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ASEXUAL PROPAGATION OF ANEMONE HUPEHENSIS
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JOAQUIN ANDRES CHONG

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ASEXUAL PROPAGATION OF ANEMONE HUPEHENSIS AND ANEMONE XHYBRIDA BY A ROOT-PLUG METHOD

By

Joaquín Andrés Chong

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ABSTRACT

ASEXUAL PROPAGATION OF ANEMONE HUPEHENSIS AND ANEMONE XHYBRIDA BY A ROOT-PLUG METHOD

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Anemones are commonly propagated by root cuttings; however, there is general uncertainty and unfamiliarity with this procedure. Studies were conducted to determine the influence of seven photoperiods and two cold treatments on the dry weight partitioning of anemone and subsequent impact on root cutting development. Shoot and root dry weights were obtained after 16.5- or 15-weeks of growth at 20 °C for *Anemone hupehensis* and *A.* x*hybrida* respectively, following either 0- or 15-weeks at 5 °C. Time to visible flower bud and to visible root bud, number of root-buds, and plant heights were determined after treatments for *A.* x*hybrida*. Root dry-weight partitioning decreased with increasing photoperiod and following cold treatment; however, cold increased root-bud number under photoperiods $\leq$ 12 h. A maximum of 60% and a minimum of 32% of dry weight were allocated to roots under 10-h photoperiods without cold, or 24-h photoperiods with cold, respectively.

A study was conducted to test a new method for more efficient propagation by root cuttings. A root-plug (RP) technique was developed that consisted of growing mother plants in a 1.5 L container with internally divided compartments, specifically a 128- and a 288-cell plug tray stacked vertically or three 288-cell plug trays horizontally or vertically oriented. Neither plant age (7.5- or 28-week) nor cold treatment (0- or 15-week) affected root plug regeneration. Top trays produced the most RPs, up to an average of 17.3. Root-plug production in plug trays decreased with increasing distance from the crown. Results with vertically oriented trays suggested that roots had to elongate to thicken, as middle trays produced more RPs than side trays.

In the memory of my father Víctor Chong Kam
because he always wanted us to study
and to my family Pilar, Maxim,
Víctor, and Viviana who
I deeply love.

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LITERATURE REVIEW

Plant Background

Anemones are members of Ranunculaceae and are commonly known as Windflower or Lily-of-the-field. Ranunculaceae contain more than 120 species distributed from zones 4 to 10. The minimum temperature range they tolerate is –35 to 5°C (Bailey, 1978).

Anemones can be divided into three main groups according to flowering season and their root morphology (Table 1).

Table 1. Anemone classification by flower timing and root morphology

Flower Timing	Spring	Early summer	Summer/autumn
Root Morphology	Tuberous rhizomes	Tuberous roots	Fibrous roots

Spring-flowering anemones are characterized by subterranean knobby, tuberous, bulblike rhizomes capable of producing new shoots. These anemones, such as *A. apennina* L., *A. blanda* Schott & Kotschy, *A. canadensis* L., *A. demissa* Hook F. & T. Thoms., *A. xlesseri*, *A. nemorosa* L., and *A. sylvestris L.*, are hardy from USDA zones 4 to 9, which have minimum temperatures from –35 to –1°C. USDA climate zones are based on the average annual minimum air temperature.

Early-summer-flowering anemones have enlarged, fleshy, subterranean tuberous roots; species include *A. coronaria L.*, *A.* x *fulgens* (DC.) Rchb. and *A. pavonina Lam.* Early summer anemones are fully hardy from USDA zones 4 to 10, which have minimum temperatures of –35 to 5°C.

Summer-autumn-flowering anemones are often called Japanese anemones and have fibrous roots. They are important perennial species used

as cut flowers and potted plants. In mid Michigan, these anemones flower from mid-August to early October. They are slow-growing perennials that can be difficult to establish and require well-drained and aerated soil, especially when first transplanted (Armitage, 1997).

Figure 1. Fibrous roots similar to summer-autumn anemones' roots (Graf, 1992)

Summer-autumn anemones have a determinate growth habit: the shoot growth stops with flowering. Leaves and shoots die back after flowering and decompose during the winter, leaving roots with root buds that overwinter. Anemones from this class are fully hardy in USDA zones 5 to 9, which have minimum temperatures from –23 to 1° C. Species include *A. hupehensis* var. *japonica* (Thunb.) Bowels & Stearn, *A. xhybrida* Paxt., *A. japonica*, *A. tomentosa* (Maxim) P'ei., and *A. vitifolia* Buch. Ham. ex DC. (Jelitto and Schacht, 1995; Armitage, 1997).

The most common anemones of the summer-autumn flowering group are *A. hupehensis* var. *japonica* and *A. xhybrida*. *Anemone hupehensis* var. *japonica* is from China (1844) although abundant in Japan, and is 60- to 75-cm tall, with five to seven rosy mauve sepals; some plants have male-sterile flowers. *Anemone xhybrida* flowers about a week later than *A. hupehensis* var. *japonica* and is 40-cm higher and 14-cm wider. Many *A. xhybrida* cultivars have semi- or double flowers. *Anemone xhybrida* 'Whirlwind' was introduced in 1887 and has

semidouble white flowers (Graham, 1990). *Anemone xhybrida* is a cross between *A. hupehensis* var. *japonica* and *A. vitifolia* (Bailey, 1978).

Plant Description

Anemone plants are composed of leaves and petioles, which form the majority of the upper plant structure. The main stem is nearly covered by the leaf stipules during the nonreproductive stage. As the plant enters its reproductive cycle, the main stem elongates producing flower shoots that carry non-base leaves and flower buds. Petioles, shoots, and leaves are pubescent. Leaves are serrated and, as with *Hedera helix*, change morphology as the plant matures (Miller and Goodin, 1976). Juvenile anemones initially produce single-lobed leaves; as the plant matures, there is a gradual change in subsequent leaves to a trilobed (mature) form. The final mature leaf form is a compound trilobed leaf (Chong et al., 2000).

Plant morphological characteristics change as plants develop (Whyte, 1938). As with many plants (O'Rourke, 1951), anemones reproduced from root cuttings undergo a reversion to juvenility, or return to a juvenile phase. The reversion has been characterized in some plants by morphological changes such as leaf shape, arrangement on the stem, leaf retention on deciduous plants, thorniness, pubescence, and more easily rooted tissues (O'Rourke, 1951; Fisher, 1961). In some cases, reversion to juvenility is false or transitory. Juvenile apple root cuttings taken from root sprouts flower significantly faster than an apple plant produced from seed. Early flowering suggests a putative juvenile stage and a

possible separation of the juvenile characteristic of easy rooting from a mature characteristic of flowering (Robinson and Schwabe, 1977a).

Plant Production

Anemones are propagated by seed, root cuttings, and division (Perry, 1998). Seed-propagated anemones are variable and not true to type. Armitage (1997) describes root-cutting propagation as more effective than shoot-division propagation. Root cuttings are used as the main source of commercially propagated material. Root buds will form from thick roots that are approximately 7-to 10-cm long and placed vertically in a moist, well-drained medium (Armitage, 1997).

Fisher (1961) described a method for root-cutting propagation of anemone and other perennials. Mature, one-year-old anemone plants are dug and harvested during mid-November. Root cuttings are placed in grape crates with soil and stored in a cold greenhouse until root buds emerge and roots grow. Then plants are planted in pots for sale in late spring or are planted in the field.

Root-Cutting Propagation

Roots perform different functions, including stabilization and water and mineral absorption. They also provide propagation material such as root cuttings, sprouts, and tubers. Some roots help certain plants survive adverse conditions by producing root buds (Peterson, 1975). Quaking aspen trees for example, are found from Arctic regions to Mexico and survive fires and icing conditions successfully because of their high root-budding capacity estimated to be one million sprouts per acre (Madison, 1999).

Root-cutting propagation depends on the ability of certain roots to produce root buds, which are defined as subterranean adventitious shoot buds located on lateral roots (Horvath, 1998). In some plants, root buds are responsible for the production of "root sprouts" or "root suckers." Root suckers emerge from subterranean roots and should not be confused with stem suckers that emerge from subterranean stems (Hudson, 1956). Root suckers emerging from a deep root push through the soil, etiolate, and often have a greater potential for rooting, even in species that do not normally root from stem tissue (Hudson, 1956). Hudson called the subterranean part of the sucker the "sucker base" and roots emerging from it "stem roots."

Macdonald (1986) described root cutting as a technique in which roots capable of developing root buds (shoots, suckers) are cut into individual pieces capable of regenerating plants. These root sprouts can be used as stem cuttings because they undergo a rejuvenility process, which makes them easy to root (O'Rourke, 1951). Stem cutting of root sprouts is not possible in *Anemone*.

Several authors (Wobst, 1868; Lindsay, 1877; anonymous, 1882; Fisher, 1961; Stoutemyer, 1968; Flemer, 1983; Macdonald, 1986; Bath and Jones, 1994; Fossel, 1998; Sanchez, 1999) have listed root bud–producing plants. Wobst (1868) found that the following families contain species that can reproduce by root cuttings: Apocynaceae, Asclepiadaceae, Bignoniaceae, Campanulaceae, Geraniaceae, Leguminosae, Papaveraceae, Passifloraceae, Plumbaginaceae, Rosaceae and Rubiaceae.

Root buds can be classified according to their mode of origin as "additional" or "reparative." Additional root buds emerge from undisturbed roots, and reparative root buds emerge from injured or senescent roots (Bosela, 1997). However, this classification system is subjective, since all roots encounter stresses and have minor injuries during their normal growth and development. In some species, these stresses or injuries might be enough to cause root bud formation (Bosela, 1999). Horticulturally, Hudson (1956) classified root buds by their position as either lateral buds that grow on the side of roots or terminal buds that grown on ends of roots that are injured or cut off. Hudson (1955b) proposed a classification

Table 2. Hudson's (1955b) approach to classification of modes of regeneration based on the study of relations between parent plants and root suckers

Type	Description
A	Root suckers growing near the parent plant, to which they normally remain attached.
B	Suckers produced naturally *from uninjured roots*, where the connecting root ultimately dies.
C	Suckers rarely produced except in response to *root injury*, though without the root's being wholly severed.
D	New plants produced from pieces of *root severed from the parent but remaining in situ*, provided the assimilating root system is not disturbed.
E	Regeneration possible from pieces of root, severed at both ends and with no undisturbed roots (the "root cutting" of horticulture).
F	Roots capable of regeneration after only special treatment (e.g., grafted with a nurse scion).
G	Plants apparently unable to produce root suckers under any circumstances.

by mode of plant regeneration based on relations between parts of the parent and the new plant. Root cuttings were classified into seven different types (Table 2).

Regeneration from root buds of types B and C occurs with a connecting root between parent shoot and root bud. The root bud receives continual supplies of water and polysaccharides from established roots and parent shoots, which fosters regeneration. In type D, connecting roots between parental shoot are eliminated, while in type E, root buds are not connected with either aerial shoot or assimilating root systems. Root cuttings (type E) depend on their own resources. Plants of types E and B can be propagated by root cuttings, but those of types C and D must be propagated in situ (Hudson, 1955b 1956).

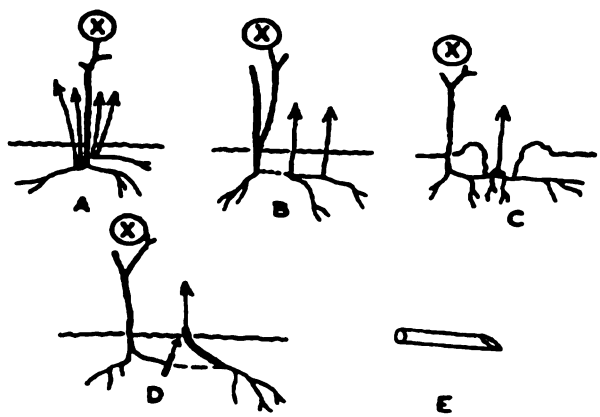


Figure 2. Types of regeneration from roots (Hudson, 1956)

Hudson (1955b) found that the response of a piece of root depends on two factors: the condition of the parent plant, which determines the *capacity* of the cutting to regenerate, and the effect of the environment and orientation of the cutting, which determine its *performance*. Plant capacity refers to required factors for regeneration success *before* removal of root cuttings from the mother plant. Plant performance refers to the environmental and hormonal factors that affect regeneration *after* root cuttings are separated from the mother plant. Root-

cutting success depends on the performance of the cutting and the capacity of the mother plant.

Advantages of Root-cutting Propagation

Root-cutting propagation is advantageous for plants that can produce root buds and are difficult to root from stems, do not bear seed, or do not produce seed that is true to type (Fisher, 1961; Macdonald, 1986). Root cuttings are easier to acclimatize than shoot cuttings (Hudson, 1955b). Root cuttings can eliminate the need for rootstocks, budding, and grafting (Robinson and Schwabe, 1977a). Root cuttings might have a longer life than grafted clones because grafting might cause scion-under-stock incompatibilities (Flemer, 1983). In general it is cheaper to propagate a root cutting than to grow an understock and then graft or bud it (Flemer, 1983).

Disadvantages of Root-cutting Propagation

There are several disadvantages to root-cutting propagation. Root cuttings have been described as one of the least frequently used and least desirable methods of vegetative propagation (Fisher, 1961; Heuser, 1977; Flemer, 1983). There are several reasons for this undesirability, among them that root-cutting propagation is a tedious and messy procedure. Root cuttings are more difficult to gather than aerial cuttings (Flemer, 1983). The method is labor intensive, the plants must be dug or the soil excavated to expose the roots, and the procedure must be carried out during the fall or winter (Heuser, 1977). There might be year-to-year differences in root material. In some years it might be difficult to obtain large quantities of root-cutting material (Flemer, 1983; Ede et

al., 1997). Chimeric material will most likely not come true to type if the mutated section is not propagated or if the mutation is in the shoot section. Little research has been aimed at the quantification of root cuttings per plant (Macdonald, 1986). Without knowledge of seasonal effects on the roots, it is impossible to morphologically distinguish roots capable of producing root buds (Hudson, 1954). Finally, there is a general uncertainty about and unfamiliarity with this procedure, making it unattractive.

Factors affecting root-buds

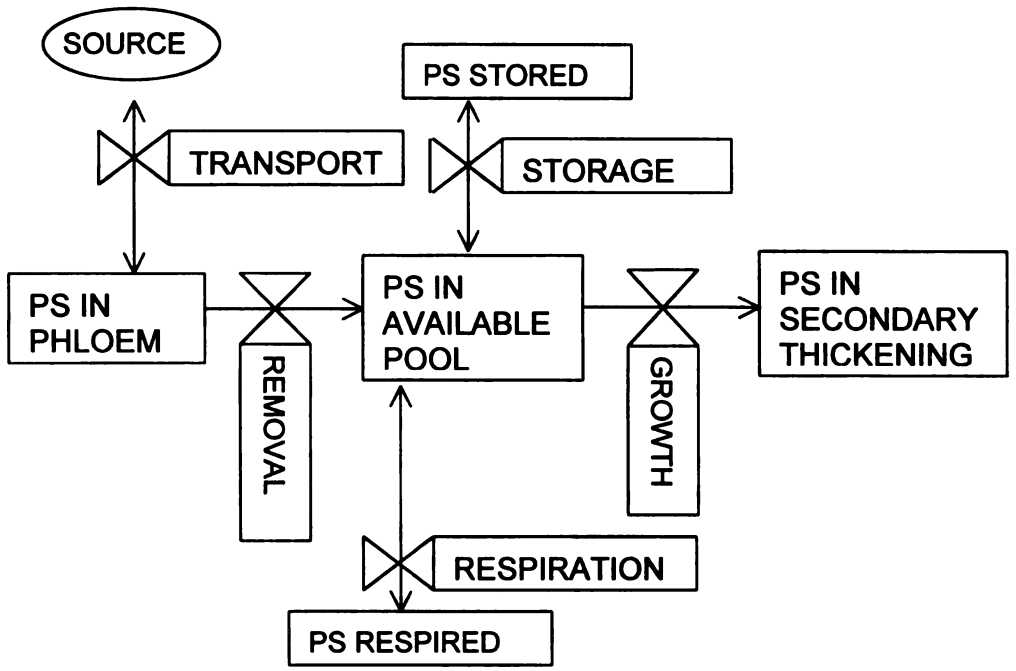
Several internal and environmental factors affect root bud initiation and development in root bud–producing plants: secondary thickening, seasonal fluctuations (cold and photoperiod), and hormonal control (Hudson, 1954; Robinson and Schwabe, 1977b).

