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Heat tolerance plays an important role in regulating remontant flowering in an F_1 population of octoploid strawberry *(Fragaria×ananassa)*

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Abstract.
BACKGROUND: Flower initiation in strawberry is often classified by photoperiod sensitivity; however, temperature also plays a major role in determining flower initiation.
OBJECTIVE: Our goal was to determine the role heat tolerance plays in regulating remontant flowering in a segregating population of strawberry, *Fragaria×ananassa.*
METHODS: Non-remontant (short day)'Honeoye' and remontant 'Tribute' were crossed and 54 progeny were grown in three temperature regimes (17, 20, and 23°C) under a long photoperiod in the greenhouse and differences in flower and runner formation among the progeny were compared. In addition, clonally replicated individuals of the same family were grown in the field in Michigan and Oregon, so that the extent of heat tolerance observed for each genotype in the greenhouse studies could be compared to their phenotype in the field (remontant vs. non-remontant).
RESULTS: A significant Genotype x Environment interaction was observed in the greenhouse studies, indicating that there was a strong genetic component regulating the response of the individuals to increasing temperature. The level of heat tolerance, as defined as the difference in flower numbers at 23°C vs. 17°C,showed a continuous distribution among the progeny, indicating polygenic control. The majority of the genotypes that were remontant in the field produced more flowers at 23°C than at 17°C in the greenhouse trials. Flower initiation in both the parents was reduced at 23°C, but 'Tribute' produced significantly more flowers than 'Honeoye' at 23°C (48.0 vs. 11.3). Most remontant progeny had few runners, although there were some notable exceptions.
CONCLUSIONS: Temperature tolerance plays an important role in the flower and runner initiation of remontant genotypes. Genotypes with high heat tolerance can be selected that will more dependably flower in environments with highly variable levels of summer heat.

Keywords: Recurrent flowering, remontancy, heat tolerance, flowering, day neutral, photoperiod

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1. Introduction

The flowering response of strawberries (*Fragaria×ananassa* Duchesne ex Rozier) is often classified as short day (SD), day neutral (DN) or long day (LD). However, the photoperiodic response of strawberries is complicated by the effect of temperature and, infact; the photoperiodic response can change with the temperature. Flower induction of SD types can occur under any photoperiod if the temperature is cool enough, generally < 15°C [1]. Nishiyama and Kanahama [2] and Sønsteby and Heide [3, 4] demonstrated that a number of cultivars were DN at low temperature (<12°C), quantitative LD at intermediate temperatures (15–21°C) and qualitative (obligate) LD at higher temperatures (>24°C). Bradford et al. [5] demonstrated that 'Tribute', which has traditionally been referred to as DN, was quantitative LD at all temperatures. Sønsteby and Heide [6] found a *F. virginiana* ssp. *glauca* population from the Wasatch Mountains in Utah to be truly DN across a wide temperature range, while other populations of *F. virginiana* ssp.*glauca* and *F. virginiana* ssp.*virginiana* behaved as quantitative SD plants.

Inhibition of flowering can also occur at high temperature under all photoperiods [7–10]. Wagstaffe and Battey [11, 12] found that the optimum temperature for flowering and fruiting in recurrent flowering 'Everest'was about 23°C, while temperatures above 26°C were inhibitory. They referred to this phenomena as thermodormancy. Karapatzak et al. [13] did not find such a strong inhibition of flowering at high temperatures in another study of 'Everest' and 'Diamante', but they did find pollen germination to be greatly inhibited at high temperatures (30/20°C). Bradford et al. [5] found the threshold temperature for floral inhibition to be 23°C in SD 'Honeoye' and 26°C in DN 'Tribute' and a wild SD selection of *F. virginiana*. Sønsteby and Heide [6] found that flowering in populations of *F. virginiana* ssp. *virginiana* was less affected by higher temperatures (27°C) than populations of *F. virginiana* ssp. *glauca*.

Because photoperiod response is so dependent on temperature, referring to a strawberry cultivar as DN is not an accurate representation of their environmental physiology. More precise terms that have been used include: 1) perpetual flowering or recurring flowering vs. seasonal flowering [3, 4, 14] and 2) remontant vs. non-remontant [5, 15]. We will use the terms remontant and non-remontant in this paper, to include those genotypes that are perpetually flowering over a long period of time and those that have multiple flowering cycles.

