Evaluation of Methods for Flowering Advancement of Herbaceous Peonies

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Abstract. Experiments to advance early production of herbaceous peony (Paeonia lactiflora Pall.) flowers were conducted over 8 years in the higher elevation, cooler regions of Israel. Anatomical studies during the summer revealed that flower bud initiation of apical buds in the crowns began at the end of July and continued also in lateral buds from mid-August until the plants became dormant in mid-November. Container-grown plants of various cultivars were moved to cold rooms maintained for 10 to 13 weeks at 2°C, from mid-August to mid-October, then drenched with 250 mL of various concentrations of GA3 and transferred to a greenhouse. The optimal GA3 concentration for flower production was 100 mg L–1. Plants treated in this way flowered 2–3 months before the natural flowering period. Field-grown plants in uncovered greenhouse structures were exposed to natural winter cold temperatures (2°C), until they had received various chill units according to a "dynamic model" (for details see Erez et al., 1988). The crowns were then drenched with 12 to 20 various amounts and concentrations of GA3, and the greenhouses were covered with plastic sheets. The optimal chill units for most cultivars was 40 and the optimal GA3 drench treatment was 250 mL of 100 mg L–1. Covered and GA3-treated field-grown plants flowered 1–2 month earlier than untreated plants grown in the open field. The GA3 treatment also greatly increased the number of produced flowers.

Herbaceous peony is primarily derived from Paeonia lactiflora Pall., native to northeast Asia. Some cultivars (cv.s) are hybrids of P. lactiflora and P. officinalis L, native to southern France (Bailey, 1916; Everett, 1981; Rogers, 1995). Peonies are widely used as garden plants in temperate climate regions, but are less common as cut flowers, in spite of Paeonia lactiflora being used as controls.

I. Experiment. Cooling of cv.s SB and KR began at the end of Aug. 1993, when most central buds had already initiated flower buds as checked by dissection and microscopic observation of three crowns per cultivar. Preliminary trials suggested that beginning cooling of plants before mid-August was not effective to enable flowering (Halevy et al., 1995).

Three-year-old crowns, field-grown in the Golan Heights, were planted one per pot in 15-L containers in a growing medium of equal volumes of volcanic gravel and peat, were cooled at 2°C for 10 weeks beginning in Aug. The crowns were then drenched with 250 mL of 250 or 500 mg·L–1 GA3 (ICI, United Kingdom) and transferred to a greenhouse heated to a minimum of 14°C.

II. Experiment 2. In the next year (1994), 4-year-old SB containerized plants were cooled at 2°C for 13 weeks, drenched with 250 mL of 100 mg·L–1 GA3 and then moved on 3 Nov. to either a heated (to 14°C) or an unheated greenhouse. Temperatures in the unheated greenhouse varied from 8 to 10°C at night and from 16 to 26°C during the day in both greenhouses. Average temperature in the heated greenhouse was 20°C and 17°C in the unheated greenhouse. Ten plants were used in each treatment.

III. Experiment 3. Forcing field-grown plants. The experiments were conducted during 1993–94 season with field-grown plants raised in a cool region of Israel—the Golan Heights. Field-grown plants grew either constantly covered or uncovered, to observe the natural flowering season, or in an unheated greenhouse covered with polyethylene sheets at two dates (14 or 24 Jan. 1994). Plants were divided into four randomized blocks, with each treatment containing five plants and treated with GA3 as detailed.
below. Three cultivars were tested: SB, KR, and ‘Duchesse de Nemours’ (DN).

a) Open field-grown plants. Temperature conditions in the open field during the winter months ranged from 2 to 8°C at night and from 8 to 28°C during the day (average temperature 17°C). The following treatments were applied:

1) Untreated control.
2) Drenched with 250 mL of 100 mg L\(^{-1}\) GA\(_3\) on 3 Mar.
3) Drenched with 250 mL of 100 mg L\(^{-1}\) GA\(_3\) on 3 and 20 Mar.
4) Drenched as in treatment 3 and sprayed with 100 mg L\(^{-1}\) GA\(_3\) on 4 Apr. Crowns were drenched with GA\(_3\) prior to natural sprouting and spray was applied 10 d after sprouting.

b) Covered field-grown plants. Plants of the same cultivars and age as those of open field were grown in uncovered greenhouse structures adjacent to the open-field plants. They were initially exposed to the cold winter weather and then the greenhouse was covered with clear polyethylene sheets either on 14 or 28 Jan. to obtain two durations of chilling before covering. Temperature in the covered greenhouses varied from 6 to 28°C (average temperature 19°C). Plants were either not drenched or drenched 2 d after they were covered with 250 mL of 100 mg L\(^{-1}\) GA\(_3\).