Secondary Thickening

Hudson (1955a) described bud–producing roots as any piece (2 to 3 cm) of secondary thickened root capable of growing and developing during a certain season. Roots have to mature before they are capable of root-bud production. A mature root has a primary cortex and has developed some secondary tissue. "Ripeness to regenerate" is the developmental stage a root has to reach before root buds can regenerate (Dore, 1955). Wilson (1975) described the distribution of secondary thickening in tree root systems.

Photosynthates (PS) are transported from the chloroplastic tissue to the entire plant (Figure 3). Some PS are trapped in sink tissues and others flow to roots, causing secondary root thickening. The flow of PS to the roots is regulated

Figure 3. Flow of photosynthates (PS; Forrester, 1968; Wilson, 1975;). Boxes are levels, or amounts, valve symbols are rates of flow, and the oval is an external source or sink.



by rate of transport, storage, respiration, growth, and hormone interaction (Wilson, 1975). Factors that affect shoot growth affect root growth, and vice versa. Plants whose growth and photosynthesis is slow because of stress will have reduced root growth because less PS and hormones are transported to the roots (Wilson, 1975). In potato, for example, short-day photoperiods promote tuberization and long-day photoperiods delay it. However, the long-run yield of potato is better under long days than under short days, probably because less PS are produced during short days due to less light availability (Gregory, 1965). Generally in order to obtain thick roots, plants must have healthy photosynthate-producing shoots and leaves.

As previously mentioned, root buds develop only from secondary thickened roots, not from short-lived thin feeding roots. Root-bud proliferation and survival increase as root diameter and length increase presumably because available carbohydrate reserves are greater. Thick and longer roots allow numerous, vigorous, longer root sprouts (Robinson and Schwabe, 1977a; Lawes and Sim, 1980). Longer thicker root cuttings produce better root sprouts than shorter roots (Robinson and Schwabe, 1977a; Ghani and Cahalan, 1991). The PS transport capacity increases as root circumference increases (Wilson, 1975). However, root thickness and sprouting capabilities might be confounded with positional effects (McMillan, 1980). Gradients in hormone concentration change within roots as root position gets farther away from the crown (Figure 4). Also, root diameter decreases with increasing distance from the crown.

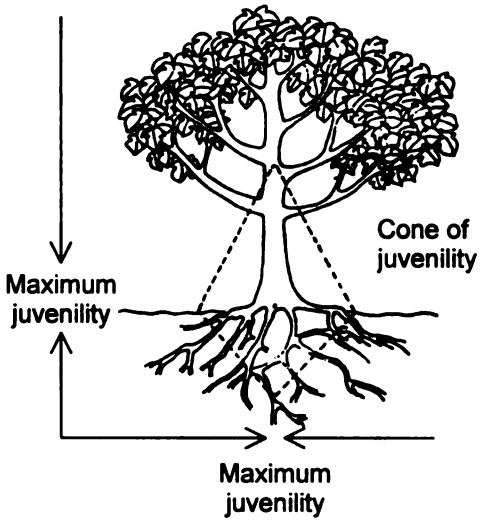


Figure 4. Juvenility cone. The closer to the crown, the better the possibility of regeneration (Dir and Heuser, 1987).

Studies on *Paulownia* spp. demonstrated that regenerative ability of root cuttings is highly correlated with root diameter and is not diminished with increasing distance (horizontally or vertically) from the root crown (Ede et al., 1997).

Seasonal Fluctuations

More than a hundred years ago, Wobst (1868) suggested that better regeneration from root cuttings occurred when cuttings were taken in early spring before the mother plant developed. He also described a method for nonhardy plants, which consisted in extracting roots in autumn and overwintering them in sand or soil in a frost-free environment, then cutting and growing them in the spring. An understanding of the mechanisms behind this empirical knowledge did not start until the mid-1950s, when Hudson published several scientific papers about the effects of seasonal fluctuations on root regeneration, growth, and development. Different physiological plant mechanisms are stressed as temperature and photoperiod change with the seasons.

Working with raspberry root cuttings (*Rubus idaeus* L.), Hudson (1954) found a strong seasonal response to root-bud regeneration. Root cuttings taken at certain periods of the year regenerated better than others did. He classified these months of good regeneration as the "on" season (Figure 5).

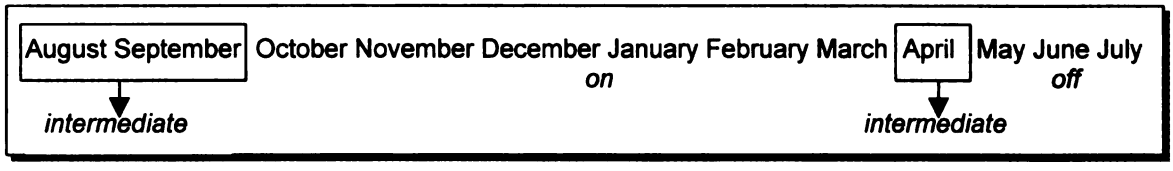


Figure 5. Hudson's (1954) description of seasonal cycles of *Rubus idaeus* L. root cuttings

The "on" season for *Rubus idaeus* is from October to March. In contrast there were months with little regeneration, which he called the intermediate season: April, August, and September. An "off" season was one in which no regeneration occurred from May through July. Further experiments by Hudson

(1955a) with horseradish (*Cochlearia armoracia* L.) and dandelion (*Taraxacum officinale* Weber ex Wiggers) demonstrated that not all plants follow a seasonal cycle, but have strong root-bud regeneration year round. However, some growth factors such as average number of shoots per cutting were affected by seasonal changes. The "on" seasons described by Hudson occurred during and after winter when cold was given to the root cuttings.

Evidence suggests that carbohydrate composition of roots has marked effects on growth and development during the next growing season. Carbohydrate reserves are important for the survival of the plant during the winter and resumption of growth in the spring. These reserves are associated with overwintering strategy in herbaceous perennials (Liu et al., 1993).

When rhubarb roots were forced before sufficient cold treatment, plants lacked vigor or did not grow (Fry, 1957). Low temperature treatment has been described as a "starter" that needs to accumulate. Fry (1957) described rhubarb plants that die back after cold as not having enough accumulated low temperatures. In 1975, Rutherford and Ali described carbohydrate changes during cold storage of rhubarb roots and found that rhubarb roots depend upon a cumulative effect of cold. Carbohydrates in the crowns and roots of rhubarb break down considerably (Rutherford et al., 1972). In general, cold storage caused a breakdown of the insoluble polysaccharides, accompanied by a corresponding increase in soluble sugars (Rutherford and Weston, 1968). Similar studies in *Panax ginseng* revealed that the glucose content in roots is higher in winter than spring, whereas starch content is lower (Liu et al., 1993).

This carbohydrate correlation with cold could explain Hudson's "on" cycle that coincided, in later experiments, with months of cold temperature and hydrolysis of polysaccharides to sugars of lower molecular weight.

Similar findings have been published on kiwifruit root cuttings that exhibited increased regeneration potential during winter and spring. Cuttings taken at other times of the year exhibit poor regeneration (Lawes and Sim, 1980). Robinson and Schwabe (1977a) observed similar seasonal fluctuation in apples.

Generally, sugars move to the roots and are converted to polysaccharides in autumn. Polysaccharides in the roots are then hydrolyzed during the winter, leaving soluble sugars that translocate to root sprouts and accelerate spring growth (Robinson and Schwabe, 1977b). Rutherford and Weston (1968) studied polysaccharide changes in roots and tubers during cold. Their studies on tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) and roots of chicory (*Cichorium intybus* L.) and dandelion showed that cold storage causes breakdown of insoluble polysaccharides, accompanied by increasing soluble sugars of lower molecular weight. Measurements of sprout height, performed by Robinson and Schwabe (1977a) on apple cultivars after one month of growth from root cuttings showed that cold-stored roots grew faster than those without cold treatment. However, after three months of growth, uncooled roots produced taller sprouts than cooled ones. Rooting of detached sprouts was faster on roots placed in cold storage for three months. Cold storage caused earlier rooting, suggesting polysaccharide hydrolysis, since cold allows better availability of soluble sugars.

Carbohydrates and hormones like indoleacetic acid (IAA) play important roles in shoot regeneration from roots of apple cultivars (Robinson and Schwabe, 1977b). Lawes and Sim (1980) suggested that a suitable balance between carbohydrates, cytokinin, and auxins affects regeneration. Heuser (1977) suggested that exogenously applied auxin and cytokinin could favorably stimulate root-cutting production.

Hormonal control

Seasonal changes have an effect on IAA concentrations. High concentrations cause root budding and inhibition of lateral stem shoots. Hormones play an important role in root-bud proliferation. Root budding in apple occurs only after roots are detached from the tree. Root cuttings exhibit polarity, where shoots develop at the proximal end, the end nearest the stem; roots, at the distal end, the end farthest from the parent plant, sometimes regeneration occurred regardless of orientation (Ede et al., 1997). Furthermore, a polar distribution of buds suggests a gradient of inhibition and promotion. Experiments on kiwifruit showed better sprout regeneration on roots placed horizontally than vertically, suggesting acropetal movement of auxins (Lawes and Sim, 1980). Root-bud production appears to be hormonally controlled because some plants produce root buds only after roots are detached from the mother plant. Robinson and Schwabe (1977b) studied endogenous auxins and exogenous application of auxins. Endogenous levels peaked during November, coinciding with the highest regeneration potential and survival of root cuttings. Exogenous application of IAA caused root-budding inhibition in apple cultivars, while 75 mg of benzylamino

purine (BAP; cytokinin) per liter increased proliferation to three to four times that of control plants (Robinson and Schwabe, 1977b). Similar studies on dwarf apple rootstocks (Kanazawa et al., 1978) and kiwifruit by Lawes and Sim (1980) showed that sucrose and benzylaminopurine applied to the root cuttings caused increased regeneration. Indolebutyric acid (IBA) application to the proximal end of the cutting caused inhibition of regeneration. Gibberellic acid and Benzylaminopurine application increased the number of shoots per cutting.

Polar distribution of buds in root cuttings suggests a hormone gradient of inhibition and promotion. Farmer (1962) working with, *Populus tremuloides* Michx., visualized aspen roots as a "physiological" extension of stems and observed that root buds on these roots develop when inhibiting influences were removed. Farmer's "apical dominance theory", described as an extension of apical dominance or correlative inhibition mechanism, was supported in his publication by the stimulation of suckering by root pruning. Furthermore, sprouting did not occur severed roots that were still attached to the stems. Maini (1968) reported similar results of inhibition of regeneration while roots were still attached to the mother plant. Correlative inhibition is a hormonal signal mechanism whose mode of action is unknown and which controls lateral bud and root-bud growth. Horvath's (1998) studies with *Euphorbia esula* L. showed correlative inhibition of root buds required the presence of leaves and meristems. The lack of root-bud emergence in *E. esula* was associated with an extension of apical dominance caused by meristematically produced auxin and possibly an unknown carbohydrate. Plants with a correlative inhibition mechanism tend to

produce reparative root buds when the roots are stressed or cut from the mother plant, because the inhibition mechanism is removed. In some varieties of apples, warmth causes a lower auxin concentration in cut roots, which promotes root buds, because correlative inhibition does not function.

Correlative inhibition is also exhibited on root sprouts; they produce hormones that can inhibit further bud initiation. Lindsay (1877) observed that, regardless of the root-cutting length, the first bud that usually regenerated in a root cutting was the one that grew, inhibiting the remaining buds. When sprouts are detached from roots, root-bud proliferation increases, probably because of active regrowth from cut surfaces and removal of shoot inhibition (Robinson and Schwabe, 1977a). Regenerated sprouts in a root cutting can more actively inhibit other root buds from proliferating than gradual depletion of reserves (Robinson and Schwabe, 1977b).

As expected in auxin regulated mechanisms, light appears to play an important role. Ghani and Cahalan (1991) showed that most of the root sprouts produced came from root parts exposed to light, which suggests a light-induced auxin translocation to the buried distal end of the root-cutting.

Conclusion

Asexual propagation by root cuttings is a labor-intensive procedure. The floriculture-perennial industry requires new methods of root-cutting propagation to achieve fast, reliable, and homogeneous material. The industry must be able to quantify root cuttings produced per plant. Many of the plant requirements described by Hudson in the 1950s, plant capacity and performance are unknown

in many perennial species. This lack of knowledge can result in unsuccessful root-cutting propagation, making it unpopular among growers. New asexual propagation methods with the proper plant environmental requirements can increase market production of root cutting–propagated plants.

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SECTION II

EFFECTS OF PHOTOPERIOD AND COLD TREATMENT ON DRY WEIGHT PARTITIONING OF *ANEMONE HUPEHENSIS* AND *ANEMONE XHYBRIDA*, AND A ROOT-PLUG METHOD FOR PROPAGATION OF ROOT CUTTINGS.

Effects of Photoperiod and Cold Treatment on Dry Weight Partitioning of *Anemone hupehensis* and *Anemone* x*hybrida*, and a Root-Plug Method for Propagation of Root Cuttings.

Joaquín A. Chong[1], Royal Heins[2], Arthur C. Cameron[2], and William Carlson[2]

Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

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[1] Graduate Student.

[2] Professor.

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Additional index words. Critical photoperiod, root bud, asexual propagation.

Abstract

The effects of photoperiod and cold treatment on dry-weight partitioning of *Anemone hupehensis* (Thunb.) Bowels & Steam and *A.* x*hybrida* (Paxt.) were determined and a root-plug method for asexual propagation of root cuttings was developed and tested. Either before or following a 15-week treatment at 5°C plants were grown under 10, 12, 13, 14, 16, or 24 hours of continuous light or 9 hours with a 4-hour night brake lighting period. Shoot and root dry weights were obtained after 16.5 or 15 weeks of growth for *A. hupehensis* and *A.* x*hybrida* respectively; following either 0 or 15 weeks of cold for *A.* x*hybrida*. Shoot-dry-weight partitioning was greater on reproductive than vegetative plants. Root-dry-weight partitioning decreased with increasing photoperiod and following cold. Cold increased root-buds and reduced root-bud appearance time. A maximum of 60% and a minimum of 32% of total dry weight was allocated to roots under 10-hr photoperiods without cold or 24-hour-photoperiods with cold, respectively.

The root-plug experiment was conducted to test a new method for more efficient root-cutting propagation. A root-plug (RP) technique was developed that consisted of growing mother plants in a 1.5 litter container with internally divided compartments, specifically a 128-cell-plug tray above a 288-cell-plug tray or three 288-cell-plug trays horizontally or vertically oriented. Neither tested plant age (7.5- or 28-week) nor cold treatment (0- or 15-week) affected root plug regeneration. Top trays produced the most RPs, up to an average of 17.3 per 25 cells. Root-plug production in plug trays decreased with increasing distance from the crown. Vertically oriented trays suggested that roots had to elongate to thicken as middle trays produced more RPs than side trays.