Runner formation is also photoperiod and temperature sensitive, with the highest number of runners generally being produced under long days and higher temperatures, with considerable variation among genotypes in the level of response [8, 16, 17]. Serçe and Hancock [10] found the remontant genotypes, 'Aromas', 'Tribute', Frederick 9, and 'Fort Laramie', did not form any runners, even under long days. Other genotypes such as CFRA0368 and 'Quinault' formed runners only under short days, while LH50-4 and RH30 formed significantly more runners under long days than under short days. Genotypes also varied in their levels of runner production at different temperatures. While some genotypes (LH40-4, RH23, and RH45) did not form any runners at all, others (LH50-4) formed runners at all temperatures; 'Tribute' produced significantly more runners under the higher temperatures. In the study by Bradford et al. [5]. 'Honeoye' produced no runners and 'Tribute' produced only a few runners under short days. However, the number of runners significantly increased with increasing temperatures under long days. 'Honeoye' had the significantly highest number of runners at 26°C and 'Tribute' had the most runners at 23°C and 16 hr photoperiod.

Genetic control of remontancy in *Fragaria* × *ananassa* has long been debated and several hypotheses have been proposed [18, 19], including single dominant gene [20, 21], single major gene with modifier genes [22], dominant complementary genes [23], and multiple gene control [24, 25]. Gaston et al. [26]identified a single major QTL (*FaPFRU*) that regulated the balance between flowering and runnering. Mezzetti et al. [27] found the *DefH9-iaaM* auxin-synthesizing gene increased number of flowers/fruits per inflorescence and increased number of inflorescences per plant in transgenic strawberry. Weebadde et al. [28] identified a number of quantitative trait loci (QTL) associated with repeat flowering in a segregating population of non-remontant 'Honeoye' × remontant 'Tribute' grown at five locations.

In the study described herein, the permissive and inhibitive temperatures identified by Bradford et al. [5] for flowering in 'Honeoye' were maintained in greenhouses to determine the importance of heat tolerance in regulating remontancy in an F_1 family of the same cross examined by Weebadde et al. [28]. This allowed us to directly determine the effects of high temperature on remontancy across the segregating individuals of this population. All previous studies of the genetics of remontancy were conducted in the field, making it difficult to separate the individual effects of photoperiod and temperature. Replicates of these progeny (produced by runners) were also grown in the field

in Michigan and Oregon to determine how a genotype's heat tolerance was related to field performance. As will be described below, we found that floral heat tolerance plays a critical role in regulating repeat flowering, and the genetic control of this phenotype is likely polygenic. The most heat tolerant genotypes identified in this study should be useful in breeding new cultivars better adapted to climates with highly variable levels of summer heat.

2. Material and methods

2.1. Selection of the segregating population

Pollen of remontant 'Tribute' was placed on emasculated flowers of non-remontant 'Honeoye' and the resulting seed was germinated to produce a family of 54 individuals. 'Tribute' and 'Honeoye' are highly heterozygous, asexually propagated cultivars that were acquired from a commercial nursery (Krohne Plant Farms, Hartford, MI). Propagules (rooted runners) of these same 54 individuals were used in the greenhouse and field studies. Replicates of each hybrid seedling were propagated by rooting runners in the greenhouse in 10 cm pots containing BACCTO High Porosity Professional Potting Mix (Michigan Peat Company, Houston, TX). After 4 weeks, when the roots of the runners were firmly established, the runners were disconnected from the parent plant.

2.2. Maintenance of plants in the greenhouse

The rooted runners were transferred to 3.8 L pots filled with the same potting mix. Three replicates of each genotype were grown in a completely randomized design in temperature controlled greenhouses at 17°C, 20°C, and 23°C, and under 16 hr photoperiod using supplemental lights (400 W high-pressure sodium lamps- P.L. Light Systems Inc., Beamsville, ON, Canada). The plants were watered using distilled water injected with 125 ppm constant feed (14–3-14–7 Ca-1 Mg MSU RO Water Special, Greencare Fertilizers Inc., Kankakee, IL). Predator mites (SPIDEX, Koppert Biological Systems, Howell, MI), Volck (petroleum oil), sulphur, Terraguard (triflumizole), and Floramite (bifenazate) were used for pest and disease control when necessary.

