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Regulation of Growth and Flowering in *Aguilegia* x *hybrida* Sims[1]

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Abstract: **Cold temperature treatments stimulated inflorescence formation of *Aguilegia* x *hybrida* at the 12-leaf stage. Long photoperiods slightly advanced flowering further, but caused petiole elongation. Sprays of butanedioic acid mono-2,2-dimethylhydrazide (daminozide) at 2,000 mg/liter effectively prevented petiole elongation.**

Aquilegia caerulea, A. longissima, A. chrysantha, A. canadensis, and *A. vulgaris* have been interbred to create the hybrid columbine commonly grown in gardens today (1). 'McKana's Giant' is perhaps the most popular hybrid because of its large flowers and 75–90-cm height. In this study the effects of photoperiod, temperature, and growth regulators were investigated in order to develop an efficient cropping schedule.

Materials and Methods

Plants were started from seed sown in peat-lite mix and germinated under mist. About 4 weeks after sowing the seedlings were transplanted into either 7.5-cm plastic or 10-cm clay pots containing a 1 soil:2 sphagnum peatmoss:2 perlite mix (by volume) with a pH of 6.5 ± 0.3. The mix was amended with 0.9 kg of treble superphosphate, 0.6 kg of potassium nitrate, 0.6 kg of magnesium sulfate, and 5.34 kg of agricultural limestone per m^3 of mix. Plants were fertilized at each watering with 200 mg/liter nitrogen and potassium. Black cloth covered the plants from 1600 to 0800 HR each day, and photoperiod was extended with 60-watt incandescent bulbs supplying 1.4 ± 0.4 μ E^{-2} sec^{-1} light. Vernalization was provided in a 4.5 ± 1°C cooler lighted with Cool White fluorescent tubes at 7.2 – 18 μ E m^{-2} sec^{-1} for 10 hr daily. Plants in the cooler were watered as needed with tap water. Pesticides were applied to plants in the greenhouse as needed. The experimental designs were randomized complete blocks.

Regulation of flowering

Photoperiod and forcing temperature. Seeds of 'McKana's Giant', 'Fairyland', and 'Crimson Star' columbine were sown Dec. 12, 1976, to determine if photoperiod could induce flowering at selected temperatures. Five-week-old seedlings were transplanted into 10-cm clay pots and 10 plants were moved into each of 6 treatments. Plants were grown in 13°C minimum night temperature (MNT), 18° MNT, and 24° MNT greenhouses under 10- and 18-hr photoperiods. Plants remained in their respective treatments for about 7 months, until July 6, 1977.

Juvenility and vernalization. Seedlings of 3 cultivars were grown in an 18°C MNT greenhouse under a 10-hr photoperiod. There were 15 treatments: 3 ages and 5 cold treatments (0, 4, 8, 10, or 12) weeks at (4.5° ± 1°) with 6 plants per treatment (Table 1). After each cold treatment, plants were moved back into an 18° MNT greenhouse under an 18 hr photoperiod. The experiment began with sowing of seeds June 30, 1977, and was terminated May 15, 1978.

The effects of light during vernalization. To determine if the presence of leaves and light or absence of leaves and darkness during vernalization had any effect on flower bud initiation or development, 'McKana's Giant' seedlings were sown June 22, 1978, transplanted into 10-cm plastic pots, and grown under a 10-hr photoperiod in an 18°C MNT greenhouse until the plants averaged 18.7 leaves. On that date, Nov. 6, 1978, leaves were cut off half of the plants and these plants were then placed in darkness under a large cardboard box in a 4.5° ± 1°C cooler. Plants with leaves were placed in the same cooler under fluorescent lamps providing 7.2 – 18 μ E m^{-2} sec^{-1} for 10 hr daily. Plants were stored for 0, 4, 8, or 12 weeks, and then greenhouse grown at 18° MNT under an 18-hr photoperiod. The experiment was terminated after 29 weeks on May 30, 1979.

