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The effect of temperature and photoperiod on floral initiation in remontant and non-remontant *Hydrangea macrophylla* cultivars

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Abstract

Hydrangea macrophylla is used as ornamental crop plant. Especially its large and colorful inflorescences make it highly attractive to consumers. The production of full-blooming plants is depending on a successful floral initiation. Naturally, floral initiation occurs in autumn under cool temperatures and short-day conditions. Shoot apical meristems change to reproductive growth and develop flower primordia, which remain within buds through winter. In the subsequent season, flower buds open and flowers appear at the top of two-year old shoots. However, *H. macrophylla* also includes remontant cultivars, which produce flowers on two-year as well as on one-year old shoots, resulting in a re-blooming phenotype. In this study, we determined the effect of four floral induction treatments based on 15 or 22°C and a photoperiod of 8 or 16 h light on the floral initiation rate of the remontant cultivars 'Diva fiore' and 'Mak20' and the non-remontant cultivar 'Libelle'. We found that floral induction at 15°C and a photoperiod of 8 h light increased the floral initiation rate of 'Libelle', whereas 'Diva fiore' and 'Mak20' showed high floral initiation rates at 1st flowering after all floral induction treatments, suggesting a temperature- and photoperiod-independent floral initiation in remontant cultivars. After subsequent pruning of all plants down to the rootstock, plants of 'Libelle' grew mostly vegetatively, while plants of 'Diva fiore' and 'Mak20' clearly showed a re-blooming phenotype, initiating on up to 77% of one-year old shoots new flowers irrespective of the previous floral induction treatments.

1. Introduction

The perennial shrub *Hydrangea macrophylla* is one of the best-known species in the genus *Hydrangea*, because of its economical importance in the ornamental sector. *H. macrophylla* develops impressively large and colorful inflorescences and attractive foliage. Therefore it is used as ornamental crop plant, e.g. for landscaping and gardening, as potted plant for indoor cultivation or for cut flower production. Full-blooming plants are in demand for marketing. In *H. macrophylla*, the transition from vegetative to reproductive growth occurs in autumn and is induced by cool temperatures and short-day conditions. After floral initiation, shoot apical meristems produce flower primordia instead of leaves. These flower primordia overwinter in buds. After winter, the flower development is completed and plants release their flowers on two-year old shoots (Orozco-Obando et al. 2005; Terfa and Torre 2019). In contrast, remontant cultivars of *H. macrophylla* produce flowers on apical tips of two-year old as well as on newly emerging, one-year old shoots within the current season. This continuous flower production results in a re-blooming phenotype and makes remontant cultivars highly attractive to consumers.

Several studies showed that temperatures from 15 to 18°C as well as 8 h photoperiod promote floral initiation in *H. macrophylla* (Litlere and Strømme 1975; Nordli et al. 2011). However, the impact of different floral induction treatments on the floral initiation of remontant cultivars was not considered. It is not yet known whether and how different floral induction treatments affect the floral initiation or flowering rate of two-year and one-year old shoots of remontant cultivars.

The objective of this study was to apply different floral induction treatments on remontant and non-remontant cultivars under controlled conditions and to determine the effect of inductive and non-inductive temperatures (15 or 22°C) and photoperiods (8 or 16 h light) on the floral initiation and flowering rate of two-year old shoots at 1st flowering time and of one-year old shoots at remontant flowering time.

2. Data, Methods and Approach

2.1 Plant material

The remontant cultivars 'Diva fiore' and 'Mak20' and the non-remontant cultivar 'Libelle' were used in this study. Potted plants were cultivated at the Erfurt Research Centre for Horticultural Crops, Erfurt University of Applied Sciences, Germany in substrate Einheitserde® CL Hortensien blau, and fertilized with Universol® blue 0.1% (Everris International BV) and irrigated as necessary. All plants were kept under non-inductive floral conditions, before the floral induction experiments started: Plants of experiment 1 (E1) were pre-cultivated until July 2016 under semi-natural summer conditions in a greenhouse without additional heat and light supply in 15 cm pots. Plants of experiment 2 (E2) were pre-cultivated until August 2020 in a greenhouse at 22°C and 16 h light photoperiod in 17 cm pots.