The plants were grown in greenhouses under 16 hr photoperiods to ensure that the differences in flowering responses were an effect of temperature and not due to inductive photoperiod. The selection of the three temperature levels was based on the study by Bradford et al. [5] where they observed that 17°C was conducive to flower formation under all photoperiods, 20°C was the critical temperature beyond which flowering was photoperiod dependent, and 23°C was the lowest temperature at which flower formation was inhibited.

2.3. Maintenance of the plants in the field

The same set of genotypes (clonally propagated by runners) was planted in the field with two replicates of each planted next to each other in MI (Southwest Michigan Research and Extension Center, Benton Harbor) on 12 Aug. 2010 and in OR (Oregon State University Vegetable Farm, Corvallis) on 16 Aug. 2010. The plants were set at a spacing of 0.9 m by 0.9 m between plants at both locations. These plants were part of a larger block that included 960 genotypes of wild selections of *Fragaria virginiana* and *F. chiloensis*, cultivars of F. × *ananassa* and other families of F. × *ananassa* [29].

2.4. Phenotypic observations in the greenhouse

The plants were maintained under treatment conditions for 45 d before data were collected to ensure that all phenotypic observations were a result of the treatment conditions, and not an effect of prior growing conditions. The total number of flowers, inflorescences, and runners were counted every week from 1 Dec. 2010 to 30 Mar. 2011. All open flowers and runners were removed after counting every week. All dead leaves present at the time of data collection were removed from the plant.

2.5. Phenotypic observations in the field

Presence/absence of flowers was recorded every week from 1 May 2011 through 15 Aug. 2011. Progeny that flowered in the spring (May-Jul) and in the long days of summer after 23 Jul. were categorized as remontant. Progeny that flowered only in the spring (May- Jul. 22) before the longest day of the year were categorized as non-remontant. A cutoff date of 23 Jul. was used to separate non-remontant from remontant plants, rather than the longest day of the year (June 22), to allow any flower buds that were induced before the longest day to complete flowering.

2.6. Data collection and analysis

The greenhouse experiment employed a split plot design with temperature as the main plot and photoperiod as the subplot, with genotypes randomly assigned within each subplot. The ANOVA analysis was done with R 2.1.2.2 [30]. Frequency distributions were generated of the number of 'Honeoye' × 'Tribute' progeny with different numbers of flowers and runners at 17, 20, 23 and 17–23°C. Whether the progeny were RT or non-RT at the field locations was compared with their ability to produce flowers under high temperature in the greenhouse. Graphs were plotted using Microsoft Excel (Redmond, WA). The field experiment was not replicated within location so an ANOVA could not be conducted on this data.

3. Results and discussion

3.1. Segregation for floral heat tolerance and remontancy

There was a significant effect of temperature ($F = 14.158$, $df = 2$; $P < 0.01$), genotype ($F = 35.755$, $df = 55$; $P < 0.01$), and genotype × temperature ($F = 2.7604$, $df = 110$, $P < 0.01$) on the total number of flowers in the greenhouse. Both of the parents had fewer flowers at 23°C than 17°C, although 'Tribute' produced more flowers than 'Honeoye' at 23°C (48.0 vs. 11.3).

The significant G × E interaction indicates that there was a strong genetic component regulating the response of the individuals to increasing temperature. At all temperatures, there was a wide range in flower production across the genotypes (Fig. 1). A number of genotypes did not flower at the highest temperature, but among those that did, the level of heat tolerance varied continuously, as measured by total number of flowers produced at 17 vs. 23°C (Fig. 2). This continuous distribution indicates that genetic control of heat tolerance is likely polygenic.