Photoperiod before and after cold storage. To determine if photoperiod before or after cold storage influenced percent flowering, rate of bud development, or flower height, 'McKana's Giant' seedlings sown on June 22, 1978 were transplanted into 10-cm plastic pots after 5 weeks, on July 17, 1978. All plants were grown to maturity (22.6 leaves) in an 18°C MNT greenhouse with half the plants grown under a 10-hour photoperiod and the other half under an 18-hr photoperiod. Cold storage at 4.5° ± 1° began on Dec. 4, 1978 for 0, 4, 6, 8, 10, or 12 weeks. After cold storage half the plants which had been raised under a 10-hr photoperiod were returned to the greenhouse under 10-hr photoperiod and the other half were placed under an 18-hr photoperiod. Similarly, plants grown under 18-hr photoperiods were placed back in both 10- and 18-hr photoperiods. There were 6 plants per treatment. The experiment was terminated after 48 weeks, in June, 1979.

Temperature and photoperiod after cold storage. To determine how temperature and photoperiod interact to affect speed of flowering after cold storage, seeds of 'McKana's Giant' were sown on June 27, 1978. Four-week-old seedlings were transplanted into 10-cm plastic pots. Plants were grown in an 18°C MNT greenhouse under a 10-hr photoperiod for 12 weeks until mature (27.3 visible leaves) and stored at 4.5° ± 1° for 10 weeks. After cold storage, plants were placed in 1 of 6 different temperature-photoperiod environments: 1) 13° MNT, 10 hr; 2) 13° MNT, 18 hr; 3) 18° MNT, 10 hr; 4) 18° MNT, 18 hr; 5) 24° MNT, 10 hr; or 6) 24° MNT, 18 hr. There were 6 plants per treatment and the experiment was terminated after 28 weeks, on June 12, 1979.

Flowering in response to gibberellic acid sprays. Gibberellic acid (GA_3) sprays were applied to determine if 'McKana's Giant' plants could be induced to flower without vernalization. Plants

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Table 1. Three plant ages as defined by number of leaves at the start of the experiment for 3 *Aguilegia* x *hybrida* cultivars.

Age	Time after transplant (wk)	Mean no. leaves		
		McKana's Giant	Fairyland	Crimson Star
I	4	6.7	7.8	7.6
II	8	11.9	12.0	14.5
III	12	17.4	14.6	26.6

from a June 27, 1978 sowing were grown until mature (20.4 leaves) under a 10-hr photoperiod in an 18°C MNT greenhouse. At the start of the experiment, April 6, 1979, half the plants were placed at an 18-hr photoperiod, while the other half remained under a 10-hr photoperiod. Plants were sprayed weekly with 0, 25, 50, 75, or 100 mg/liter (± 0.01 mg) GA_3 for 10 weeks with 5 plants per treatment. Distilled water was used to prepare solutions; the surfactant, Triton B-1956, was added at 5000 mg/liter to facilitate wetting of the foliage. Plants were sprayed using a plastic hand-mister bottle until all leaves were wet. The experiment terminated after 11 weeks, on June 22, 1979.

Regulation of growth

Effect of various photoperiods and temperatures on petiole length. On Oct. 28, 1978, all leaves were removed from 60 mature 'McKana's Giant' which had been growing under a 10-hr photoperiod in an 18°C MNT greenhouse. Ten plants were then placed in each of 6 different temperature-photoperiod environments: 13°, 18°, or 24° MNT at 10- or 18-hr photoperiod. Three months later, 3 fully expanded leaves from each plant were randomly selected from the whorl of similar-appearing, mature leaves at the plant base. Petioles were measured from the top of the stipule to the junction of the 3 petioles.

Effect of growth retardants. 'McKana's Giant' plants were grown to maturity under a 10-hr photoperiod in an 18°C MNT greenhouse. On June 1, 1978 the older, outermost leaves on each plant were removed until only 5 of the youngest, innermost leaves of each rosette remained. All plants were then moved to an 18-hr photoperiod and sprayed until runoff with daminozide, (2-chloroethyl)trimethylammonium chloride (chloromequat), or α-cyclopropyl-α-(4-methoxyphenyl-5-pyrimidinemethanol (ancymidol), each at 3 concentrations. The surfactant, Triton B-1956, was added at 5000 mg/liter to facilitate wetting of the foliage. There were 5 plants per treatment. An overall height measurement from soil level to the top of the highest leaf was taken from each plant after 6 weeks, on July 14, 1978.