2.2 Experimental design

Two independent floral induction experiments were performed, E1 starting on 12 July 2016 and E2 starting on 31 August 2020. The experimental setup is shown in Figure 1. Both of these experiments started by pruning the plants down to the rootstock, followed by pre-cultivation in a greenhouse under non-inductive floral conditions at 22°C and 16 h light photoperiod (additional light supply at 15 klux) for 5 and 7 weeks, respectively, in order to initiate new shoot growth. As next, 3-5 plants per cultivar in E1 and 6 plants per cultivar in E2 were transferred for 12 weeks in 4 climate chambers to run 4 different floral induction treatments in parallel under following conditions:

- Chamber 1: 22°C and 16 h light (complete floral non-inductive conditions)
- Chamber 2: 22°C and 8 h light (non-inductive temperature but floral inductive short-days)
- Chamber 3: 15°C and 16 h light (floral inductive temperature but non-inductive long-days)
- Chamber 4: 15°C and 8 h light (complete floral inductive conditions)

After floral induction treatment, the plants were transferred to a cold room and kept at constantly 5°C and darkness, in order to break bud dormancy, which was induced simultaneously under floral inductive conditions. After 9 (E1) and 6 weeks (E2) cold storage, respectively, all plants were transferred to a greenhouse and forced at 22°C and under 16 h light photoperiod. After 11 (E1) and 10 weeks (E2), flowering of two-year old shoots (1st

flowering) was recorded. After 1st flowering record, all plants were pruned down to the rootstock and cultivated continuously under the same forcing conditions at 22°C and 16 h light. After 18 (E1) and 14 weeks (E2), flower development on one-year old shoots was recorded to determine remontant flowering.

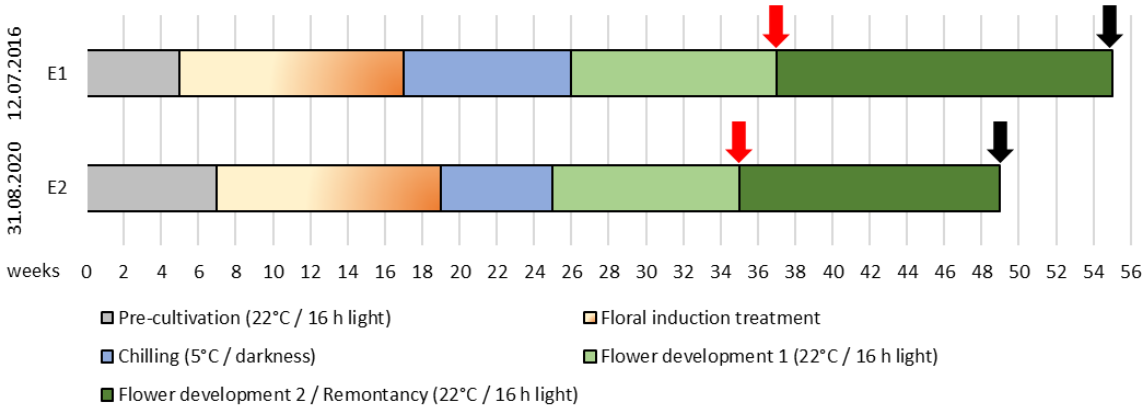

Figure 1: Experimental setup of floral induction experiment 1 (E1) and 2 (E2). Red arrows show the time points of phenotyping 1st flowering on two-year old shoots, followed by pruning of all shoots down to the rootstock. Black arrows indicate the time points of phenotyping remontant flowering of newly developed, one-year old shoots.

2.3 Determination of flowering rates at first and remontant flowering

For all floral induction treatments, the flowering or floral initiation rates of two-year old shoots at 1st flowering time and of newly emerged, one-year old shoots at remontant flowering time were determined. In E1, 3-5 plants per cultivar with 5-24 shoots at 1st flowering time and with 12-33 shoots at remontant flowering time were phenotyped. In E2, 6 plants per cultivar with 4-17 shoots at 1st flowering time and with 6-22 shoots at remontant flowering time were analyzed. Per plant, all shoots were phenotyped for apical flower development. In E1, only flowers visible per eyes were scored and the flowering rate was determined as percentage of flowering shoots per plant. In E2 visible flowers as well as flowers in buds were scored and the floral initiation rate per plant was calculated as percentage of generative shoots per plant. The flowering or floral initiation rates per cultivars at 1st and remontant flowering time are given as mean of plants per experiment and floral induction treatment.

2.4 Statistical Analysis

Descriptive statistics, one-way ANOVA and Bonferroni post hoc tests ($p < 0.05$) were performed using the software program IBM SPSS Statistic, Armonk, USA.