Overall, 54% of the genotypes in the field were remontant in Michigan and 51% in OR. Twenty-one genotypes were remontant at both locations, while five were remontant in OR and non-remontant in MI, and seven were remontant in MI and non-remontant in OR. Maximum temperatures from Jun to Aug averaged 23°C in Oregon and 27°C in Michigan, both above the threshold temperature for floral induction observed by Bradford [5] for short day plants. The majority of the remontant genotypes at both locations were also heat tolerant in the greenhouse screens, although there were exceptions (Fig. 2).This indicates that while floral heat tolerance plays an important role in determining remontancy in the field, there may be other factors. It may be that not only maximum daily temperature, but also the amplitude of temperature change, plays a role in the expression of remontancy. Wagstaff and Battey [11, 12] has observed that cool night temperatures (13°C) can offset the negative effects of high temperatures during the day (>26°C) on floral initiation.

3.2. Segregation for runner production

There was a significant effect of temperature ($F = 384.17$, $df = 2$; $P < 0.001$), genotype ($F = 15.384$ $df = 55$; $P < 0.001$), and genotype × temperature ($F = 7.75$, $df = 110$, $P < 0.001$) on number of runners (Fig. 3). This significant G × E interaction indicates that there was a strong genetic component regulating the runnering response of genotypes to increasing temperature. Sønsteby and Heide [6] also found a significant temperature × population interaction in runner formation of *F. virginiana*, as did Serçe and Hancock [10] in the three octoploid species.

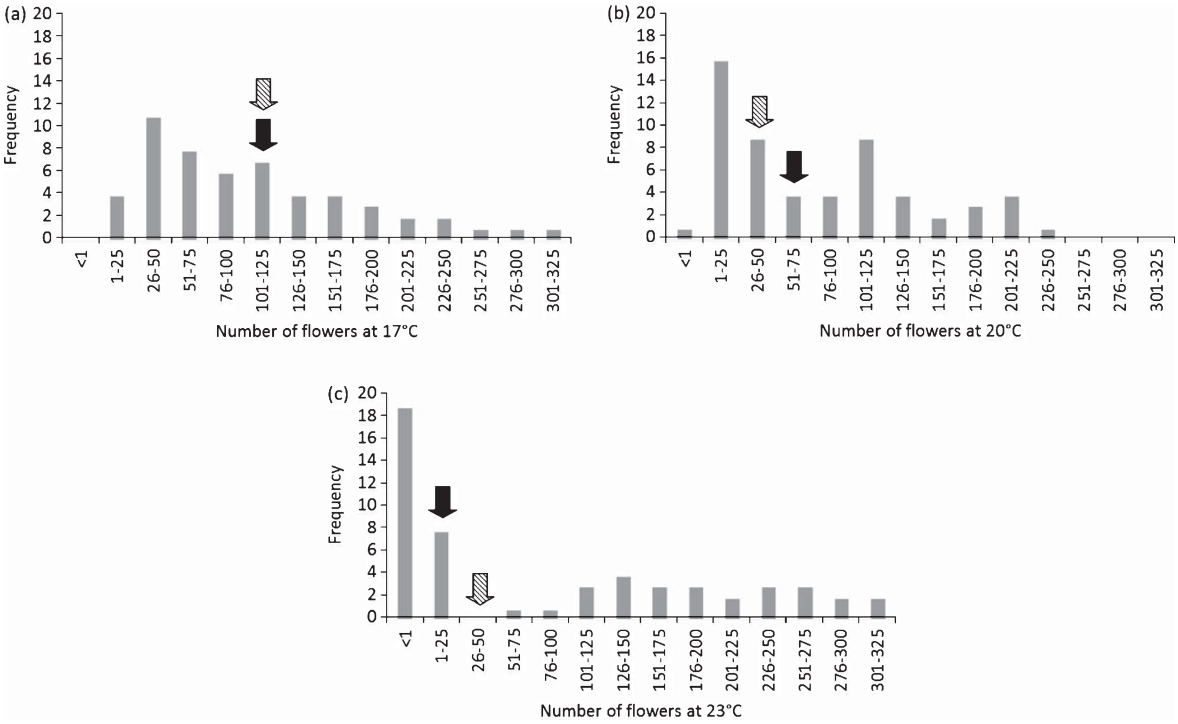

Fig. 1. a-c. Frequency distribution of progeny with different numbers of flowers in the 'Honeoye' × 'Tribute' population. (a) Distribution of total flowers at 17°C, (b) Distribution of total flowers at 20°C, (c) Distribution of total flowers at 23°C. Number of flowers in the parents are indicated: Honeoye: Dark arrow, Tribute: Shaded arrow.