The following parameters were recorded: number of flowers per plant, flowering period, stem length, and strength (means of all flowering stems per treatment). Stem strength was determined by measuring the angle of stem bending when held horizontally, determined by measuring the angle of stem bending when held horizontally.

### Experiment 4. Evaluation of optimal GA\(_3\) concentration.
Four-year-old SB and DN plants were drenched with 250 mL of GA\(_3\) at various concentrations (as detailed in Fig. 1) on 16 Jan. 1995, the day the greenhouse was covered with plastic sheets. Twenty plants were used for each cv. and treatment arranged in randomized blocks of five plants per treatment and cv. The number of shoots per plant and the number of flowers produced per plant were recorded.

### Experiment 5. Forcing field-grown plants.
In the next year, 4-year-old SB containerized plants were grown after cooling in either heated or unheated greenhouses. The data of Table 2 shows that it was possible to obtain very early flowering of marketable quality SB flowers in January by combined treatments of cooling and GA\(_3\). Growing in unheated greenhouses was preferable, because flowering percentage was higher and stems were stronger (data not shown).

### Results

#### Flower initiation and development.
Until mid-July, all buds were vegetative. The first flower initials were observed in apical (central) buds at the end of July in SB and 2 weeks later in KR. Lateral buds remained vegetative until mid-August. During August, ~85% of the central buds initiated flowers, first in the buds having large apical meristems and later also in the smaller central buds, and they continued to develop during the summer. Starting from late August, flower initiation began also in large lateral buds, and it continued until the plants became dormant in November. About 35% of these lateral buds did not complete their differentiation before dormancy.

#### Flowering of container-grown plants.

**Experiment 1.** Early flowering was achieved in containerized plants by combined treatments of cooling and GA\(_3\) (Table 1). Drenching with 500 mg L\(^{-1}\) GA\(_3\) was excessive, since it caused flower malformation and weak stems (data not shown). Most plants that were recorded as not flowering did form flower buds that aborted later. We suspected that growing in a closed heated greenhouse promoted flower abortion, as shown in an earlier study in California (Byrne and Halevy, 1986).

**Table 1.** Effect of cooling and GA\(_3\) application on flowering of 3-year-old container-grown plants of ‘Sara Bernhardt’ (SB) and ‘Karl Rosenfeld’ (KR) peonies. Plants were either not cooled (–) or cooled (+) for 10 weeks at 2°C, and they were either not treated (–) or drenched with 250 mL of GA\(_3\) (+). Plants were grown in a greenhouse heated to 14°C. Means of 10 replicates ± SE.

| Treatment  | GA\(_3\) (mg L\(^{-1}\)) | Flowering period | No. of flowers per plant (%) | Length of flowering period (cm) |
|------------|--------------------------|------------------|-----------------------------|-----------------------------|
| SB         | –                        | 50               | 1.5 ± 0.3                   | 55.2 ± 3.3                  |
|            | + 0                      | 50               | 3.4 ± 1.1                   | 59.6 ± 3.5                  |
|            | + 500                    | 40               | 3.1 ± 1.0                   | 35.9 ± 4.1                  |
| KR         | –                        | 60               | 1.8 ± 0.4                   | 51.2 ± 2.4                  |
|            | + 250                    | 70               | 3.2 ± 1.3                   | 54.0 ± 3.6                  |
|            | + 500                    | 50               | 4.8 ± 1.6                   | 58.9 ± 3.9                  |

| Sig. of interaction | GA\(_3\) (mg L\(^{-1}\)) | Flowering period | Heating |
|---------------------|--------------------------|------------------|---------|
|                       | –                        | 50               | NS      |
|                       | + 100                    | 50               | <0.00001|

