes after the heat treatments. The decline between initial ovules and fertilized ovules indicates failed fertilization. The decline between fertilized ovules and mature seeds indicates seed abortion. The error bars represent the standard error.
Herbivory Experiment

**Figure 16** – Average flower deployment through time for each treatment. The vertical rectangular blocks on each graph indicate the period on the x-axis when herbivory occurred. The coloured lines indicate the response of each genetic line used, with colours corresponding to the genetic lines indicated on legend at the bottom of the figure. The 6 genetic lines that were also used in the heatwave experiment are in colour, while the 8 other genetic lines are in greyscale.
**Figure 17** – Average length of the flowering season for each treatment. The treatment is denoted by the colour of the boxplots, corresponding to the labels to the x-axis.
Figure 18 – Average aggregate number of A) total flowers produced B) remaining flowers and C) removed flowers per plant during the herbivory treatments. The colour of each bar represents a different herbivory treatment, as indicated on the x-axis. Error bars show the standard error.
Figure 19 – Average aggregate number of total flowers produced per plant in each treatment and genotype. Treatment is indicated on the x-axis and is represented by a differently shaded background colour. The genotypes correspond to the legend on the right side of the figure. This figure does not include flowers removed during the herbivory treatment. Coloured boxplots represent each genetic line, with colours corresponding to the legend on the right.
Figure 20 – Average number of primary, secondary, and tertiary branches produced by plants in each treatment. The treatment corresponds with the legend to the right. The error bars represent the standard error.
Figure 21 — Average aggregate seed yield per plant in each treatment. Yield is measured in aggregate seed weight (g). Treatment is indicated on the x-axis. The error bars represent the standard error.
Figure 22- Average aggregate seed yield per plant in each treatment and genotype. Yield is measured in aggregate seed weight (g). Treatment is indicated on the x-axis and is represented by a differently shaded background colour. The genotypes correspond to the legend on the right side of the figure. The error bars represent the standard error.
References


Tayo, T. O., & Morgan, D. G. (1979). Factors influencing flower and pod development in oil-


Appendix

Flower deployment was similar between control and experimental groups before heat or herbivory treatments occurred. Differences observed (damage*day) were likely because plants did not begin flowering on the same date; staggered flowering leads to differences in flower deployment (Figure 3, Figure 16). Below are the summary results of the GLMM models for damage, genotype and date before each treatment.

Heatwave Experiment

Table A – 1 – Effects of heat damage, genotype, and date in the flowering period on flower deployment before the early heat treatment.

<table>
<thead>
<tr>
<th></th>
<th>First Temporal Block</th>
<th>Second Temporal Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>Chisq</td>
</tr>
<tr>
<td>Damage</td>
<td>4</td>
<td>618.5***</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>124.4***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>4</td>
<td>6.9</td>
</tr>
<tr>
<td>Damage:Day</td>
<td>7</td>
<td>18.4*</td>
</tr>
<tr>
<td>Genotype:Day</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>Damage:Day:Genotype</td>
<td>5</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table A – 2 – Effects of heat damage, genotype, and date in the flowering period on flower deployment before the peak heat treatment.

<table>
<thead>
<tr>
<th></th>
<th>First Temporal Block</th>
<th>Second Temporal Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>Chisq</td>
</tr>
<tr>
<td>Damage</td>
<td>7</td>
<td>801.8***</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>110.4***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>7</td>
<td>11.8</td>
</tr>
<tr>
<td>Damage:Day</td>
<td>19</td>
<td>236.1***</td>
</tr>
<tr>
<td>Genotype:Day</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Damage:Day:Genotype</td>
<td>15</td>
<td>15.7</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Table A – 3 – Effects of heat damage, genotype, and date in the flowering period on flower deployment before the late heat treatment.

<table>
<thead>
<tr>
<th></th>
<th>First Temporal Block</th>
<th></th>
<th>Second Temporal Block</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>Chisq</td>
<td>DF</td>
<td>Chisq</td>
</tr>
<tr>
<td>Damage</td>
<td>11</td>
<td>1039.2***</td>
<td>48</td>
<td>3034.2***</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>15.2***</td>
<td>29</td>
<td>69.7*</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>14.8*</td>
<td>49</td>
<td>280.3***</td>
</tr>
<tr>
<td>Damage:Genotype</td>
<td>11</td>
<td>190.8***</td>
<td>24</td>
<td>59.1*</td>
</tr>
<tr>
<td>Damage:Day</td>
<td>40</td>
<td>596.3***</td>
<td>44</td>
<td>202.8***</td>
</tr>
<tr>
<td>Genotype:Day</td>
<td>5</td>
<td>92.0***</td>
<td>24</td>
<td>42.2</td>
</tr>
<tr>
<td>Damage:Day:Genotype</td>
<td>31</td>
<td>90.2***</td>
<td>25</td>
<td>32.8</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001
Herbivory Experiment

Table A – 4 – Effects of herbivory damage, genotype, and date in the flowering period on flower deployment before the herbivory treatments. Damage was inflicted early in the flowering period, during peak flowering, and late in flowering. For this reason, the dates of Pre-damage periods differed. This necessitated three different GLMM analyses, each comparing one of the damage treatments to a single control group.

<table>
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<th>Damaged Early</th>
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<th>Damaged at Peak</th>
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<th>Damaged Late</th>
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<td>468.4***</td>
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<td>5.7*</td>
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*P<0.05; **P<0.01; ***P<0.001