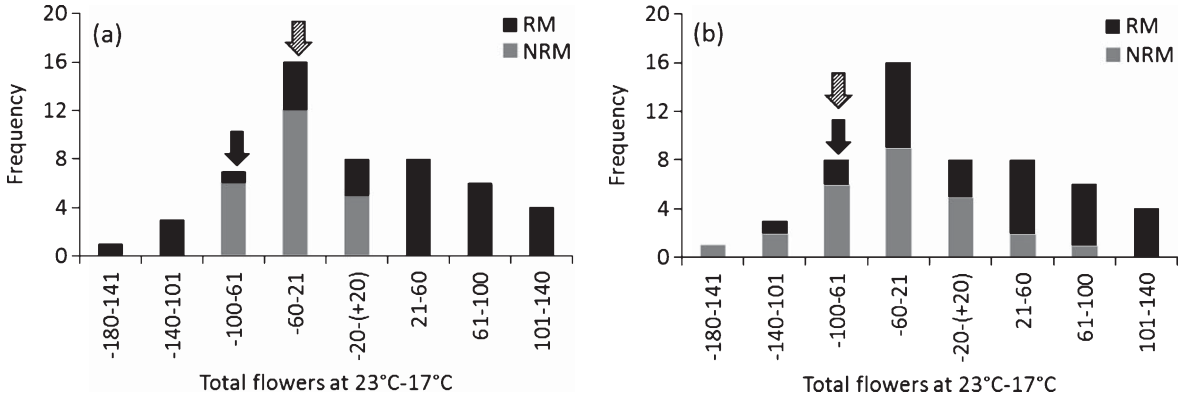

Fig. 2. a-b. Frequency distribution of total flowers at 23°C minus total flowers at 17°C in the 'Honeoye' × 'Tribute' population and their flowering phenotype in the field in (a) MI and (b) OR. RM: Remontant, NRM: Non-remontant; Dark arrow: 'Honeoye' (NRM); shaded arrow: 'Tribute' (RM).

The majority of the non-remontant genotypes produced significantly more runners at 23°C than at 17°C, as did their parents 'Honeoye' and 'Tribute' (Fig. 4). However, most of the remontants produced only limited numbers of runners at 23°C, except for a few genotypes. These outlying individuals could be a very useful in breeding remontant cultivars that produce sufficient runners for propagation.