### Table 2. Effect of cooling container-grown 4-year-old ‘Sara Bernhardt’ peony plants for 13 weeks at 2°C, drenched with 250 mL of 100 mg L\(^{-1}\) GA\(_3\) (+) and grown in a heated (to 14°C) (+) or unheated (–) greenhouse, on flowering parameters. Means of 10 replicates ± SE.

| GA\(_3\) (mg L\(^{-1}\)) | Greenhouse days to flowering | Flowering period | No. of flowers per plant (%) | Length of flowering period (cm) |
|--------------------------|----------------------------|------------------|-----------------------------|-----------------------------|
| +                        | 31 Dec.–12 Jan.            | 90 ± 1.1         | 46.0 ± 0.9                  | 47.3 ± 3.7                  |
| +                        | 6–12 Jan.                 | 96 ± 2.6         | 1.0 ± 0.2                   | 70.6 ± 3.0                  |
| +                        | 8–16 Jan.                 | 98 ± 2.7         | 1.6 ± 0.8                   | 66.8 ± 2.6                  |
| −                        | 31 Dec.–19 Jan.           | 90 ± 1.9         | 2.0 ± 0.3                   | 68.5 ± 2.8                  |

\(\text{NS}\) = Nonsignificant \( P > 0.05.\)
were longer. GA3 treatment advanced flowering what lower than in the open field, but the stems adjacent to the covered plants (compare data in (Table 4). Flower yield was some-

Repeated GA3 applications (drench or spray) reduced flower production of SB by promot-
ing flower abortion. Stem strength was gener-
ally reduced by repeated GA3 applications (data not shown).

Covering field plants in unheated plastic structures (Table 4) advanced flowering by ≈1 week and greatly increased flower yield. In all cases, one GA3 drench greatly in-
creased flower production. However, exces-
sive GA3 was detrimental for SB (Table 3). The next test was therefore performed to evalu-
ate the optimal GA3 drench concentration re-
quired for flower production.

Evaluation of optimal GA3 concentration. Experiment 4. The test was conducted with 4-

year-old SB and DN plants. They were drenched and the greenhouse was covered on 16 Jan. Results in Fig. 1 show that a drench with 100 mg·L−1 GA3 was optimal. This con-
centration was, therefore, used in all the fol-

owing trials and is recommended for com-

mercial applications.

Experiment 5. Since GA3 treatment has-
tened flowering and increased flower produc-
tion, untreated plants were not included in the tests conducted in the following years. In the following year the winter climate was warmer than in the previous years and, therefore, cov-
ering the plants was delayed to let them obtain more days of cool weather (Table 5).

Two GA3 treatments were included in this year: 1) GA3, drench at 100 mg·L−1 on the day the greenhouse was covered, and 2) GA3, drench as in (1) and 100 mg·L−1 GA3, spray 10 d later. The results (Table 5) show that double GA3 treatment of drench and spray was in most cases not better than drench alone, and in some cases reduced flowering percentage by pro-
moting flower abortion.

Experiment 6. Evaluation of the number of chill units required before covering. Results of Table 5 indicate a possible difference in chilling requirement of the various cvs. The lower chilling requirement was in DN and the higher in KR, which did not bloom at the early covering date without a GA3 drench (Table 5). These results also indicate that the date is not a reliable criterion for the appropriate time to cover the plants after receiving sufficient chill-
ing. We, therefore, decided to examine the use of the chill units according to the “dynamic model” (Erez et al., 1988) as a more objective criterion for covering the greenhouses.

Results in Fig. 2 show that chilling satura-
tion was reached at 42 chill units for cv. SB and at 36 chill units for cv. DN. These criteria are used commercially now to determine the ap-

propriate time to cover the greenhouses with plastic in the winter to obtain early flowering.

Discussion

The developmental behavior of herbaceous peonies is similar in some ways to that of deciduous fruit trees. They bloom in the spring, initiate their flowers during the summer, and shed their leaves and enter dormancy in late autumn. However, unlike deciduous trees, foliage senescence and dormancy are not in-
duced by short days in peonies and flower formation and development are also unaf-

The factors inducing flower formation, leaf abscission, and bud dormancy of peonies are unknown. Breaking of bud dormancy requires exposure to a certain chilling period, as known for most deciduous trees (Erez et al., 1998). In peonies, this requirement can be achieved not only by freezing temperatures, as assumed previously (Post, 1952), but also by low tem-

perature a little above freezing (Byrne and Halevy, 1986; Wilkins and Halevy, 1985).