A second experiment was performed using a greater number of application rates of the 2 effective chemicals, ancymidol and daminozide. Plants were grown to maturity under a 10-hr photoperiod in an 18°C MNT greenhouse. On Jan. 15, 1979, photoperiod was changed to 18 hr and leaf area was made similar as before by removing the older, outermost leaves until only 6 of the youngest, innermost leaves of each rosette remained. The youngest expanding leaf was notched with scissors so that the leaf could be identified at later dates. The growth-retardant chemicals were each sprayed at 4 concentrations until all foliage was wet with the aid of Triton B-1956 added at 5000 mg/liter. There were 10 plants per treatment. On Feb. 26, 1979, petioles of the first 3 leaves to expand after the notched leaf were measured as previously described.

Effect of daminozide. On Jan. 4, 1979, leaves were cut off 18 'McKana's Giant' plants which had been grown to maturity (more than 15 leaves) under 10 hr in an 18°C MNT greenhouse. The plants were subsequently placed in a 4.5° ± 1° cooler for 12 weeks and back in an 18° MNT greenhouse under 18 hr. By April 18, 1979, the plants had attained an average of 8.5 leaves and were sprayed with 0, 4000, or 8000 mg/liter daminozide, using 6 plants per treatment. The surfactant, Triton B-1956, was added to each spray solution at 5000 mg/liter to facilitate wetting of the foliage. The chemical was applied with a small hand-sprayer until all foliage was wet.

Results

Photoperiod and forcing temperature. None of the cultivars flowered regardless of treatment. Petioles elongated more under 18- than 10-hr photoperiods.

Juvenility and vernalization. Age I plants of 'McKana's Giant' (6.7 leaves), 'Fairyland' (7.8 leaves), and 'Crimson Star' (7.6 leaves) did not flower 100% at any duration of cold, including 12 weeks (Table 1 and 2). Nonflowering plants remained rosettes. 'McKana's Giant' (11.9 leaves) and 'Crimson Star' (14.5 leaves) at age II flowered 100% after 10 and 8 weeks of cold storage, respectively. Complete flowering was not induced at Age II (12.0 leaves) of 'Fairyland'. All age III plants flowered with as little cold treatment as 4 weeks for 'Fairyland' (14.6 leaves) and up to 10 weeks for 'McKana's Giant' (17.4 leaves).

Light effect during vernalization. Plants cold-stored without leaves in darkness grew and flowered like plants cold-stored with leaves in light. All plants in both treatments flowered after 12 weeks cold storage, formed visible buds in 48–52 days, and bloomed 14–15 days after visible bud (Table 3). Height of the plants to first flower was 47–48 cm. Older leaves of plants with intact foliage during vernalization began to yellow after 2–3 weeks at 4.5°C and were removed during growth in the greenhouse.

Photoperiod before and after cold storage. Photoperiod before cold storage had no consistent effect on the number of days to visible bud; however, an 18-hr photoperiod after cold storage slightly promoted development of visible buds, flowering, and plant height (Table 4). In all cases, half of the control plants, which received no cold, flowered. The time from end of cold to visible bud was less as the duration of cold increased regardless of the photoperiod combination. Days from visible bud to

Table 2. Flowering of 3 *Aguilegia* cultivars when stored at 4.5°C for various durations at 3 plant ages; 6 replications per treatment.

Cultivar	No. of leaves at start of cold storage	Flowering (%) 4.5°C storage					
		0 wk	4 wk	8 wk	10 wk	12 wk	Mean
McKana's Giant	7	17	0	0	17	50	21
	12	0	17	67	100	100	57
	17	17	33	50	100	100	60
	Mean	11	13	39	72	83	
Fairyland	8	0	0	0	0	0	0
	12	17	0	83	83	83	53
	15	0	100	100	100	100	80
	Mean	6	33	61	61	61	
Crimson Star	8	0	0	0	0	0	0
	15	17	0	100	100	100	63
	27	0	17	100	100	100	63
	Mean	6	6	67	67	67	

Table 3. Growth and flowering of 'McKana's Giant' plants vernalized with leaves in light or without leaves in darkness; 6 replications per treatment.