Introduction

Asexual propagation allows clonal propagation and perpetuation of germplasm. Sterile plants only can be propagated asexually, sometimes by root cuttings. Macdonald (1986) described root cutting as a technique in which roots capable of developing root buds (shoots, suckers) are cut into individual pieces capable of regenerating plants. Adventitious buds develop from roots that have been cut into individual pieces and planted upright in a growing medium. Root-cutting propagation depends on the ability of certain roots to produce root buds that subsequently form shoots.

Root buds are defined as subterranean adventitious shoot buds located on lateral roots (Horvath, 1998). Hudson (1955) described bud-producing roots as a piece (2 to 3 cm) of secondary thickened root capable of growing buds during a certain season. Roots must be mature or have reached the "ripeness to regenerate" developmental phase (Dore, 1955), before they are capable of root-bud production. A mature root has a primary cortex and has developed some secondary tissue.

More than a century ago, Wobst (1868) suggested that better regeneration from root cuttings occurred when cuttings were taken in early spring before the mother plant developed. An understanding of the mechanisms behind this empirical knowledge did not start until the mid-1950s when Hudson published several scientific papers about the effects of seasonal fluctuations on root regeneration, growth, and development. Hudson (1954) classified months of good regeneration as "on" season, months with little

regeneration as intermediate season, and months with no regeneration as "off" season. He also found that not all species followed a seasonal cycle (Hudson, 1955).

Later studies suggested that carbohydrate composition of roots had marked effects on growth and development of root cuttings (Robinson and Schwabe, 1977b). Controlling the partitioning of carbohydrates becomes important for proper root-cutting development. Larger carbohydrate reserves can increase survival of plants during winter and promote growth resumption in the spring. Reserves are associated with the overwintering strategy in herbaceous perennials (Liu et al., 1993). Reserves partition between shoots and roots and changes in partitioning are probably stimulated by photoperiod and the plant developmental phase. In potato, for example, it is generally agreed that short days (SD) promote tuberization and long days (LD) delay it (Gregory, 1965). A common parameter for evaluating partitioning between shoots and roots is the shoot : root ratio (Wolfgang, 1979) or, inversely, the root : shoot ratio.

Generally, sugars move to the roots and are converted to polysaccharides in autumn. The polysaccharides are then hydrolyzed during the winter, leaving soluble sugars that translocate to root sprouts and accelerate spring growth (Robinson and Schwabe, 1977b). Rutherford and Weston (1968) studied polysaccharide changes in roots and tubers exposed to cold. Their studies on tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) and roots of chicory (*Cichorium intybus* L.) and dandelion showed that cold

storage causes breakdown of insoluble polysaccharides, accompanied by an increase in soluble sugars of lower molecular weight.

Polar distribution of buds in root cuttings suggests a hormone gradient of inhibition and promotion. Farmer (1962) working with *Populus tremuloides* Michx., visualized aspen roots as a "physiological" extension of stems and observed that root buds on these roots develop when inhibiting influences were removed. Farmer's "apical dominance theory" is described as an extension of apical dominance or a correlative inhibition mechanism was supported in his publication by the stimulation of suckering by root pruning. Furthermore, sprouting did not occurred on severed roots that were still attached to the stems. Maini (1968) reported similar results of inhibition of regeneration while roots were still attached to the mother plant. Correlative inhibition is a hormonal signal mechanism that controls lateral bud and root-bud growth and whose mode of action is unknown.

Correlative inhibition is also exhibited on root sprouts: they produce hormones that can inhibit further bud initiation. As early as 1877, Lindsay observed that, regardless of the root-cutting length, the first bud that regenerated in a root cutting was the one that grew first, and it inhibited the remaining buds. Root-bud proliferation increases when sprouts are detached from roots, probably because of active regrowth from cut surfaces and removal of shoot inhibition (Robinson and Schwabe, 1977a). Regenerated sprouts in a root cutting can more actively inhibit other root buds from proliferating than gradual depletion of reserves (Robinson and Schwabe, 1977b).

Root-cutting propagation is important when the plant has difficult-to-root stems, does not bear seed, or does not produce seed that is true to type (Fisher, 1961; Macdonald, 1986). Root cuttings can eliminate the need for rootstocks, budding, and grafting (Robinson and Schwabe, 1977a). Root cuttings might have a longer life than grafted clones because grafting might cause scion understock incompatibilities (Flemer, 1983). There are several disadvantages to root-cutting propagation. Root cuttings have been described as one of the least frequently used and least desirable methods of vegetative propagation (Fisher, 1961; Flemer, 1983; Heuser, 1977) for several reasons. Root-cutting propagation is tedious and messy. Root cuttings are more difficult to gather than aerial cuttings (Flemer, 1983). The method is labor intensive, the plants must be dug or the soil excavated to expose the roots, and the procedure must be carried out during the fall or winter (Heuser, 1977). There might be yearly differences in root material. In some years it might be difficult to obtain large quantities of root-cutting material (Ede et al., 1997; Flemer, 1983). Chimeric material might not come true to type if the mutated section is not propagated or if the mutation is in the shoot section. Little research has been aimed at the quantification of root cuttings per plant (Macdonald, 1986). Without knowledge of seasonal effects on the roots, it is impossible to morphologically distinguish roots capable of producing root buds (Hudson, 1954). Finally, there is a general uncertainty about and unfamiliarity with this procedure.

Anemones can be propagated by seed, root cuttings or division (Perry, 1998). Seed propagated anemones are variable and not true to type. Armitage (1997) described anemone root-cutting propagation as more effective than shoot division propagation. Root cuttings are used as the main source of commercially propagated material even though root-cutting propagation varies yearly and protocols are unavailable.

The goal of our research was to understand how photoperiod and cold treatment affect anemone dry-weight (DW) partitioning and control these factors to increase root mass and root-bud regeneration. Stimulating root mass or dry weight partitioning to roots can facilitate root-bud regeneration as larger root reserves can improve regeneration (Robinson and Schwabe, 1977a). The second goal of our research was to develop a procedure to facilitate, accelerate and homogenize asexual propagation by root cuttings.

Materials and methods

Plant material

Dry Weight Partitioning Experiment. Asexually propagated *Anemone hupehensis* and *A.* x*hybrida* 'Whirlwind' plants arrived in 36-cell plug trays from a commercial greenhouse (Greenleaf Enterprises, Inc., Leola, Penn.) on September 17, 1998, and on November 11, 1998, respectively. Plants varied in size and number between plug cells and were therefore singulated.

Root-plug Experiment. Anemone x*hybrida* 'Whirlwind' and *Anemone hupehensis* were propagated by root cuttings from one-year-old plants

received in one-gallon containers on June 18, 1998. When propagated, all plants from root cuttings were planted in 32-cell plug trays (130 mL per cell) from Summit Plastic Co. (Tallmadge, Ohio) in the same medium used in the DW partitioning experiment.

Plant culture

Plants were top-watered and fertilized at every irrigation throughout all experiments. The nutrient solution was well water (EC of 0.70 $mS \cdot cm^{-1}$ and 105, 35, and 23 mg Ca, Mg, and $S \cdot L^{-1}$, respectively; pH 6.0) acidified with H_2SO_4 to a titratable alkalinity of 130 mg $CaCO_3 \cdot L^{-1}$and water soluble fertilizer providing 125-12-125-13 N-P-K-Ca ($mg \cdot L^{-1}$; 30% ammoniacal N) plus 1.0, 0.5, 0.5, 0.5, 0.1, and 0.1 Fe, Mn, Zn, Cu, B, and Mo, respectively ($mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago, Ill.).

Dry weight partitioning experiment

In the DW partitioning experiment, plants were randomly divided into two groups, a 0- and 15-week cold treatment. Uncooled plants were transplanted to 13-cm square plastic containers (1.1 L) filled with a commercial soiless medium composed of pine bark, vermiculite, Canadian sphagnum peat, and coarse perlite with a wetting agent, lime, and starter fertilizer charge (High Porosity Mix, Strong-Lite Products, Pine Bluff, Ark.) and moved to photoperiod treatments.

Photoperiods were provided by pulling opaque black cloth from 1700 until 0800 HR every day over all benches; incandescent lamps ($\approx 2\ \mu mol \cdot m^{-2} \cdot s^{-1}$) were turned on to complete each photoperiod. Ten plants were placed per

bench and incandescent lights treatments set to complete 10-, 12-, 13-, 14-, 16- or 24-h photoperiods as day extension, and one was set as a 4-h night interruption (NI) from 2200 to 0200 HR.

Cold consisted in 15 weeks of 5 °C in a cooler. After cold treatment, plants were transplanted to the 13-cm square plastic containers (1.1 L) and placed under photoperiods as described for plants without cold.

Root-plug experiment

Two cultivars were tested in the root-plug (RP) experiment, *Anemone* x*hybrida* and *A. hupehensis.* The *A.* x*hybrida* experiment was a factorial with three factors: plant age, tray treatment and cold. For *A.* x*hybrida*, two plant ages were tested; plants propagated on September 21, 1998, or February 12, 1999. At the start of the experiment on April 6, 1999, all plants propagated on September 21 had trilobate compound leaves, and plants propagated on February 12 had monolobate simple leaves.

Plants were planted in 1.5-L square black containers 13 cm wide and 13 cm tall (Super Square, National Polymers, Inc., Lakeville, Minn.) with plug trays cut to fit inside each pot (Fig. 1). Two trays sizes were tested, a 288-cell plug tray consisting in 2-cm-wide and 2.6-cm-tall cells with 6 mL per cell, and a 128-cell plug tray consisting in 2.7-cm-wide and 5-cm-tall cells with 25 mL per cell (Blackmore Co., Belleville, Mich.).

The tray-treatment factor for *A.* x*hybrida* plants had two levels: the first had one cut-to-fit 128-cell plug tray set above a cut-to-fit 288-cell plug tray inside the pot (128/288 treatment; Fig. 1A). The top 128-cell tray had nine

complete cells and seven half cells, and the bottom 288-cell plug tray was cut to fit in the bottom of the container, leaving 20 complete cells and five half cells. The second treatment had three 288-cell plug trays stacked horizontally inside the container (288/288/288 treatment; Fig. 1B). The top two trays had 25 complete cells; the bottom tray, 20 complete and five half cells. The bottoms of all 128-cell plugs were cut off in the 128/288 treatment, and drainage holes of the top two 288-cell plugs trays in the 288/288/288 treatment were enlarged with a 1-cm file to allow root growth into the next trays.

Photoperiod for the RP experiment remained at 16 h throughout the experiment and was provided by day extension lighting at a minimum of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux *(PPF)* and an average of 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height with high-pressure sodium (HPS) lamps from 1700 to 2400 HR.

The cold factor had two levels, 0 and 6 weeks. After 15 weeks of plant growth from the transplant day (April 6), half of the *A.* x*hybrida* and *A. hupehensis* plants were transferred to a 5 °C controlled-environment chamber for 6 weeks. The other half was cut to determine shoot DWs; for the cooled plants, they were determined after cold. The roots and plug trays were removed from the 13-cm container, and plug trays were cut with a sharp knife to separate them from the plant soil mass and trays. Plug trays were placed under the 16-h photoperiod for root-bud formation. Root plugs regenerated after 5 weeks of growth were counted on each tray.

The *A. hupehensis* experiment was a factorial with two factors: tray treatment and cold. The *A. hupehensis* experiment started on June 9, 1999, with plants propagated by root cutting on March 3, 1999. The tray treatment factor had three levels, the first two as previously described for the *A. xhybrida* experiment. The third level, 288-288/288 treatment (Fig. 1C), consisted in using three 288-cell cut-to-fit plugs trays placed vertically inside a container. The plants were transplanted between a 288-cell tray and two, 288-cell vertically placed trays, bottoms to the sides. The drainage holes of the 288-cell center plug tray were enlarged with a 1-cm file to allow roots to grow to the adjacent tray.

Tray treatments occupied ≈0.9 L of the volume of the container, leaving 0.6 L where the plant was transplanted. Trays and containers were filled with a commercial soilless medium composed of peat (70%), coarse perlite (21%), vermiculite (9%), a wetting agent, lime (calcitic and dolomitic), and a starter fertilizer charge (Suremix Perlite, Sure Michigan Growers Products, Inc., Galesburg, Mich.).

Experimental settings

All experiments were conduced in the Plant and Soil Sciences Research Greenhouses at Michigan State University, East Lansing, Michigan. Greenhouse temperatures were set the same for DW and RP experiments: 20 °C. The average daily temperature (ADT) and daily light integral were monitored with a CR-10 datalogger (Campbell Scientific, Logan, Utah) by using 36-gauge type-E thermocouples and quantum sensors (LI-COR),

respectively. The datalogger collected temperature and light measurements every 10 s and recorded the hourly average. Under each black cloth in the DW experiment, heat was provided when needed to maintain a night temperature of 20 °C by using a 1500-W electric heater (Model T771, Rival Manufacturing Co., Sedalia, Mo.).

Supplemental lighting for both the DW and RP experiments was switched on from 0800 to 1700 HR if the ambient greenhouse *PPF* was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and switched off if it exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Four-hundred-watt HPS lamps provided a minimum of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* and an average of 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height.

Lighting in the cooler was the same for both experiments and was from cool-white fluorescent (F96T12/CW/VHO, Philips, Somerset, N.J.) lamps from 0800 to 1700 HR. The *PPF* from the cool-white fluorescent lamps was ≈100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height. Plants in the chamber were watered as needed (approximately two times per week) with well water acidified with sulfuric acid (H_2SO_4) to a pH of approximately 6.0.

Data collection

Dry weight partitioning. Dates the root bud was first visible were recorded approximately three times a week for *A. xhybrida*. Root buds were considered first visible when they could be seen in soil mass after it was lifted from the container. Shoot and root DWs were obtained after 15 weeks of forcing for 'Whirlwind' and 16.5 weeks for *A. hupehensis*. Shoots and roots were dried for 3 days in a NAPCO model 630 oven (National Appliance Co.

Portland, Ore.) at a setting of 65 °C. For *A.* x *hybrida*, all the soil was separated from the roots before plants were dried, and root buds longer than 0.75 cm were counted. The DW experiments were analyzed as a factorial design with two factors, cold and photoperiod treatment: cold had two levels and photoperiod has seven levels. Ten plants per treatment were used and data were analyzed with SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and a general linear model (GLM) procedure.

Root-Plug Experiment. Shoot DWs were obtained from mother plants after 15 weeks of forcing for plants not treated with cold and after 6 weeks for cold-treated mother plants. Number of regenerated RPs per tray level was counted 5 weeks after cutting.

The *A.* x*hybrida* and *A. hupehensis* experiments were analyzed as a three- and two-way factorial for DWs, respectively. Dry weights, total RPs, and weeks of cold were analyzed with SAS's (SAS Institute, Cary, N.C.) ANOVA and a GLM procedure. Tray levels were analyzed separately with SAS's mixed procedure, repeated variables with a compound symmetry type.

Results

Dry weight partitioning experiment

Anemone hupehensis. Dry weight partitioning was affected by photoperiod. Root DW decreased when photoperiod exceeded 14 h, the same photoperiod that induced flower formation (Fig. 2A). Shoot DW, however, increased with increasing photoperiod, with the greatest increase occurring from

the 10- to 14-h photoperiod treatment. Plant variability as shown by 95% confidence intervals was greater for shoots than for roots. The maximum visible flower bud percentage was 60 under the 24-h photoperiod. Dry weight root : shoot ratio linearly decreased statistically with increasing photoperiod from 10 to 24 h, although most change occurred from 10- to 16-h photoperiod (Fig. 2B).