Table 3. Effect of 100 mg·L−1 GA3 treatments, applied as one (3 Mar.) or two (3 and 20 Mar.) soil drenches of 250 mL per plant or as two drenches and spray (4 Apr.), on flowering of 3-year-old, open field-grown plants. Means of 20 replicates ± SE.

| Cultivars     | Flowers per plant | Flowering period | Stem length (cm) | Flowers per plant | Flowering period | Stem length (cm) | Flowers per plant | Flowering period | Stem length (cm) |
|---------------|-------------------|------------------|------------------|-------------------|------------------|------------------|-------------------|------------------|------------------|
| Control       | 6.0 ± 0.6         | 29 Apr.–12 May   | 48.6 ± 3.4       | 5.7 ± 0.1         | 27 Apr.–14 May   | 40.0 ± 2.3       | 5.7 ± 0.4         | 26 Apr.–14 May   | 47.8 ± 3.4       |
| One GA3, drench | 9.8 ± 1.1         | 20 Apr.–6 May    | 54.4 ± 3.6       | 11.8 ± 2.2        | 20 Apr.–6 May    | 37.3 ± 2.1       | 13.0 ± 2.1        | 24 Apr.–10 May   | 32.6 ± 2.8       |
| Two GA3, drench | 5.3 ± 0.9         | 22 Apr.–10 May   | 44.7 ± 2.9       | 19.8 ± 3.6        | 21 Apr.–8 May    | 37.6 ± 1.9       | 12.3 ± 1.9        | 23 Apr.–7 May    | 42.5 ± 3.1       |
| Two GA3, drenches + spray | 3.5 ± 0.5         | 24 Apr.–11 May   | 41.6 ± 2.6       | 20.2 ± 3.8        | 20 Apr.–5 May    | 37.2 ± 2.6       | 13.7 ± 2.2        | 23 Apr.–8 May    | 41.3 ± 3.2       |

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and with GA 3, but it also caused multiple cytokinins at various concentrations, alone Fig. 1). We have also tried application of sprouting of many shoots but many of them concentrations, or excessive applications, induced too high concentrations to sprout and too high con-

optimal. Too low concentrations caused a low number of shoots to sprout and too high con-
ncentrations, or excessive applications, induced sprouting of many shoots but many of them aborted and did not produce flowers (Table 5, Fig. 1). We have also tried application of cytokinins at various concentrations, alone and with GA3, but it also caused multiple sprouting of shoots with aborted flower buds (Haley et al., 1995).

The method developed in the present study for advancing flowering of field-grown peony

is based on exposure of the plants to ambient cold winter weather until they obtain a sufficient duration of cold temperature. This exposure is supplemented by drenching with GA3 to hasten growth and flowering is promoted by covering the greenhouse and raising the growing temperatures.

Another method for obtaining very early flowering (during January–February) is with container-grown plants. They are artificially cooled at 0–2 °C for 10–13 weeks, drenched with GA3, and transferred to the greenhouse. No light is given during cooling, since only crowns without above-ground parts are present during cooling.

The chilling requirement for breaking bud dormancy was found to vary in the various cvs. (Fig. 2). Weather conditions vary at various seasons and locations. Therefore, the time of closing the greenhouses could not be deter-
mined by a fixed date. Requirement of cold period was estimated by using chill units, according to the “dynamic model” developed by Fishman et al. (1987a, 1987b) and Erez et al. (1988) for deciduous fruit trees. Attainment of ~40 chill units is now used by growers to determine the time for closing the greenhouses for most cvs. (Fig. 2). In conclusion, this study formed the basis for developing practical methods for the advancement of flowering in herbaceous peonies, which are used to greatly extend the flowering period.