Time at 4.5°C (wk)	With leaves & light	Without leaves & darkness
	Flowering (%)	
0	---	---
4	50	50
8	83	67
12	100	100
	Days from end of cold storage to visible bud	
0	---	---
4	101 ± 27[z]	81 ± 28
8	60 ± 12	66 ± 13
12	48 ± 13	52 ± 19
	Days from visible bud to bloom	
0	---	---
4	24 ± 4[z]	21 ± 2
8	16 ± 3	18 ± 2
12	14 ± 3	15 ± 3
	Plant height to first flower (cm)	
0	---	---
4	45 ± 13[z]	46 ± 10
8	45 ± 19	32 ± 17
12	47 ± 17	48 ± 19

[z] ± SD

first bloom were not affected by photoperiod either before or after cold storage.

Temperature and photoperiod after cold storage. All plants flowered under both photoperiods at 13°C after 10 weeks of cold storage. However, 10 weeks of storage did not induce 100% flowering in plants forced at 18° or 24° (Table 5). Plants flowered fastest under 18 hr at 24°. Flower bud development was also slightly faster in that environment. Neither temperature nor photoperiod had a marked effect on the number of days from visible bud to bloom. Plant height to first flower was greatest under the long photoperiod regardless of temperature.

Flowering in response to gibberellic acid sprays. GA_3 induced some plants to flower, but also caused weak, elongated growth. Flower stalks were weak and some collapsed before florets opened, especially at higher concentrations. All plants flowered under 18-hr photoperiod and 50 mg/liter GA_3 sprays (Table 6). Higher or lower GA_3 concentrations were less effective in promoting flowering. Several plants which did not flower formed perched rosettes.

Petiole length at various photoperiods and temperatures. Photoperiod had a greater effect on petiole length than did temperature (Table 7). Petioles were shortest under the 10-hr photoperiod at 13°C. At each temperature, the petioles were always longer under the 18-hr photoperiod.

Effect of retardants. In the first experiment, chlormequat caused chlorosis of the leaf margins of older leaves and was ineffective in reducing petiole elongation. Daminozide at 7500 mg/liter caused some marginal burn of younger leaves and occasional upward cupping of mature leaves. All 3 concentrations tested significantly reduced petiole length (Table 8). Ancymidol at the highest concentration (100 mg/liter) also caused slight marginal leaf burning and upward cupping on a few mature leaves. Both the 50 mg/liter and the 100 mg/liter concentrations significantly retarded petiole elongation.

Table 4. Growth and flowering of 'McKana's Giant' at 4 photoperiod combinations before and after 4.5°C storage: 10 hr photoperiod before and after, 10 hr photoperiod before and 18 hr photoperiod after, 18 hr before and 10 hr after, and 18 hr before and after; 6 replications per treatment.

	Photoperiod combinations (hr)			
Weeks at 4.5°C	10/10	10/18	18/10	18/18
	Flowering (%)			
0	50	50	50	50
4	50	83	50	83
6	50	100	17	67
8	67	100	67	67
10	83	83	100	83
12	67	100	100	100
Mean	61	86	64	75
	Days from end of cold to visible bud			
0	159 ± 25[z]	104 ± 10	164 ± 19	105 ± 14
4	94 ± 29	55 ± 13	119 ± 13	78 ± 17
6	65 ± 11	69 ± 26	90	59 ± 20
8	64 ± 21	55 ± 11	76 ± 18	81 ± 34
10	56 ± 17	53 ± 9	72 ± 14	52 ± 15
12	46 ± 8	36 ± 8	58 ± 18	37 ± 9
Mean	81	62	97	69
	Days from visible bud to bloom			
0	16 ± 4[z]	14 ± 2	18 ± 2	23 ± 8
4	15 ± 0.6	18 ± 5	14 ± 2	21 ± 6
6	15 ± 1	19 ± 4	10	15 ± 3
8	25 ± 8	22 ± 4	21 ± 9	14 ± 5
10	20 ± 4	17 ± 3	17 ± 8	15 ± 3
12	23 ± 6	20 ± 5	17 ± 3	15 ± 2
Mean	19	18	16	17
	Plant height to first flower (cm)			
0	19 ± 13[z]	44 ± 14	23 ± 5	65 ± 7
4	40 ± 8	46 ± 13	24 ± 9	35 ± 21
6	40 ± 8	59 ± 12	14	35 ± 12
8	49 ± 5	59 ± 9	31 ± 8	28 ± 25
10	46 ± 8	46 ± 13	31 ± 19	38 ± 21
12	43 ± 9	52 ± 12	31 ± 11	45 ± 4
Mean	40	51	26	41

[z] ± SD

In the second experiment, all concentrations of daminozide and only the highest concentration of ancymidol significantly reduced petiole elongation (Table 9, Fig. 1). Marginal burning of leaves was observed on plants sprayed with 100 mg/liter ancymidol, and the 2 highest concentrations of daminozide, 6000–8000 mg/liter. Lengths of the 3 petioles measured on each plant did not differ significantly.