Anemone xhybrida. As for *A. hupehensis* (Fig. 2A), not all uncooled *A. xhybrida* plants flowered, even under continuous light (Fig. 3A). All plants cold-treated for 15 weeks initiated flowers under ≥14-h photoperiods or NI. Dry weight in the uncooled plants increased linearly with increasing photoperiod from 10 to 24 h. Dry weight for plants cold-treated for 15 weeks increased from the 10- to 13-h photoperiod, then decreased (Fig. 3B). The 14-h photoperiod data point was excluded from the analysis, since plants were closer to the cooling pads and were apparently affected by the lower greenhouse temperature. The root : shoot ratio for plants with a cold treatment was lower than for plants without cold (Fig. 3C), however, cold treated plants had grater total DW (Fig. 3A and 3B), up to 13.8 g, except for 24-h photoperiod plants.

Without cold, DW partitioning to roots decreased as photoperiod increased from 10 to 14 h (Fig. 3C) and then remained the same. Following cold, root DW partitioning was least favored by photoperiods longer than 12 h. The root : shoot ratio decreased linearly as photoperiod increased from 10 to 24 h.

Root bud production appears earlier under 13-h photoperiod cold treated plants, however no horticulturally important trends were shown (Fig. 4A). For cold treated plants, root-bud number was highest in the 10- to 12-h photoperiod,

then decreased as photoperiod increased (Fig. 4B). Root bud numbers were similar across all photoperiods for uncooled plants. Plant height increased linearly for cold treated and untreated plants as photoperiod increased (Fig. 4C). Cold treated plants were taller than uncooled plants.

Root-plug experiment

Anemone xhybrida. Shoot DW for plants grown for 15 weeks or 15 weeks following 6 weeks of cold averaged 30.4 g and was not significantly different for plant age or tray treatment (data not shown). Weeks of cold did not significantly affect total RPs produced for any tray level: therefore, data were pooled for further analysis. Total RPs per 0.9 L container volume was calculated to assess RPs produced per volume for both tray treatments (Fig. 5A and 5B). In the 128/288 treatment (Fig. 5A), average RP production was 10.6 and 12.1 for the 7.5- and 28-week-old mother plants, respectively. The 7.5-week-old plants in the 288/288/288 treatment (Fig. 5B) produced an average of 27.5 RPs, with the 28-week-old plants producing an average of 11.4 RPs.

The most RPs produced in both plug tray treatments were in the top level (Fig. 5C and 5D). In the 128/288 treatment, average RP production was a statistically similar 10.3 and 11.8 for 7.5- and 28-week mother plants, respectively. The top, middle, and bottom of the 7.5-week-old plants in the 288/288/288 treatment produced an average of 17.3, 7.7, and 0.5 RPs, respectively (Fig. 5D). Few or no RPs were produced at the bottom levels. For the 28-week mother plants, the average RP production was 9.4, 2.0, and 0, for top, middle, and bottom trays, respectively (Fig. 5D).

Anemone hupehensis. Only one plant age was tested for *A. hupehensis.* Total RP production in the 128/288 treatment averaged 9 (Fig. 6A); in the 288/288/288 treatment, 13.5 (Fig. 6B). As with the *A.* x*hybrida* experiment, upper levels produced the most RPs, averaging 8.5 and 10.3 for top levels of the 128/288 and 288/288/288 treatments, respectively.

The 288-288/288 treatment produced a combined average of 9.4 RPs for all levels (Fig. 6C). The left, middle, and right trays averaged 2.2, 5.2, and 2.0 RPs, respectively.

Discussion

Dry weight partitioning experiment

In 1955, Hudson stated that root cutting behavior depended on two factors, plant capacity and plant performance. Plant capacity refers to required factors required for regeneration success before root cuttings are taken from the mother plants. Plant performance refers to the environmental and hormonal factors that affect root cuttings after the roots are separated from the mother plant. The purpose of the DW partitioning experiment was to understand how photoperiods and cold treatment affect *A. hupehensis* and *A.* x*hybrida* root-to-shoot DW partitioning, a plant capacity issue. Dry weight partitioning between roots and shoots can help us identify the optimal environmental conditions for mother-plant growth. Larger root DWs are synonymous with larger storage reserves, which presumably mean more photosynthates that increase root-bud regeneration (Robinson and Schwabe, 1977a).

The data showed that photoperiod and cold influenced DW partitioning between shoots and roots. The data suggested that as the plant enters its reproductive development, plant shoots and flowers become strong sinks and reduce partitioning to the roots. In general, photoperiods above 14 h caused bolting, flowering, and the shift of DW partitioning to the flower producing organs (Fig. 2A, 2B; Fig. 3A, 3B, 3C; Fig. 4C). According to data from 15- and 16.5-week-old plants, the best photoperiod for root weight partitioning is under short, noninductive photoperiods. It is possible, however, that a longer growth duration under LD could yield more root DW than under SD, because less photosynthates are produced during short days (Gregory, 1965).

Anemone has been classified as a cold-beneficial LD plant; i.e., after a cold treatment, plant growth is more vigorous (E. Clough, unpublished data). Cold generally decreased DW partitioning to roots and increased DW of shoots (Fig. 3C).

Cold increased visible flower-bud percentage (Fig. 3B) and increased the number of root buds formed per plant under photoperiods shorted than 12 h (Fig. 4B). Root bud formation on anemone does not appear to be directly controlled by a correlative inhibition mechanism, as Maini (1968) described, but rather conforms to Hudson's (1955) description of bud-producing roots, any piece (2 to 3 cm) of secondary thickened root capable of growing and developing during a certain season. In this experiment, regenerated root buds were produced from roots ≈0.2 mm or larger in diameter.

Root-plug experiment

The purpose of the RP experiment was to devise a new method of root-cutting propagation to ease root handling, avoid polarity problems, and make propagation more efficient and quantifiable. As expected, more regeneration occurred closer to the crown where the roots were thicker (Fig. 5C, 5D, 6D and 6E), results in agreement with those of other studies (Dir and Heuser, 1987; Ghani and Cahalan, 1991; Robinson and Schwabe, 1977a; Wilson, 1975). The plant crowns sat on the first tray inside the pots, except for those in the 288-288/288 tray treatment, and as roots grew into the top plug trays, their ability to get into middle or bottom trays was reduced because of the funneled architecture of the trays. This architecture forced some roots to grow alongside the plug trays; these roots expanded freely to the bottom of the container, where secondary thickening occurred, and some were capable of root-bud production. The need for expansion for root thickening is suggested by the 288-288/288 treatment (Fig. 6F) in which the middle tray produced more RPs than the left tray, presumably because the center tray's could expand to the right tray. To avoid the root funneling problem, we are conducting another experiment in which plants are grown over light egg crates that are positioned vertically and horizontally (unpublished data) under a 13- and 16-h photoperiod.

Root plugs can be defined as plantlets produced from roots that grow into internal divided compartments inside a container; the compartments are later cut into layers and root buds are regenerated from them. Root plugs might prevent polarity problems; increase time efficiency; homogenize the crop, since root buds

are regenerated at the same time; reduce root handling; and help quantify the plantlets produced per pot. However, there is a need for a manufactured container with internal unfunneled divisions that would facilitate the setup of the mother plant transplanting.

In conclusion, as any asexual propagation, anemone root-cutting propagation requires disease free mother plants preferably grown over a season to increase root thickness. For industry production plugs should be uniform and homogeneous root cuttings should preferably be regenerated at the same time or sorted according to size when separated form the root mass. Although, short photoperiods can increase root dry weight partitioning, long photoperiods without cold increased total root mass (Fig. 3A). Future investigation shall include a combination of weeks of long then short photoperiods, which might increase root mass and root partitioning, respectively. Other possibilities might include spray applications of ethephon to chemically remove flower buds and force a sink partitioning to roots.

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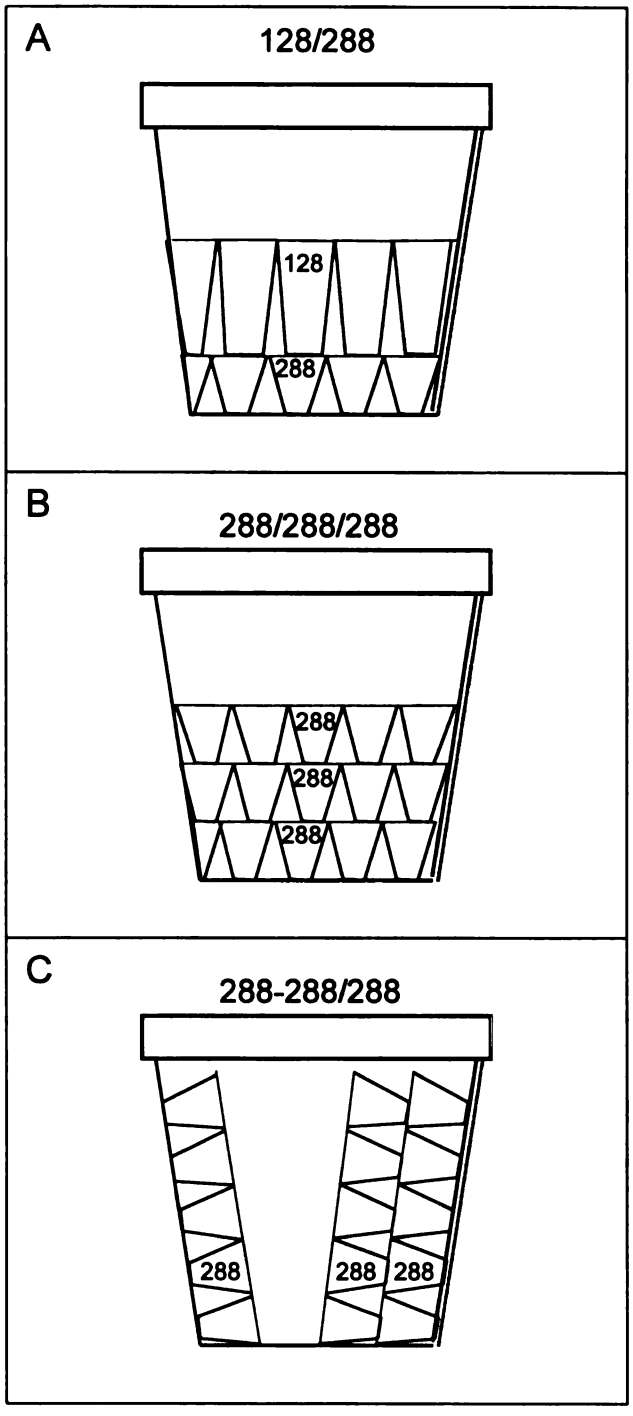

Figure 1. Drawing of root-plug tray treatments for *Anemone hupehensis* (A and B) and *A. xhybrida* (A, B and C) root-plug experiment. Tray treatments 128/288, 288/288/288, and 288-288/288 are represented in figures A, B, and C, respectively.

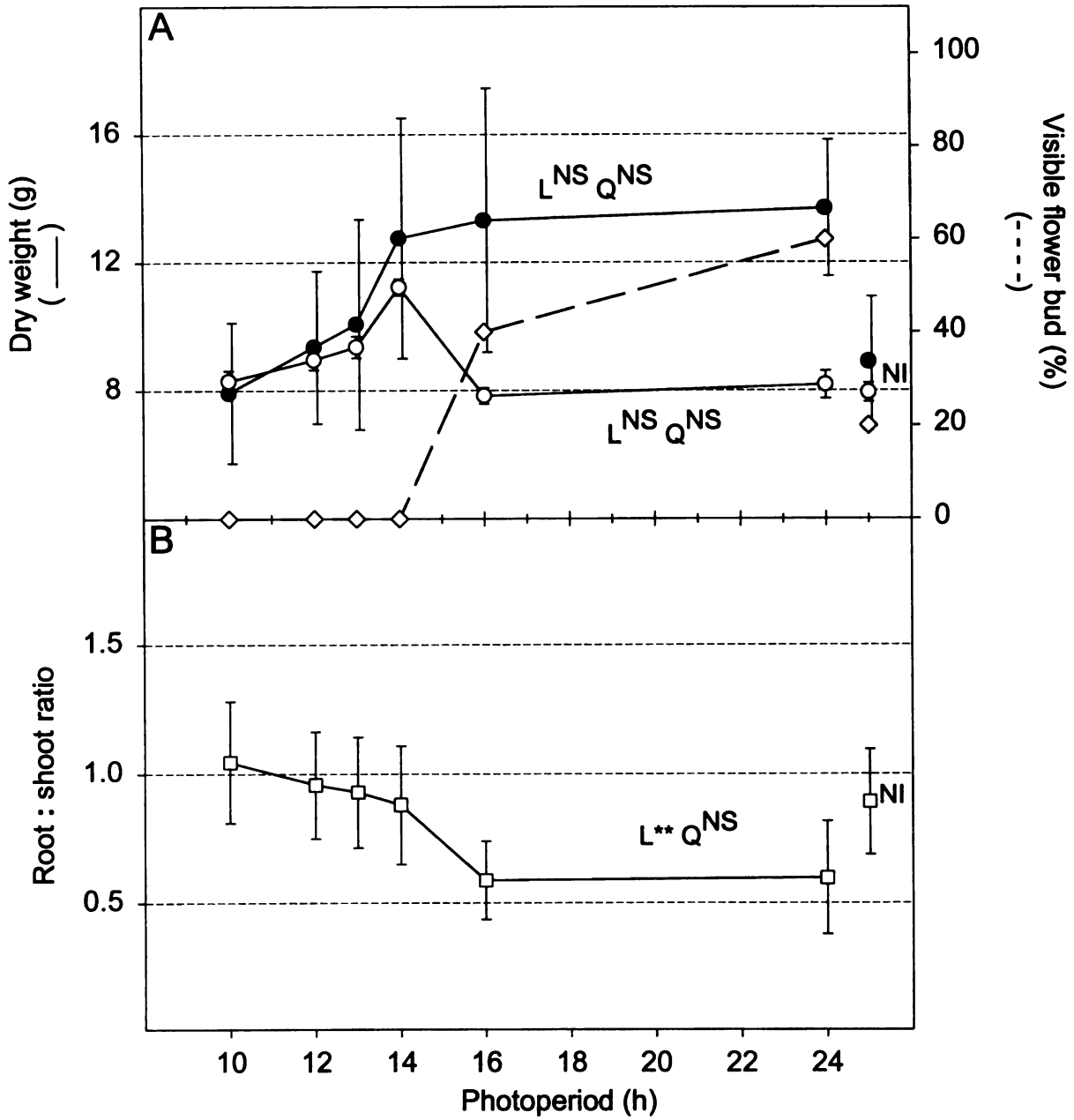

Figure 2. A: The effects of photoperiod on *Anemone hupehensis* root (unfilled circles) and shoot (filled circles) dry weights and visible flower-bud percentage (unfilled diamonds). B: Dry root to shoot ratios in response to photoperiod. Error bars are 95% confidence intervals and for clarity are offset to the right of data points for shoot dry weights. L = linear; Q = quadratic trends. ^{NS,**} Nonsignificant or significant at $P \leq 0.01$.