### Table 4. Effect of 250 mL of 100 mg·L−1 GA3 drench to 3-year-old, field-grown peony cvs.—‘Sara Bernhardt’ (SB), ‘Karl Rosenfeld’ (KR), and ‘Duchesse de Nemours’ (DN)—grown in an unheated greenhouse, covered on two dates, 14 and 28 Jan. Means of 20 replicates ± se.

| Treatment | GA3 (µg·g−1) | Flowering period | No. of flowers per plant | Length of flowering stem (cm) |
|-----------|----------------|------------------|-------------------------|-----------------------------|
| Cover date | Cultivar | 14 Jan. | | | |
| SB | – | 25 Mar.–10 Apr. | 3.4 ± 0.4 | 56.6 ± 3.3 |
| KR | + | 19 Mar.–6 Apr. | 2.5 ± 0.3 | 43.4 ± 2.8 |
| DN | + | 23 Mar.–8 Apr. | 10.4 ± 1.1 | 55.0 ± 3.1 |
| DN | – | 29 Mar.–16 Apr. | 2.0 ± 0.2 | 59.6 ± 3.4 |
| Cover date | Cultivar | 28 Jan. | | | |
| SB | + | 31 Mar.–16 Apr. | 5.9 ± 0.6 | 63.3 ± 3.6 |
| SB | – | 4–24 Apr. | 1.3 ± 0.2 | 67.4 ± 3.6 |
| KR | + | 28 Mar.–15 Apr. | 2.5 ± 0.3 | 54.8 ± 3.9 |
| KR | – | 4–24 Apr. | 0.6 ± 0.1 | 60.6 ± 3.4 |
| DN | + | 29 Mar.–13 Apr. | 7.2 ± 0.9 | 57.7 ± 3.1 |
| DN | – | 4–24 Apr. | 1.1 ± 0.2 | 57.5 ± 3.0 |

Significance of:

- Cultivar × GA3 <0.0001
- Cultivar <0.0001
- GA3 <0.0001
- Cover date <0.0001

### Table 5. Effect of 100 mg·L−1 GA3 treatment to 4-year-old, field-grown peony cvs.—‘Sara Bernhardt’ (SB), ‘Karl Rosenfeld’ (KR), and ‘Duchesse de Nemours’ (DN), grown in greenhouses covered on two dates, 24 Jan. and 6 Feb. Two GA3 treatments at 100 mg·L−1 were included—drench (D) of 250 mL and drench and spray (D + S). Means of 20 replicates ± se.

| Treatment | GA3 (µg·g−1) | Flowering period | No. of flowers per plant | Length of flowering stem (cm) |
|-----------|----------------|------------------|-------------------------|-----------------------------|
| Cover date | Cultivar | 24 Jan. | | | |
| SB | – | 30 Mar.–14 Apr. | 2.2 ± 0.2 | 66.4 ± 3.6 |
| SB | + | 1–13 Apr. | 5.2 ± 0.7 | 63.3 ± 3.2 |
| KR | D | 25–16 Apr. | 1.2 ± 0.2 | 53.0 ± 2.8 |
| KR | + | 30 Mar.–15 Apr. | 0.5 ± 0.1 | 52.8 ± 2.7 |
| DN | D | 28 Mar.–14 Apr. | 14.5 ± 1.3 | 62.4 ± 3.1 |
| DN | + | 29 Mar.–15 Apr. | 16.8 ± 1.4 | 59.0 ± 3.0 |
| Cover date | Cultivar | 6 Feb. | | | |
| SB | D | 5–24 Apr. | 8.2 ± 0.9 | 60.3 ± 3.0 |
| SB | + | 3–22 Apr. | 4.9 ± 0.6 | 61.4 ± 3.1 |
| KR | D | 2–22 Apr. | 4.3 ± 0.6 | 47.3 ± 2.6 |
| KR | + | 1–21 Apr. | 7.6 ± 0.9 | 53.6 ± 2.8 |
| DN | D | 3–22 Apr. | 14.7 ± 1.4 | 62.7 ± 3.2 |
| DN | + | 4–22 Apr. | 14.6 ± 1.4 | 58.4 ± 3.0 |

Significance of:

- Cultivar × GA3 NS NS
- Cultivar NS NS
- GA3 <0.0001 <0.0001
- Cover date <0.0001 NS

*Non-significant.
Fig. 2. Effect of covering greenhouses with polyethylene sheets after obtaining various numbers of chill units (see Materials and Methods) on number of flowering shoots per plant of 'Sara Bernhardt' and 'Duchesse de Nemours' peonies.