Effect of daminozide. Time from the end of cold storage to first visible bud was unaffected by daminozide spray, but days from first visible bud to the first bloom were decreased slightly with increasing daminozide concentrations. Daminozide reduced the height of the foliar canopy (Table 10). Flower size was reduced on the plants sprayed with daminozide, especially at the 8000 mg/liter level.

Discussion

Aquilegia x *hybrida* seedlings flowered poorly without cold storage regardless of photoperiod. A few plants flowered after an extended time under photoperiod treatment, usually 18 hr. Thus, vernalization probably is the primary natural environmental factor required to induce flowering. Neither the presence

Table 5. Growth and flowering of 'McKana's Giant' at various greenhouse temperatures and photoperiods after 10 weeks storage at 4.5°C; 6 replications per treatment.

Min. night Temp. (°C)	Photoperiod 10 hr	18 hr	Mean
	Flowering (%)		
13	100	100	100
18	83	83	83
24	83	67	75
Mean	89	83	
	Days from end of cold to visible bud		
13	65 ± 20[z]	45 ± 6	55
18	63 ± 24	53 ± 7	58
24	61 ± 10	37 ± 5	49
Mean	63	45	
	Days from visible bud to bloom		
13	15 ± 3[z]	20 ± 3	18
18	14 ± 7	19 ± 2	17
24	14 ± 6	13 ± 0.6	14
Mean	14	17	
	Plant height to first flower (cm)		
13	27 ± 15[z]	47 ± 6	37
18	37 ± 14	42 ± 4	40
24	19 ± 8	39 ± 4	29
Mean	28	43	

[z] ± SD

Table 6. Flowering of 'McKana's Giant' after gibberellic acid sprays under 2 photoperiods; 5 replications per treatment.

GA_3 (mg/liter)	Flowering (%) 10 hr	18 hr	Mean
0	0	17	9
25	17	17	17
50	33	100	67
75	0	33	17
100	33	17	25
Mean	17	37	

Table 7. Petiole lengths (cm) of 'McKana's Giant' under 6 temperature—photoperiod treatments; 10 replications per treatment.

Night temp (°C)	Petiole length (cm) 10 hr	18 hr	Mean
13	4.5 ± 1.9[z]	12.2 ± 2.3	8.3
18	7.7 ± 1.3	15.1 ± 2.9	11.4
24	6.4 ± 1.0	11.6 ± 1.8	9.0
Mean	6.2	13.0	

[z] ± SD

Table 8. 'McKana's Giant' heights 6 weeks after a growth retardant spray; 5 replications per treatment.

Growth retardant	Concn (mg/liter)	Plant ht (cm)
None	0	29
Daminozide	2500	20
	5000	14
	7500	13
Chlormequat	1500	28
	2500	31
	5000	27
Ancymidol	25	25
	50	21
	100	19
LSD 5%		6

Table 9. Petiole lengths for 'McKana's Giant' plants sprayed with several concentrations of 2 growth retardants; 10 replications per treatment.

Retardant (mg/liter)	Petiole length (cm)
Control	
0	11.9
Ancymidol	
25	11.4
50	9.1
75	8.9
100	7.7
Daminozide	
2000	7.0
4000	7.9
6000	5.7
8000	4.5
LSD 5%	2.4

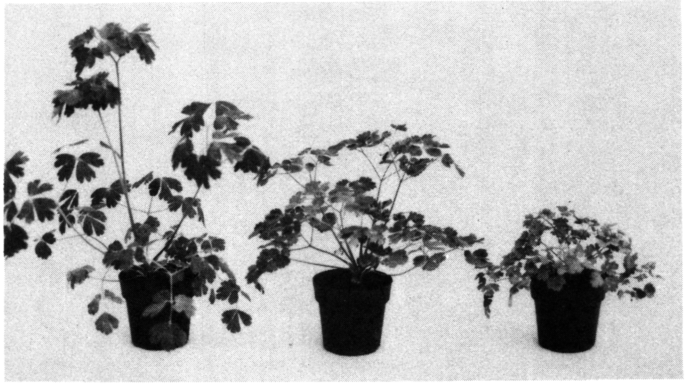

Fig. 1. 'McKana's Giant' plants (left to right) 0, 4000, and 8000 mg/liter daminozide spray.

of leaves nor light during cold storage was necessary for flower induction (Table 3), apparently because the central growing point, not leaf tissue, perceives the cold stimulus (2).