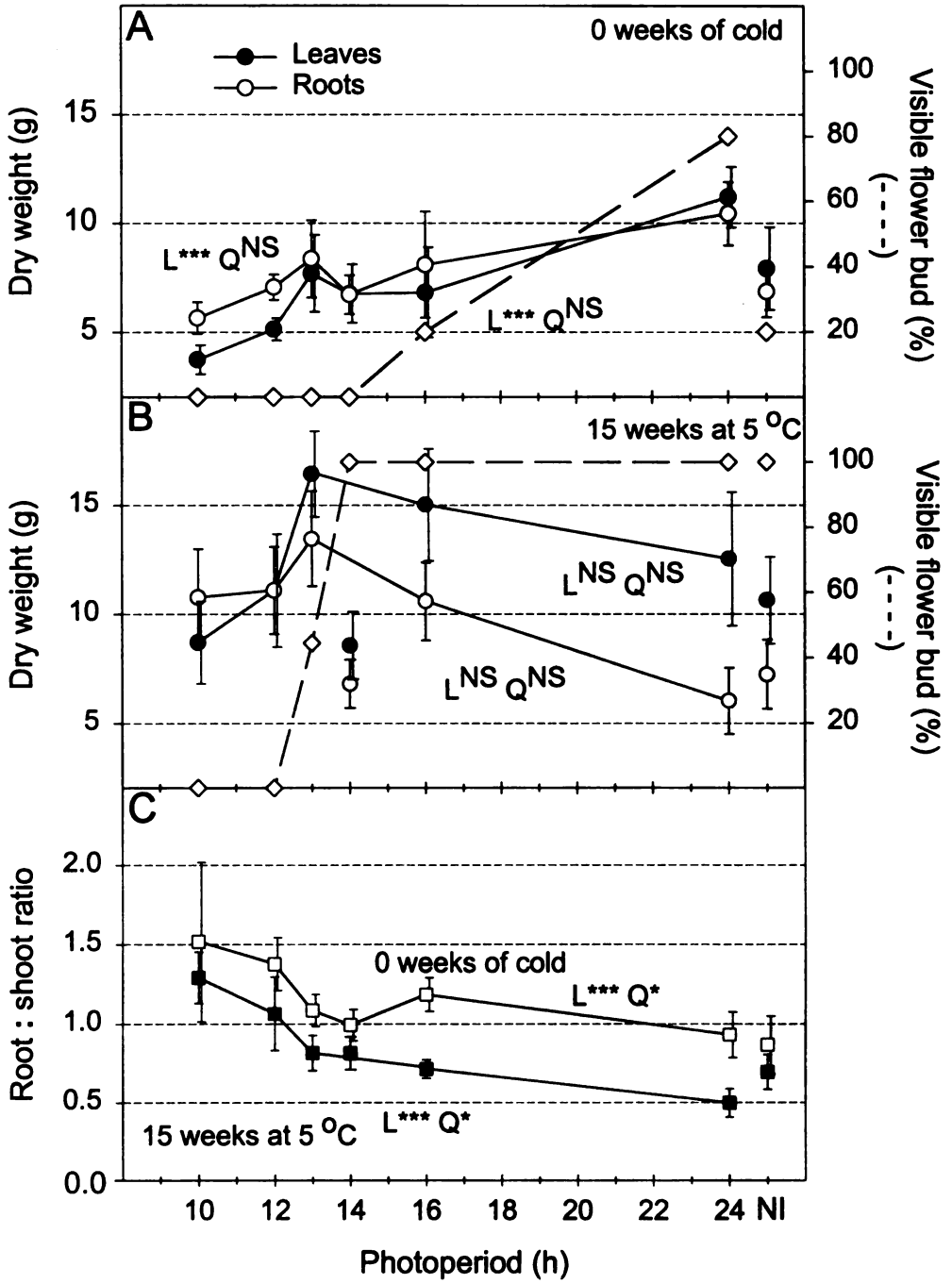

Figure 3. The effects of photoperiod on the growth of *Anemone xhybrida* 'Whirlwind'. A: Dry weight of uncooled plants and visible flower bud percentage measured at 15 weeks. B: Dry weight of plants cooled for 15 weeks and visible flower bud percentage. In A and B filled circles represent shoot dry weight; unfilled circles root dry weight. C: Unfilled squares represent root : shoot ratios of uncooled plants; filled squares, root : shoot ratios of plants cooled 15 weeks. Error bars are 95% confidence intervals and for clarity are offset to the right. L = linear; Q = quadratic trends. ^{NS,*,***} Nonsignificant at $P \leq 0.05$ or 0.001

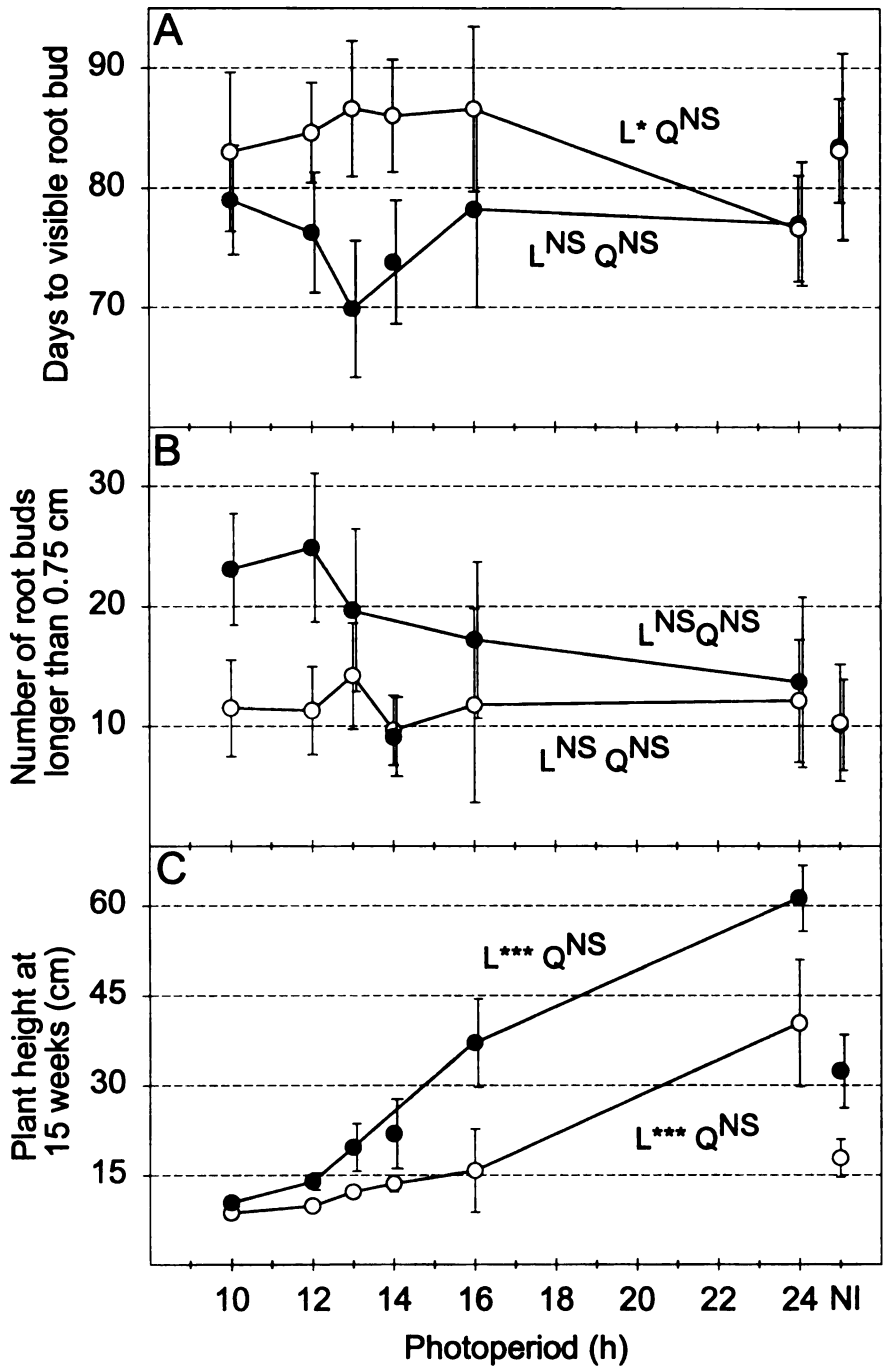

Figure 4. The effects of photoperiod on days to visible root bud (A), root bud number (B), and canopy height (C) of *Anemone xhybrida* 'Whirlwind'. Unfilled symbols represent uncooled plants grown for 15 weeks. Filled symbols represent plants after 15 weeks of 5 °C and 15 weeks' growth. Error bars are 95% confidence intervals and for clarity are offset to the right of data points for 5 °C treated plants. L = linear; Q = quadratic trends. ^{NS,*,***} Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

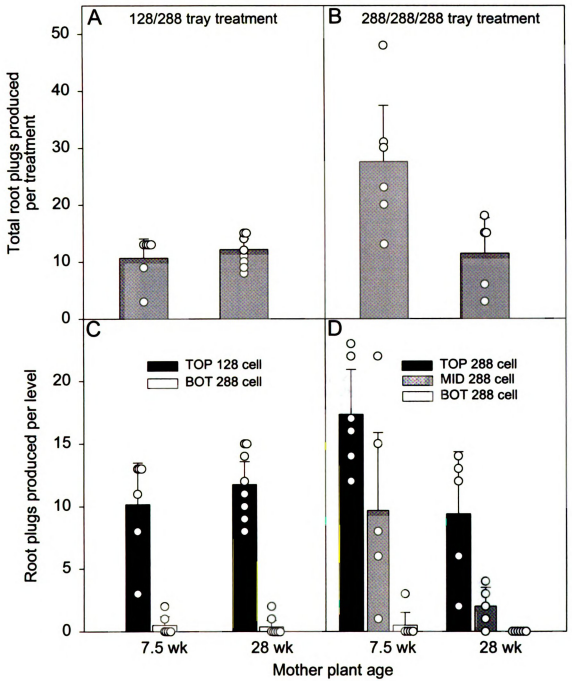

Figure 5. The regeneration of *Anemone xhybida* cut after 15 weeks of growth and measured after 5 weeks of root-bud regeneration. Total root plugs produced from 128/288 (A) and 288/288/288 (B) tray treatments. Root plugs produced per level from 128/288 (C) and 288/288/288 (D) tray treatments. Error bars are 95% confidence intervals. Unfilled circles represent raw data and for clarity are offset to the right when the same value is repeated.

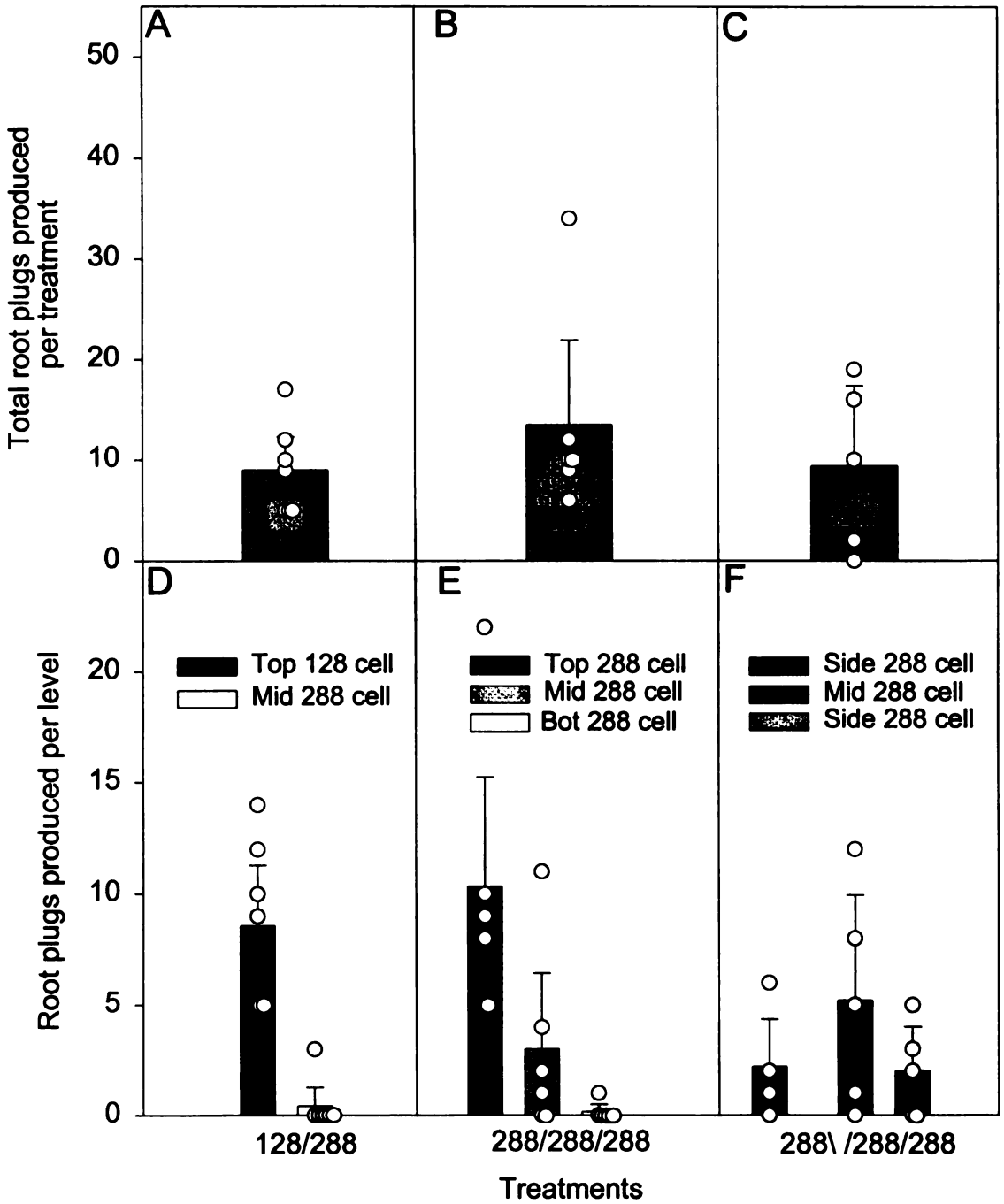

Figure 6. The regeneration of *Anemone hupehensis* cut after 16.5 weeks of growth and measured at 5 weeks of root-bud regeneration. Total root plugs produced from 128/288 (A), 288/288/288 (B), and 288-288/288 (C) tray treatments. Root-plugs produced per level from 128/288 (D), 288/288/288 (E), and 288-288/288 (F) tray treatments. Error bars are 95% confidence intervals. Unfilled circles represent raw data and for clarity are offset to the right when the same value is repeated.

APPENDIX

EFFECTS OF PHOTOPERIOD TRANSFERS AND COLD TREATMENTS ON THREE ASTERACEAE SPECIES, *COREOPSIS GRANDIFLORA* 'EARLY SUNRISE', *LEUCANTHEMUM XSUPERBUM* 'SNOW LADY' AND *RUDBECKIA FULGIDA* 'GOLDSTURM'.

Effects of Photoperiod Transfers and Cold Treatments on Three Asteraceae Species, *Coreopsis grandiflora* 'Early Sunrise', *Leucanthemum xsuperbum* 'Snow Lady' and *Rudbeckia fulgida* 'Goldsturm'.

Joaquín A. Chong[1], Royal Heins[2], Arthur C. Cameron[2], and William Carlson[2].

Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

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[1] Graduate Student.

[2] Professor.

Abstract

The effects of photoperiod transfers between short and long days or cold treatment on flowering, height, and bud number of three Asteraceae plants, *Coreopsis grandiflora* Hogg ex. Sweet 'Early Sunrise', *Leucanthemum* x*superbum* L. 'Snow Lady', and *Rudbeckia fulgida* Ait. 'Goldsturm' were determined. Plants were grown to maturity under 9- (SD) or 16-h (LD) photoperiods, and thereafter were treated with an opposite photoperiod (SD or LD) duration or a cold period of 0, 1, 2, 3, 4, 5, 6, 8, or 10 weeks. After completion of the opposite photoperiod duration, plants were transferred to their initial photoperiod or placed under a 16-h photoperiod after cold. To flower, plants did not require SD or cold before LD. All plants under continual LD flowered faster than either SD- or cold-treated plants, with the exception of *Rudbeckia*, which flowered the fastest with 6 weeks of LD intercalated between SD. *Rudbeckia* exhibited abnormal lateral flowering. *Rudbeckia* plants of the SD-LD-SD treatment or SD before cold had less abnormal flowering than plants under LD-SD-LD treatment or LD before cold. Bud number and plant height on *Coreopsis* and *Rudbeckia* increased with increasing LD and decreased with increasing SD. In *Leucanthemum*, SD-LD-SD treatments did not affect plant height or flower bud number.