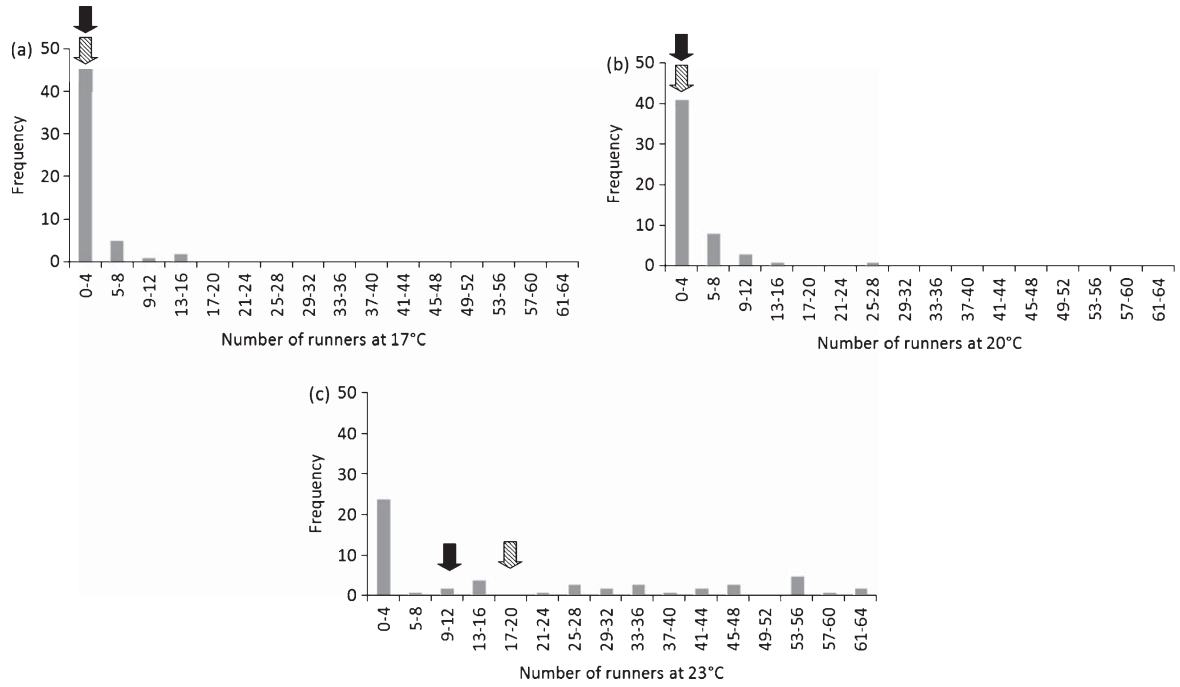

Fig. 3. a-c. Frequency distribution of progeny with different numbers of runners in the 'Honeoye' (H)×'Tribute' (T) population grown in a greenhouse at 17°C, 20°C, and 23°C. (a) Distribution of total runners at 17°C, (b) Distribution of total runners at 20°C, (c) Distribution of total runners at 23°C. Numbers of runners in the parents are indicated: Honeoye: Dark arrow, Tribute: Shaded arrow.

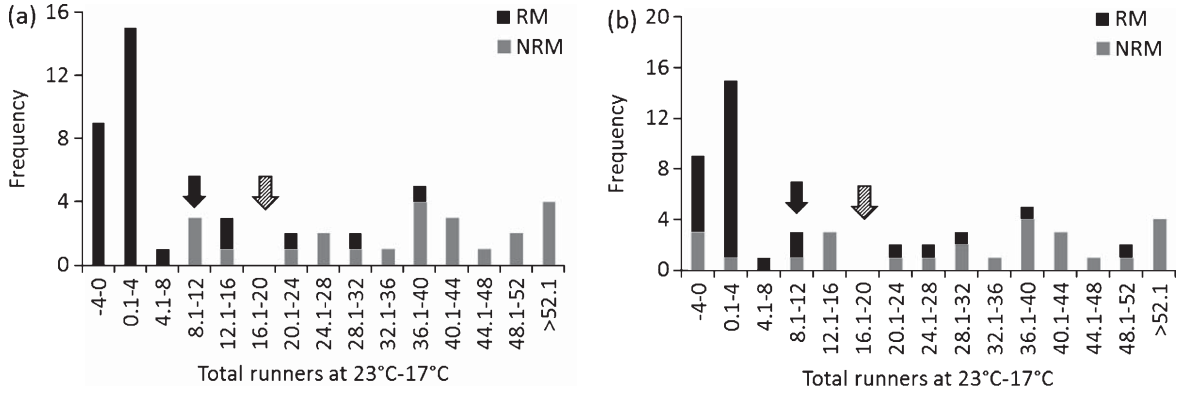

Fig. 4. a-b. Frequency distribution of total runners at 23°C minus total runners at 17°C in the 'Honeoye'×'Tribute' population and their flowering phenotype in the field in (a) MI and (b) OR. RM: Remontant, NRM: Non-remontant; Dark arrow: 'Honeoye' (NRM); shaded arrow: 'Tribute' (RM).

4. Conclusions

Our experiment demonstrates that flower formation can be significantly impacted by a strawberry genotype's level of floral heat tolerance. The degree of heat tolerance is likely quantitatively controlled as a significant G × E interaction was observed and the difference between flowers produced at 23°C vs. 17°C varied continuously among genotypes. The progeny of 'Honeoye' × 'Tribute' segregated widely for degree of floral heat tolerance in the

greenhouse and most of the genotypes that were remontant under field conditions in MI and OR, also had high levels of heat tolerance. Most remontant progeny produced few runners at high temperature but there were a few exceptions. The most heat tolerant, remontant genotypes from this study could be used to breed cultivars better adapted to hot summer conditions.

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