3. Results and Discussion

In two independent floral induction experiments, we analyzed the effect of floral inductive or non-inductive temperatures (15 or 22°C) and floral inductive or non-inductive photoperiods (8 or 16 h light) on the flowering and floral initiation rates of the remontant cultivars ‘Diva fiore’ and ‘Mak20’ and the non-remontant cultivar ‘Libelle’. All results are shown in Figure 2. In ‘Libelle’, the floral inductive parameters 15°C and 8 h light photoperiod resulted in the highest flowering rate (E1) and floral initiation rate (E2) on two-year old shoots at 1st flowering time, while the lowest flowering and floral initiation rates were found after non-inductive conditions at 22°C and 16 h light photoperiod. Floral induction

treatments based on one inductive and one non-inductive parameter resulted in intermediate flowering and floral initiation rates, suggesting an additive effect of temperature and photoperiod on floral initiation. Also Litlere and Strømme (1975) found that cool temperatures from 15 to 18°C during floral induction treatment resulted in higher flowering rates compared to temperatures between 21 and 24°C. In addition, the authors found an accelerated floral bud development at 18°C and photoperiods of 10 and 12 h light in comparison to 21 and 24°C and photoperiods of 14 to 20 h light. Based on our results, the non-remontant cultivar ‘Libelle’ seems to respond tendentially to temperature and photoperiod although the floral initiation rates did not differ significantly between the different floral induction treatments. In contrast, the remontant cultivars ‘Diva fiore’ and ‘Mak20’ showed high flowering and floral initiation rates on two-year old shoots at 1st flowering time independent from different temperatures and photoperiods (Figure 2). Also Adkins and Dirr (2003) detected 5 out of 10 cultivars that initiated flowers independent from 8 h inductive short-day and 24 h non-inductive extended-day photoperiods and proposed a remontant flowering potential for these cultivars. However, the impact of cool temperatures in combination with different photoperiods were not described in this study. Based on our results, floral initiation seems to occur independent from photoperiod as well as temperature in the remontant cultivars ‘Diva fiore’ and ‘Mak20’.

After evaluation of 1st flowering, the plants were pruned down to the rootstock and cultivated continuously under non-inductive floral conditions at 22°C and 16 h light to promote new shoot growth. After 18 (E1) and 14 weeks (E2), plants of ‘Libelle’ had produced one-year old shoots with predominantly vegetative apical buds (Figure 2). Surprisingly, 1 to 3 plants showed slight re-blooming after 3 out of 4 floral induction treatments of E1, but none in E2. Reasons for this unexpected re-blooming are unknown. In contrast, all plants of ‘Diva fiore’ and nearly all plants of ‘Mak20’ had initiated flowers on newly emerging shoots at remontant flowering time. In both of these experiments, ‘Diva fiore’ constantly showed best re-blooming performance. Thereby, ‘Diva fiore’ and ‘Mak20’ showed similar flowering rates in E1 depending on the previous induction treatments. In contrast, floral initiation rates of ‘Diva fiore’ and ‘Mak20’ differed significantly in E2, but not between different floral induction treatments. Reasons for these contradictory results for ‘Diva fiore’ and ‘Mak20’ might be that in E2 the phenotyping of remontancy started 4 weeks earlier. It is possible, that the floral initiation in ‘Mak20’ is delayed in comparison to ‘Diva fiore’ and results in a lower floral initiation rate at an earlier time point, but in a similar floral initiation rate at later time points. Furthermore, phenotyping in E2 included visible flowers as well as closed flower buds. Closed flower buds were not considered in E1 but might homogenize the flowering rates according to the results in E2. Further experiments are necessary.

4. Conclusions

The non-remontant cultivar ‘Libelle’ showed the highest floral initiation rate on apical tips of two-year old shoots in combination of 15°C and a photoperiod of 8 h light, whereas the remontant cultivars ‘Diva fiore’ and ‘Mak20’ initiated flowers independent from temperature and photoperiod. An unexpected re-blooming of one-year old shoots was partly found in ‘Libelle’ after 3 out of 4 previous floral induction treatments in E1 but no remontant flower development in E2. In contrast ‘Diva fiore’ and ‘Mak20’ showed remontant floral initiation on one-year old shoots for all previously applied floral induction treatments in all experiments.