Introduction

For growers to successfully force herbaceous perennials into flower for specific market dates, specific photoperiodic and cold requirements as well as the cultural information to control plant height and architecture are required. Species of Asteraceae such as *Coreopsis grandiflora* 'Early Sunrise', *Leucanthemum xsuperbum* 'Snow Lady', and *Rudbeckia fulgida* 'Goldsturm' are popular perennials, ranking among the top 10 best-selling species (Rhodus, 1995). Plant architectural changes could allow many new marketing opportunities, since plant height specifications are important to meet shipping requirements.

In 1996, *C. grandiflora* 'Early Sunrise' and *L. xsuperbum* 'Snow Lady' were seeded in early July and grown for sale in late summer in a commercial greenhouse. Most plants in the population failed to set flower buds and had to be discarded. Throughout the crop's growth, the growing environment was characterized by high temperatures and a photoperiod of continual natural long days (LD). Traditionally *C. grandiflora* 'Early Sunrise' and *L. xsuperbum* 'Snow Lady' species are seeded under natural short days (SD) and then transferred to LD for flowering. The failed crop was never exposed to SD because of its seeding date. Summer temperatures during 1996 were very high in Michigan. Failure to flower could have been due to *Coreopsis* and *Leucanthemum*'s having the dual photoperiod requirement of SD followed by LD; alternatively, flowering could have been delayed and inhibited by high summer temperatures.

Three main photoperiodic categories for flowering in plants are common, short day, long day, and day indifferent or day neutral. Less common photoperiodic categories include intermediate-day plants and dual-daylength plants. Intermediate-day plants flower when the day is neither too short nor too long (Brian and Vince-Prue, 1997). Dual-daylength plants require a two step flower induction process consisting in a primary induction photoperiod of either SD or LD followed by a secondary induction photoperiod for flowering (Blondon, 1972; Heide, 1984). These inductive steps represent plant adaptations developed in distinct latitudinal ecotypes in response to seasonal changes (Heide, 1995). Dual-photoperiodic plants have adapted to seasonal changes to germinate, grow, overcome juvenility, flower, bear seed and continue their life cycle under specific advantageous environmental conditions.

There are two main subdivisions in dual-photoperiodic plants. Plants that require an SD followed by an LD are called short-long-day plants (SLDP), and those that require an LD followed by a SD are called long-short-day plants (LSDP). LSDP flower in the secondary induction, SD, after having had LD exposure for primary induction. SLDP flower in the secondary induction, LD, after having had SD for primary induction. A dual-photoperiod plant exposed to either continual LD or SD should fail to initiate flower. Photoperiodic categories are not fixed, but are modifiable by environmental conditions such as temperature, light irradiance and age (Brian and Vince-Prue, 1997).

Some species of Asteraceae such as *C. grandiflora*, which are native from Arizona (lat. 32° N, long. 111° W) to Minnesota (lat. 44° N, long. 95° W), might

require a dual photoperiod to flower. Ketellapper and Barbaro (1966) found *C. grandiflora* Nutt. offspring of var. Single Mayfield Giant required an SD before LD for flowering induction and that the SD could be replaced by low temperatures. Experiments with *L. vulgare* Lam. var. Oxeye daisy grown from seeds collected in northern Norway showed that the plants required dual photoperiodic induction for flowering (Heide, 1995).

As mentioned, photoperiodic categories are not fixed, but modifiable. Requirements for vernalization can sometimes be substituted partially or entirely by SD photoperiods (Evans, 1987; Heide, 1986 and 1995). Plants whose SD inductive treatments can substitute for vernalization treatments can be classified as SLDP or as a cold-requiring LDP (Heide, 1995; Brian and Vince-Prue, 1997). Evans (1987) showed low temperature vernalization could be replaced entirely by SD in some European winter wheat varieties, especially Templar. Unvernalized SD-treated wheat varieties Templar and Huntsman had faster inflorescence initiation than vernalized plants during secondary inductive LD (Evans, 1987). Plants whose SD cannot replace vernalization requirements are consider not SLDP but vernalization-requiring LD plants (Brian and Vince-Prue, 1997). '*Oxeye*' daisy is such a plant (Heide, 1995).

Long day vernalization is also possible and is referred to as the substitution of cold requirement with LD. Long day vernalization occurs in *Scabiosa columbaria* L., and *Campanula longistyla* Fomin. (Mathon, 1960). Little is known about the mechanism of LD vernalization.

Requirements of cold and photoperiod and the effects these have on flowering and plant height are pertinent the perennial greenhouse industry. The goal of our research was to define whether three species from Asteraceae, *C. grandiflora* 'Early Sunrise', *L.* x*superbum* 'Snow Lady' and *R. fulgida* 'Goldsturm', required an SD or cold treatment before LD for rapid and uniform flowering and to assess the effects of these treatments on plant height.

Materials and methods

Plant material

Experiments were conducted in the Plant and Soil Science Research Greenhouses at Michigan State University, East Lansing, Michigan.

Seed propagated *C. grandiflora* 'Early Sunrise', *L.* x*superbum* 'Snow Lady' and *R. fulgida* 'Goldsturm' arrived from a commercial greenhouse (C. Raker & Sons, Inc., Litchfield, Mich.) on January 17, 1998, in size 288 plug trays (6 ml per cell). Plant material had two false leaves (cotyledons) and zero to two nodes. Plants for replicates were at the same stage of development when received on March 1, 1998.

Initial Photoperiod Treatments

Plug trays were placed under a 9- or 16-h photoperiod upon arrival and were singulated, leaving a single plant per cell. Plants under the 16-h photoperiod were lighted from 0800 to 2400 HR with 400-W high-pressure sodium (HPS) lamps. Plants under 9-h photoperiods were covered with opaque black cloth from 1700 until 0800 HR. Photoperiod (1700 to 2400 HR) and supplemental (0800 to 1700 HR) lighting were switched on during the indicated part of the day if the ambient greenhouse photosynthtic photon flux (*PPF*) was below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and switched off if it exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The HPS lamps provided a minimum *PPF* of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an average of 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height.

Plants were transplanted to size 32 plug trays, 130 ml per cell (Summit Plastic Co. Tallmadge, Ohio, after approximately two weeks. Trays were filled

with a commercial soilless medium composed of pine bark, vermiculite, Canadian sphagnum peat, and coarse perlite with a wetting agent, lime, and starter fertilizer charge (High Porosity Mix, Strong-Lite Products, Pine Bluff, Ark).

Photoperiod transfers and cold treatments

Coreopsis grandiflora plants bulked under the 9- or 16-h photoperiod had developed ≈8 nodes by March 17, 1998, and *R. fulgida* had developed ≈10 by March 30, 1998. Photoperiod transfers and cold treatments began on these dates. *Leucanthemum* x*superbum* plants under 9-h photoperiod reached ≈13 nodes by March 17, 1998, and were treated with cold and photoperiod transfers for the same duration as *C. grandiflora* and *R. fulgida*. However, *L.* x*superbum* plants under 16-h photoperiods induced flowers, and all plants developed visible flower buds; therefore, they were not treated with cold or photoperiod transfer.

In the replicate experiment, *C. grandiflora* bulked under the 16-h photoperiod had developed ≈8 nodes by April 20, 1998, and those under the 9-h photoperiod developed ≈8 nodes by April 29, 1998. *Rudbeckia fulgida* in both photoperiods reached ≈10 nodes by May 13, 1998, and *L.* x*superbum* in 9-h photoperiods reached ≈13 nodes by April 23, 1998. All *L.* x*superbum* plants under 16-h photoperiods induced flowers and were not treated with cold or photoperiod transfer.

Photoperiod transfer

Photoperiod-treated plants were transplanted from size 32-cell plugs to 13-cm square plastic containers (1.1 L) with the same medium the cold-treated plants had. Eighty plants bulked under 9-h photoperiods were transferred to a

16-h photoperiod for 1, 2, 3, 4, 5, 6, 8, or 10 weeks, and 10 plants were kept under 9-h photoperiods as a control (Fig. 1). After each 16-h photoperiod duration, plants were returned to the 9-h photoperiod. Similarly, 80 plants bulked under 16-h photoperiods were transferred to a 9-h photoperiod for 1, 2, 3, 4, 5, 6, 8, or 10 weeks, and 10 plants were kept under the 16-h photoperiod as a control. After each 9-h photoperiod duration, plants were returned to the 16-h photoperiod.

In the replicate experiment, plants under the 16-h photoperiod were transferred to 9-h photoperiods for 1, 2, 3, 4, or 5 weeks and plants under 9-h photoperiods were transferred to 16-h photoperiods for 1, 2, 3, 4, or 5 weeks. Ten plants were left under each initial photoperiod treatment as controls.

Cold treatments

Cold consisted of either no cold or transfer of plants to a 5 °C controlled-environment chamber for 1, 2, 3, 4, 5, 6, 8, or 10 weeks. In the replicate experiment, cold treatments were no cold or 1, 2, 3, 4, or 5 weeks. The controlled environment chamber was divided, and plants were exposed to a photoperiod of 9 or 16 h. Lighting was from cool-white fluorescent (F96T12/CW/VHO, Philips, Somerset, N.J.) lamps from 0800 to 1700 HR for both photoperiods. Incandescent lamps completed the 16-h photoperiods as a day extension from 1700 to 2400 HR. The *PPF* from the cool-white fluorescent lamps was ≈100 $\mu mol \cdot m^{-2} \cdot s^{-1}$; from the incandescent lamps ≈1 to 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants in the chamber were watered as needed (approximately two times per

week) with well water acidified with sulfuric acid (H_2SO_4) to a pH of approximately 6.0.

Plant node counts were made after cold treatments, and plants were transplanted to 13-cm square terracotta plastic containers (1.1 L) filled with the same medium (High Porosity Mix, Strong-Lite Products, Pine Bluff, Ark.). After transplant, plants were forced under a 16-h photoperiod with daylength extension from 1700 to 2400 HR with HPS lamps, as described in the initial photoperiod protocol. Natural daylength was supplemented from 0800 to 1700 HR, as previously discussed. Settings and *PPF* were as described for the initial photoperiod treatments.

Plant culture

Plants were top-watered and fertilized at every irrigation throughout all experiments. The nutrient solution was of well water (EC of 0.70 mS cm^{-1} and 105, 35, and 23 [mg L^{-1}] Ca, Mg, and S, respectively; pH 6.0) acidified with H_2SO_4 to a titratable alkalinity of 130 mg $CaCO_3$ L^{-1} and water soluble fertilizer providing 125-12-125-13 N-P-K-Ca (mg L^{-1}; 30% ammoniacal N) plus 1.0, 0.5, 0.5, 0.5, 0.1, 0.1 (Fe, Mn, Zn, Cu, B, Mo; mg L^{-1}; MSU Special, Greencare Fertilizers, Chicago, Ill.

Temperature Settings

Plants in the first experiment were bulked at an average daily temperature (ADT) of 23 °C for three weeks and 20 °C thereafter. Plants of the repetition experiment were bulked at a constant 20 °C ADT from the beginning of the experiment. Average daily temperature and daily light integral were monitored

with a CR-10 datalogger (Campbell Scientific, Logan, Utah) using 36-gauge type E thermocouples and quantum sensors (LI-COR), respectively. The datalogger collected temperature and light measurements every 10 and recorded the hourly average. Under each black cloth, heat was provided when needed to maintain a night temperature of 20 °C by using a 1500-W electric heater (Model T771, Rival Manufacturing Co, Sedalia, Mo.).

Data Collection

In the cold experiment, node counts were made after the end of each cold treatment. In the photoperiod transfer experiment, node counts were made when plants were transferred from their bulking photoperiod to the 9- or 16-h photoperiod. All plant nodes were counted, and the final leaf node produced was marked with Liquid Paper correction fluid ('Bond White'; Gillette Co., Boston, Mass.). At anthesis final node count was taken on all plants from the marked leaf to the flower or flower shoot.

Days to visible bud and flower were calculated on all reproductive plants. In the cold experiment, calculations were made from the seeding date and from the day cold duration ended. In the photoperiod transfer experiment, days to visible bud and days to flower were calculated from seeding. Flowering percentages were calculated by dividing the number of plants that flowered by the plants that were forced. Plant height was recorded on all three species.

The cold duration experiment was analyzed as a factorial design with three factors, initial photoperiod, cold duration and photoperiod during cold. The photoperiod transfer experiment was analyzed as a factorial with two factors,

initial photoperiod and transfer duration. Ten plants per treatment were used in all experiments, except for *L.* x*superbum* which had eight plants per treatment during the first experiment.

Initial and final node number; days to visible bud, flower, and from visible bud to flower; plant height; and flower bud number data were analyzed with SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA) and a general linear model (GLM) procedure.

Results

Dual-photoperiod experiment

Coreopsis grandiflora. All plants bulked under LD (10 weeks) from seeding flowered, irrespective of SD treatment duration (Fig. 2A). However, time to visible bud and flower increased as SD duration increased. Plants bulked under SD from seeding required only two weeks of LD for all plants to flower (Fig. 2B); 80% of plants grown under continual SD flowered, but flowering was delayed about 50 days compared with that of grown under continual LD. Days to visible bud and flower decreased as LD duration increased for SD-LD-SD plants. Flower bud number (Fig. 2C) and plant height (Fig. 2D) generally increased as LD duration increased in SD-LD-SD plants. In contrast, flower bud number and plant height decreased as SD duration increased in LD-SD-LD plants. Total leaf number differed significantly between initial photoperiod treatments but was not statistically significant for weeks of transfer photoperiod (data not shown).

Leucanthemum xsuperbum. All plants bulked under LD initiated flowers after forming approximately 20 nodes and flowered before SD treatment; thus, no data are reported. All SD-bulked plants flowered under SD following only 1 week of LD (Fig. 3A). Days to visible bud and flower decreased as LD duration increased from 0 to 2 weeks; thereafter, they remained the same. Plant height (Fig. 3B) and flower bud number (Fig. 3C) increased as LD duration increased from 0 to 5 weeks by 7.5 cm and 19 buds, respectively.

Rudbeckia fulgida. All plants bulked under continual LD flowered (Fig. 4A). All LD-bulked plants exposed to 8 or fewer weeks of SD flowered; only 60%

of plants flowered when exposed to 10 weeks of SD following LD bulking. Days to visible bud and flower increased as SD duration increased up to 6 weeks for LD-SD-LD plants. Further increases in SD duration did not increase days to visible bud or flower. Flower bud number (Fig. 4C) increased on LD-SD-LD plants as SD increased to 4 weeks; thereafter, flower bud number decreased, especially between 6 and 8 weeks of SD. Plants bulked under SD required at least 4 weeks of LD for all plants to flower (Fig. 4B). Days to visible bud and flower decreased as LD duration increased to 6 weeks. Flower bud number (Fig. 4C) and plant height (Fig. 4D) generally increased with LD duration increase in SD-LD-SD plants. Node counts were not statistically significant between initial photoperiods and weeks of transfer photoperiod (data not shown).