Very young (juvenile) seedlings did not respond to vernalization. The phase change to maturity occurred at slightly different ages depending on cultivar. For example, after 10 weeks of cold storage started at the 12-leaf stage, 'McKana's Giant' flowered 100% while only 83% of 'Fairyland' flowered (Table 2). The vernalization requirements also varied between cultivars. While 10 weeks of 4.5°C storage were required to induce 100% flowering of 'McKana's Giant', only 4 weeks were required for 'Fairyland' (Table 2).

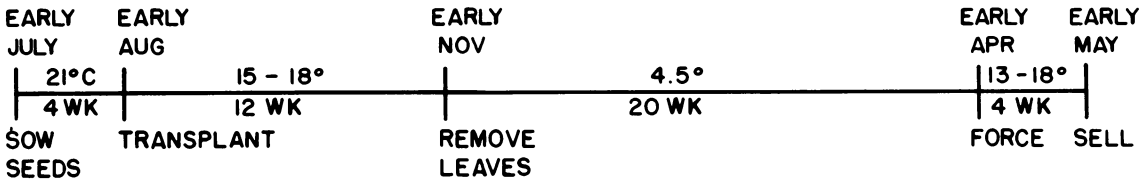

Fig. 2. Fuel efficient production scheme for *A.* x *hybrida* bedding plants.

Table 10. Growth and flowering data of 'McKana's Giant' plants after 12 weeks at 4.5°C followed by a daminozide spray; 6 replications per treatment.

Daminozide (mg/liter)	Time from end of cold to visible bud (days)	Time from visible bud to first bloom (days)	Plant ht (cm)	
			Vegetative growth	To first flower
0	49 ± 12[z]	20 ± 2[z]	25 ± 3[z]	44 ± 7[z]
4000	42 ± 5	15 ± 3	16 ± 2	23 ± 3
8000	52 ± 10	12 ± 4	10 ± 2	13 ± 5

[z] ± SD

While gibberellic acid promoted flowering without vernalization, weekly 50 mg/liter sprays weakened and elongated growth. Thus, there is no indication that GA_3 will be useful in cultural practice.

Long photoperiods promoted flower bud development and plant spread (petiole elongation), but temperature after cold storage had no marked effect on ability to flower or vegetative elongation. Thus, an optimum forcing environment promotes flowering by the use of long photoperiods, while resulting elongation is counteracted by applications of daminozide. A daminozide spray at 2500 mg/liter, applied as soon as the vernalized plants develop new leaf area, would prevent elongation.

Due to the small size of columbine rosettes at maturity (12–15 leaves), production of plants in small cell packs (48 cells per 28 × 53-cm flat) is feasible. Plants could be transplanted directly into the cell packs, grown to maturity, vernalized, forced, and sold in the same pack. Production could begin with a September 1 sowing with transplanting by October 1, after which the plants would be allowed to grow for 3 months at 18°C under natural photoperiods. Maturity at the 12–15 leaf stage would be attained by January 1, when the leaves could be cut off and 4.5° storage begun. Vernalization could be given in a refrigerated cooler, cold frame, or polyethelene-covered house heated to 4.5°. Flats could be shelved on racks, since light is not required during cold and the plants are leafless. Slow forcing at 13° to 18° could begin after the 12 weeks of cold on April 1, when the flats would have to be placed on benches or spread out on the floor in adequate light. Small plants, vegetative or in bud, would be ready for sale from May 1–15, but a colored picture would probably be necessary to enhance sales. This production scheme has a major drawback in that it utilizes 13 to 18° greenhouse space through November and December, a high fuel consumption period. An alternative scheme is longer but more fuel efficient (Fig. 2). Sowing in July would place the vegetative growth period earlier, in the warmer months from August 1 to November 1. The lengthened cold storage period would not be a problem in many climates.

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