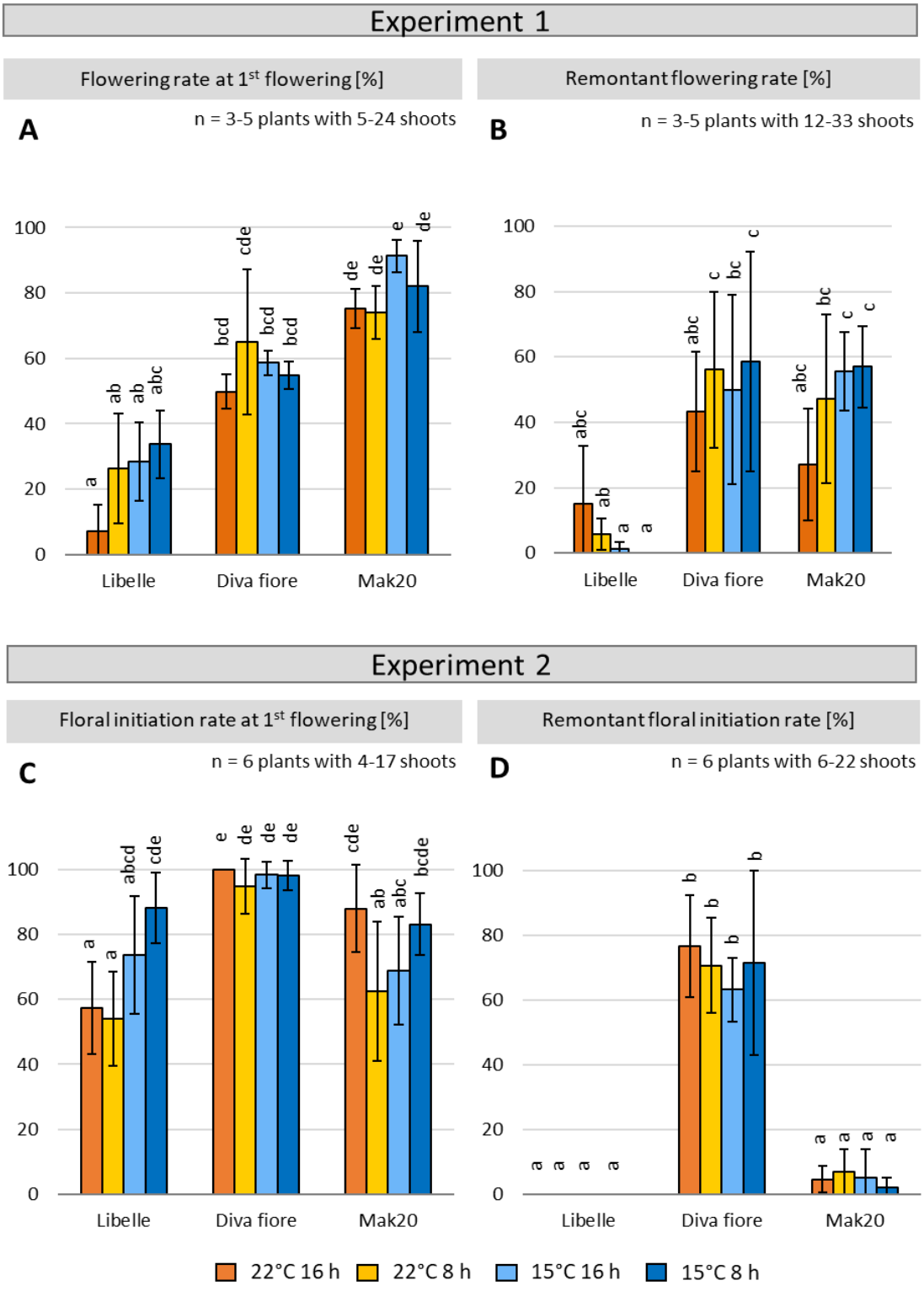

Figure 2: Flowering rates and floral initiation rates of the non-remontant cultivar 'Libelle' and the remontant cultivars 'Diva fiore' and 'Mak20' at 1st flowering time (A, C) and at remontant flowering time (B, D) in experiment 1 (A, B) and experiment 2 (C, D). The flowering rate was determined based on the number of shoots with visible flowers, whereas the floral initiation rate was calculated based on the number of generative shoots with visible flowers or flower buds. Different letters indicate significant differences based on Bonferroni post-hoc test ($p < 0.05$).

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