Cold treatment experiment

Coreopsis grandiflora. All plants flowered irrespective of cold treatment (data not shown). Photoperiod during cold treatment had no significant effect on time to flower; therefore, data from both photoperiods were pooled for further analysis. The time from the end of cold treatment to flower progressively decreased as cold duration increased (Fig. 5A). However, when time to flower was calculated from seeding, total time increased as cold duration increased. Plants bulked under LD before cold flowered up to 6 days faster than those bulked under SD ($P \leq 0.0001$). Flowering was relatively homogeneous within treatments, as shown by very tight 95% confidence intervals. Plant height at flower increased about 10 cm as cold duration increased from 0 to 6 weeks (Fig. 5B); photoperiod before cooling had no effect on flowering height. Flower

number increased 38 or 41% as cold duration increased from 0 to 3 weeks or 0 to 4 weeks for SD and LD pretreated plants, respectively (Fig. 5C). Further increases in cold duration did not increase bud number. Total node counts were insignificant between initial photoperiods but significant with cold treatment. Total node count generally decreased quadratically ($P \leq 0.0001$) and linearly ($P \leq 0.0008$) with increasing in cold, suggesting earlier induction caused by cold treatment.

Leucanthemum xsuperbum. All plants bulked under LD before cold exhibited flower buds before cold; thus, only days to visible bud and flower are reported (Fig. 6A). All SD bulked plants flowered, irrespective of cold treatment duration (data not shown). Time to flower following cold treatment decreased 13 days as cold duration increased to 10 weeks for SD-bulked plants (Fig. 6A). However, total time to flower from seeding increased 57 days as cold duration increased from 0 to 10 weeks. Time to flower was significantly affected by photoperiod during the cold treatment. However, since the cold-treated plants under LD flowered only 1 to 2 days faster than plants under SD, data were pooled: there was no statistical interaction, and the 1- to 2-days difference was judged horticulturally insignificant. There was no consistent trend between cold duration and plant height or flower bud number (Fig. 6B and Fig. 6C). Plant height averaged 24 cm and flower bud number averaged 26 buds across all cold durations. Total node number had a linear trend ($P \leq 0.001$).

Rudbeckia fulgida. All plants flowered, irrespective of cold treatment (data not shown). Photoperiod during cold treatment, as with *Coreopsis*, was

insignificant, and data were pooled for further analysis. The time to flower from the end of cold treatment progressively decreased by up to 29 days as cold duration increased (Fig. 7A). However, when calculated from seeding, total time to flower increased by up to 39 days. Plants bulked under SD before cold flowered up to 11 days faster than those bulked under LD ($P \leq 0.0001$). Both plant populations had homogeneous flowering within treatments, as shown by tight 95% confidence intervals. The percentage of plants flowering from a lateral shoot generally decreased as cold duration increased; flowering from lateral shoots was also less prominent in plants bulked under SD before cold (Fig. 7B). Plants bulked under LD before cold tended to have more flower buds than plants bulked under SD for cold durations shorter than 8 weeks (Fig. 7C). Plant height at flower was similar on plants from all cold treatments, and average height was 51 cm (Fig. 7D). Total node number was significant ($P \leq 0.002$) between initial photoperiods and decreased quadratically and linearly for 9-h photoperiods and linearly for 16-h photoperiods (data not shown).

Discussion

The main purpose of this experiment was to determine whether SD or cold was required before LD for rapid uniform flowering of *C. grandiflora* 'Early Sunrise' and *L.* x*superbum* 'Snow Lady'. The experiment was initiated because during the summer of 1996, plants of these species grown under natural continual LD and high temperature in a commercial greenhouse failed to flower uniformly. In our experiment all *C. grandiflora*, *L.* x*superbum* and *R. fulgida*, grown under continual LD flowered with or without cold. The data showed that neither SD nor cold was required before LD for flower induction of these species, disproving our hypothesis. However cold treatment decreased time to flower from start of inductive LD in all species tested (Fig. 5A, 6A, and 7A). As Harkess and Lyons (1994) found with *Rudbeckia*, our *Coreopsis* and *Rudbeckia* plants flowered when transferred to SD following LD induction, and plants were shorter compared with those grown under continual LD.

Based on these results, it seems more probable that plants grown during the summer of 1996 were exposed to supra-optimal temperatures, causing flowering delay and improper plant growth. In a preliminary experiment, we found that plants grown under continual 35°C demonstrated similar characteristics: some failed to flower, and all grew abnormally.

The most rapid flowering *C. grandiflora* and *L.* x*superbum* occurred under continal LD without cold or SD. However, *R. fulgida* flowered fastest under the SD-LD-SD treatment consisting in 6 weeks of LD intercalated between SD. The plants flowered 12 to 14 days earlier than those in continual LD or LD-SD-LD,

respectively (Fig. 4 and 7). The data suggest that *R. fulgida* 'Goldsturm' is the only species in these experiments that benefits from SD before LD treatment for most rapid flowering.

Plant architecture and height can be controlled with photoperiod treatments (Damann and Lyons, 1993). *Rudbeckia* and *Coreopsis* plants bulked under 9-h photoperiods had a smaller leaf area than those bulked under 16-h photoperiods. As plants bulked under SD were transferred to LD new developed leaves increase its size. Conversely, new developed leaves of plants bulked under LD decreased in size when plants were transferred to SD. *Rudbeckia* had abnormal lateral-shoot flowering, which meant the apical meristem failed to develop or development was slower than that of lateral shoots, which reached anthesis first. In general, SD pretreated *Rudbeckia* plants had less abnormal flowering than LD pretreated plants. In the photoperiod-transfer experiment, SD-LD-SD plants had ≈28% abnormal flowering compared to ≈54% abnormal flowering in LD-SD-LD plants averaged across all treatments (data not shown). Similarly, plants bulked under SD before cold had less abnormal flowering than plants bulked under LD before cold (Fig. 7B).

Although SD and cold are not required before LD for flowering, and photoperiods during cold treatment had no horticulturally significant effect on time to flower, treatment combinations of SD and LD duration changed plant morphology and architecture. Effects were greatest on *C. grandiflora* and *R. fulgida* whose plant height and bud number, both horticulturally important

variables, were affected. Generally plant height and bud number increased with increasing LD duration and decreased with increasing SD duration.

Damann and Lyons (1993) expanded on Murneek's (1936) concept of "photoperiodic inhibition," defining limited inductive photoperiod (LIP) as a plant's being given a minimum number of LD inductive cycles to initiate flowering and then being transferred to noninductive conditions. Our data are similar to those from studies of Damann and Lyons (1993), where an increase in inductive LD increased plant height of two *Coreopsis* species.

In *L.* x*superbum*, SD-LD-SD treatments did not affect plant height or flower bud number; however, LD intercalated between SD increased *C. grandiflora* and *R. fulgida* plant height and flower bud number and decreased days to visible bud and flower with increasing LD duration. The reverse of SD-LD-SD were LD-SD-LD treatments, or what we call limited noninductive photoperiods (NIP). Noninductive SD photoperiod treatments were intercalated between LD inductive photoperiods. Our data, like that of Harkess and Lyons (1994) in their publication on *Rudbeckia hirta*, show that increasing SD durations generally decreased *Rudbeckia* and *Coreopsis* plant height and bud number and increased days to visible bud and flower. In *C. grandiflora* and *R. fulgida*, LD-SD-LD plants (NIP) had greater mass than SD-LD-SD plants (LIP), which were generally smaller.

The LIP and NIP methods of photoperiod control are potentially important for the perennial industry, since they provide nonchemical height-control methods capable of changing plant architecture, especially height.

In summary, *C. grandiflora* 'Early Sunrise', *L.* x*superbum* 'Snow Lady', and *R. fulgida* 'Goldsturm' did not require an SD or cold before LD for flowering. However, SD or cold before LD in *Rudbeckia* and a cold treatment in *Leucanthemum* and *Coreopsis* decreased time to flower following the cold treatment.

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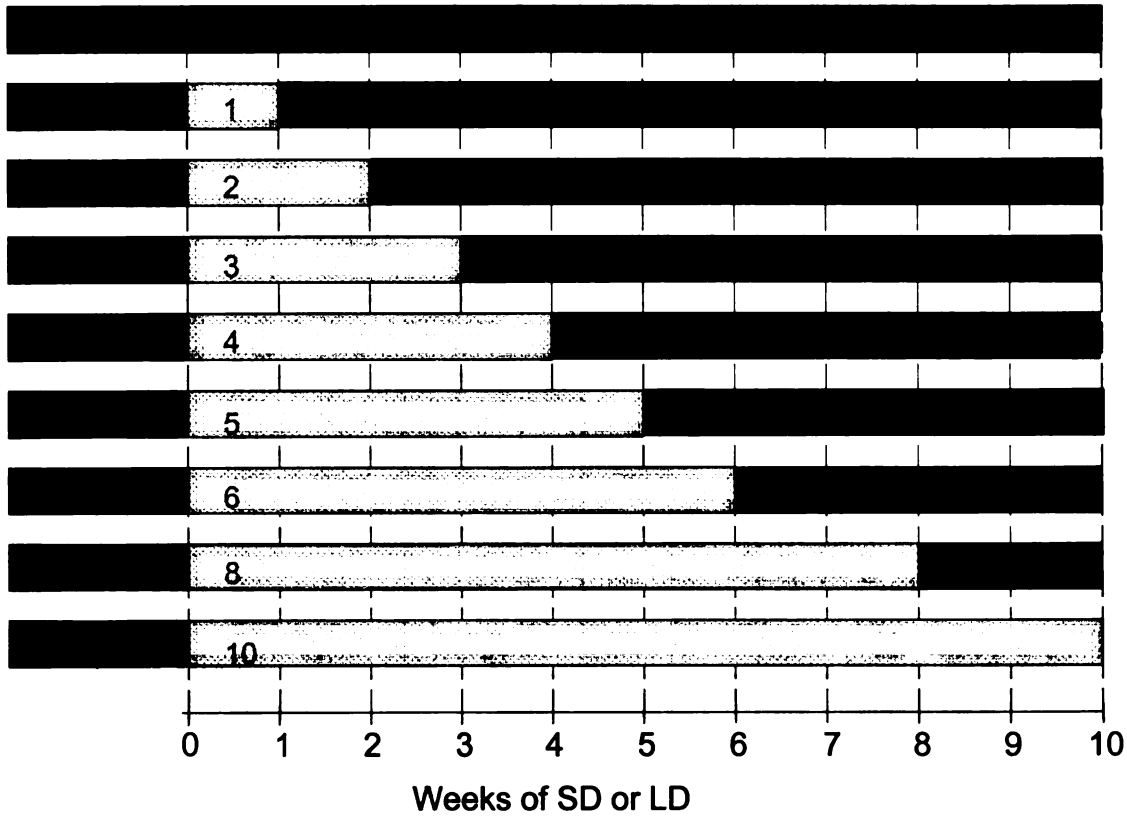

Figure 1. Schematic representation of photoperiod transfer experiment. Black filled bars represent initial photoperiod under 9- (SD) or 16-h (LD) photoperiods and gray bars represent the transfer photoperiod duration. Plants were under the initial photoperiod for 10, 11, or 13 weeks for *Coreopsis*, *Leucanthemum*, and *Rudbeckia*, respectively. After the initial photoperiod treatment, plants were moved to the opposite photoperiod for 0, 1, 2, 3, 4, 5, 6, 8, or 10 weeks and then transferred back to the initial photoperiod if anthesis was not reached.

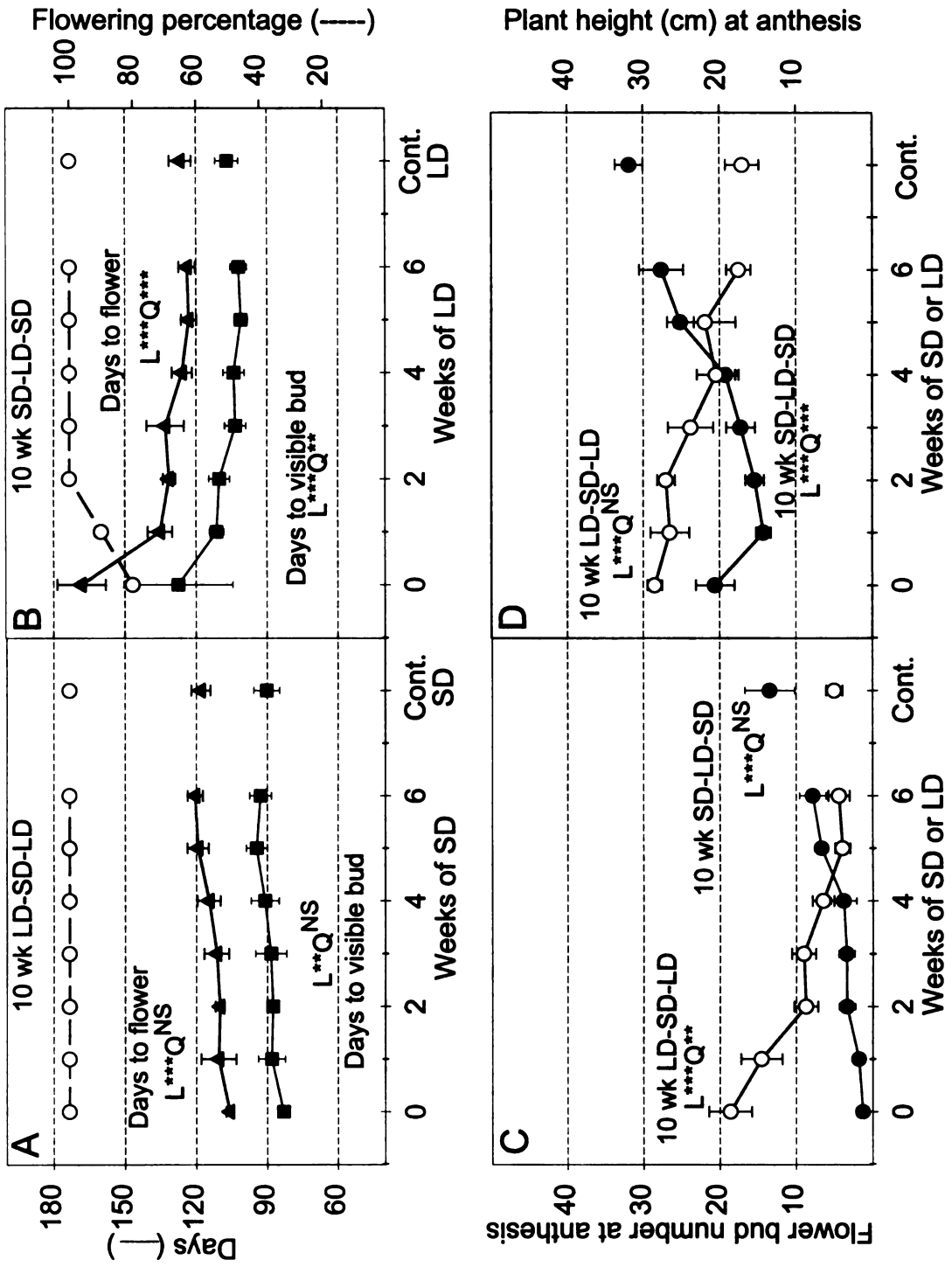

Figure 2. The effects of LD-SD-LD (A ; SD = short days; LD = long days) and SD-LD-SD (B) on days to visible bud, days to flower, flowering percentage, flower bud number (C), and plant height (D) of *Coreopsis grandiflora* 'Early Sunrise'. Unfilled circles represent LD-SD-LD (NIP) treatment and black-filled circles represent SD-LD-SD (LIP) treatments in (C) and (D). Error bars are 95% confidence intervals. L = linear; Q = quadratic trends. NS,**,*** nonsignificant or significant ot $P \leq 0.01$ or 0.001, respectively.

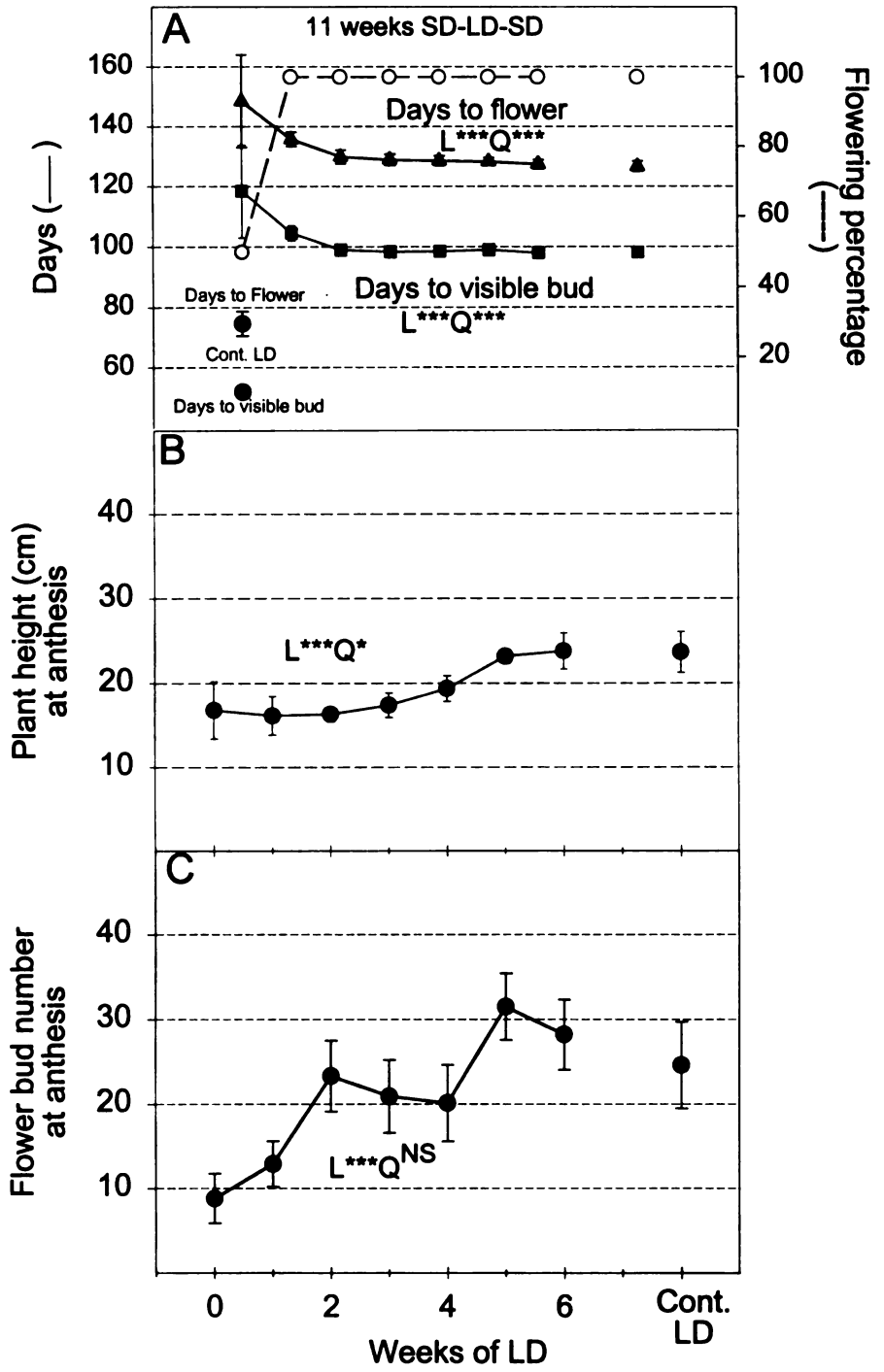

Figure 3. The effects of SD-LD-SD (SD = short days; LD = long days) on days to visible bud, days to flower, flowering percentage (A), plant height (B), and flower bud number (C) of *Leucanthemum xsuperbum* 'Snow Lady'. L = linear; Q = quadratic trends. NS,*,*** nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

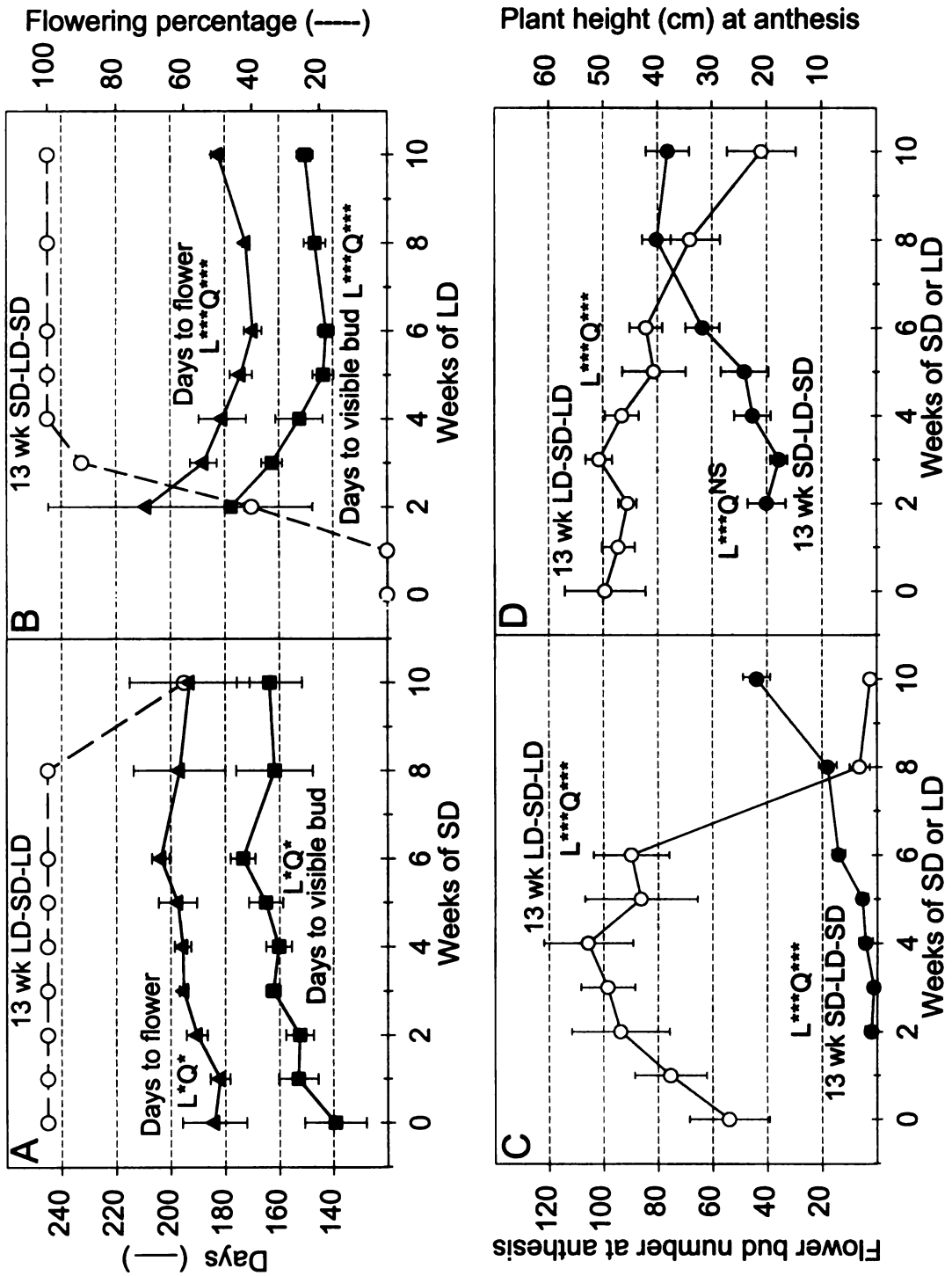

Figure 4. The effects of LD-SD-LD (A; SD = short days; LD = long days) and SD-LD-SD (B) on days to visible bud, days to flower, flowering percentage, flower bud number (C), and plant height (D) of *Rudbeckia fulgida* 'Goldsturm'. Unfilled circles represent LD-SD-LD (NIP) treatment and black-filled circles represent SD-LD-SD (LIP) treatments in (C) and (D). Error bars are 95% confidence intervals. L = linear; Q = quadratic trends. NS,*,*** nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

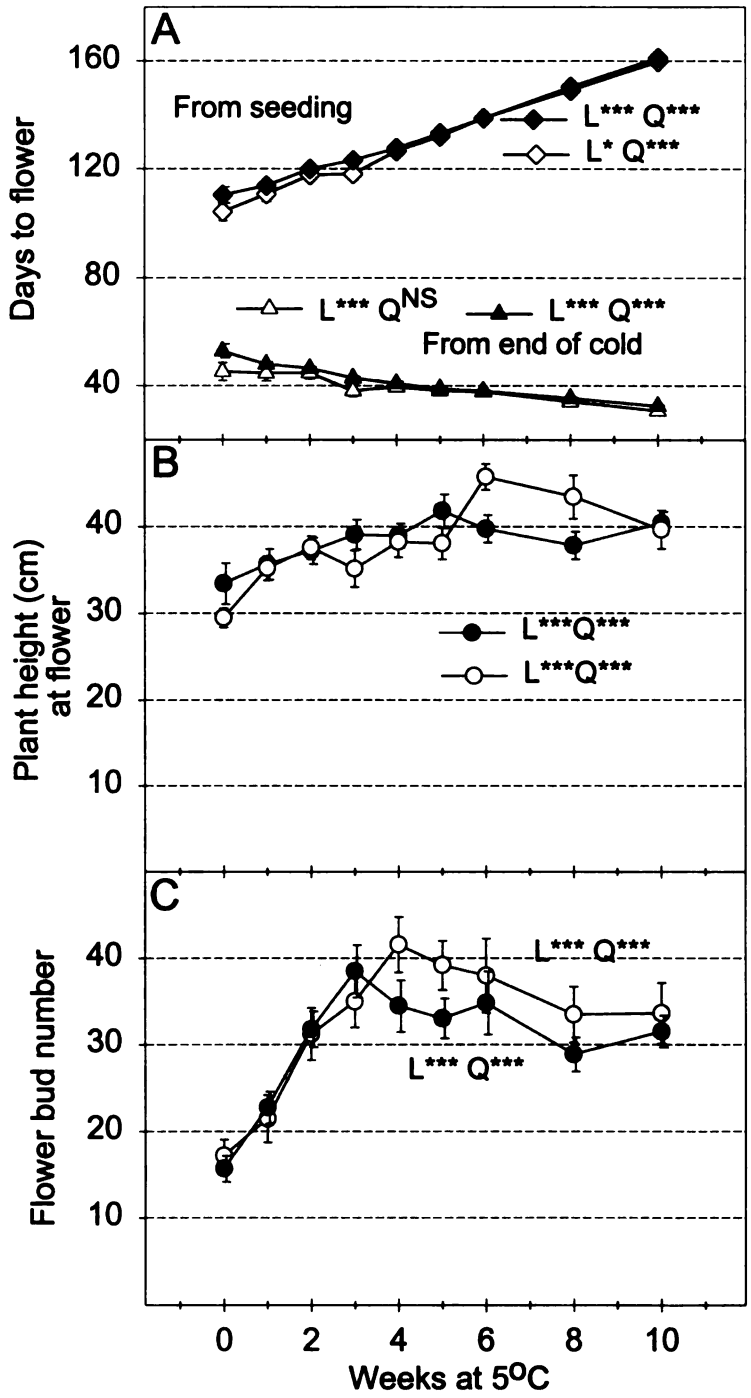

Figure 5. The effects of cold treatment on days to flower (A), plant height (B), and flower bud number (C) of *Coreopsis grandiflora* 'Early Sunrise'. Days to flower were calculated from seeding and the end of cold. Black-filled symbols represent SD before cold; unfilled symbols, LD before cold. Error bars are 95% confidence intervals. L = linear; Q = quadratic trends. NS,*,*** nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

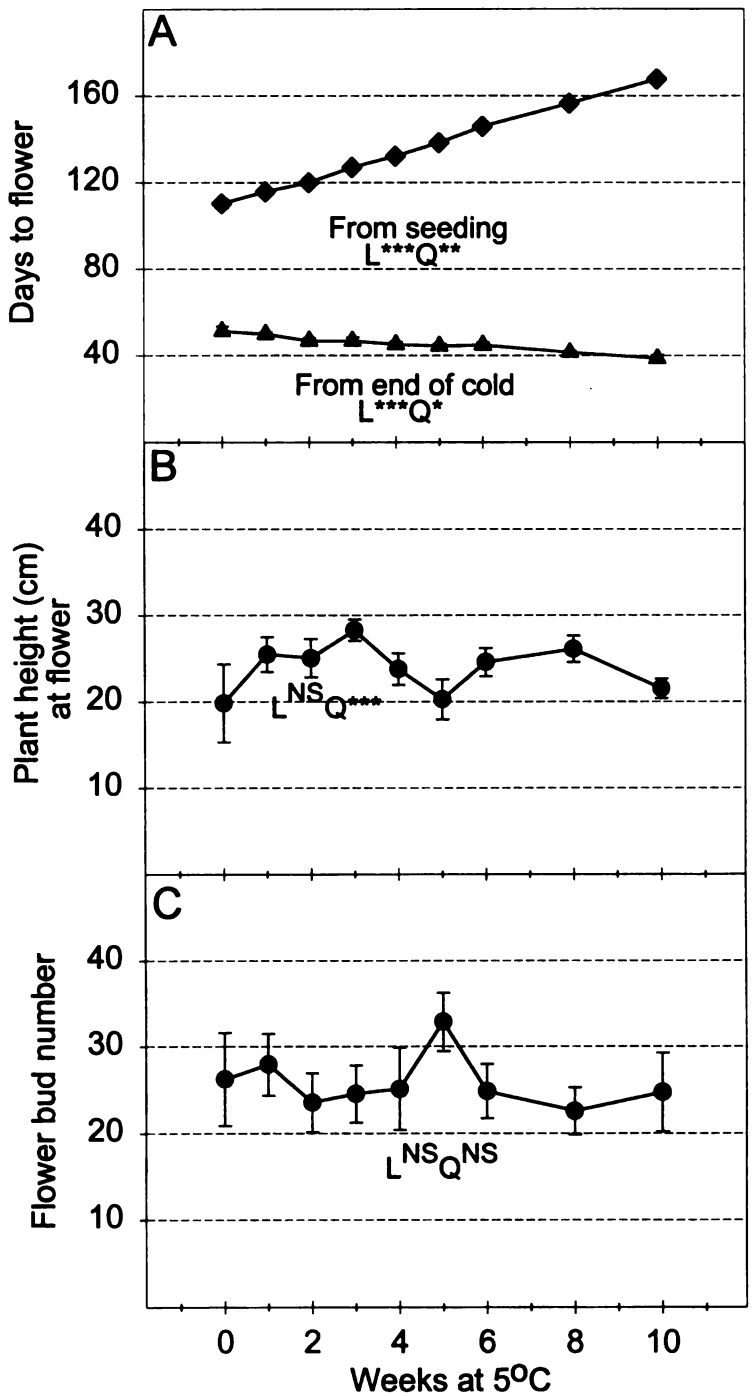

Figure 6. The effects of cold treatment on days to flower (A), plant height (B) and flower bud number (C) of *Leucanthemum* x*superbum* 'Snow Lady'. Days to flower were calculated from seeding and the end of cold. Black-filled symbols represent SD before cold. Error bars are 95% confidence intervals. L = linear; Q = quadratic trends. NS,*,**,*** nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

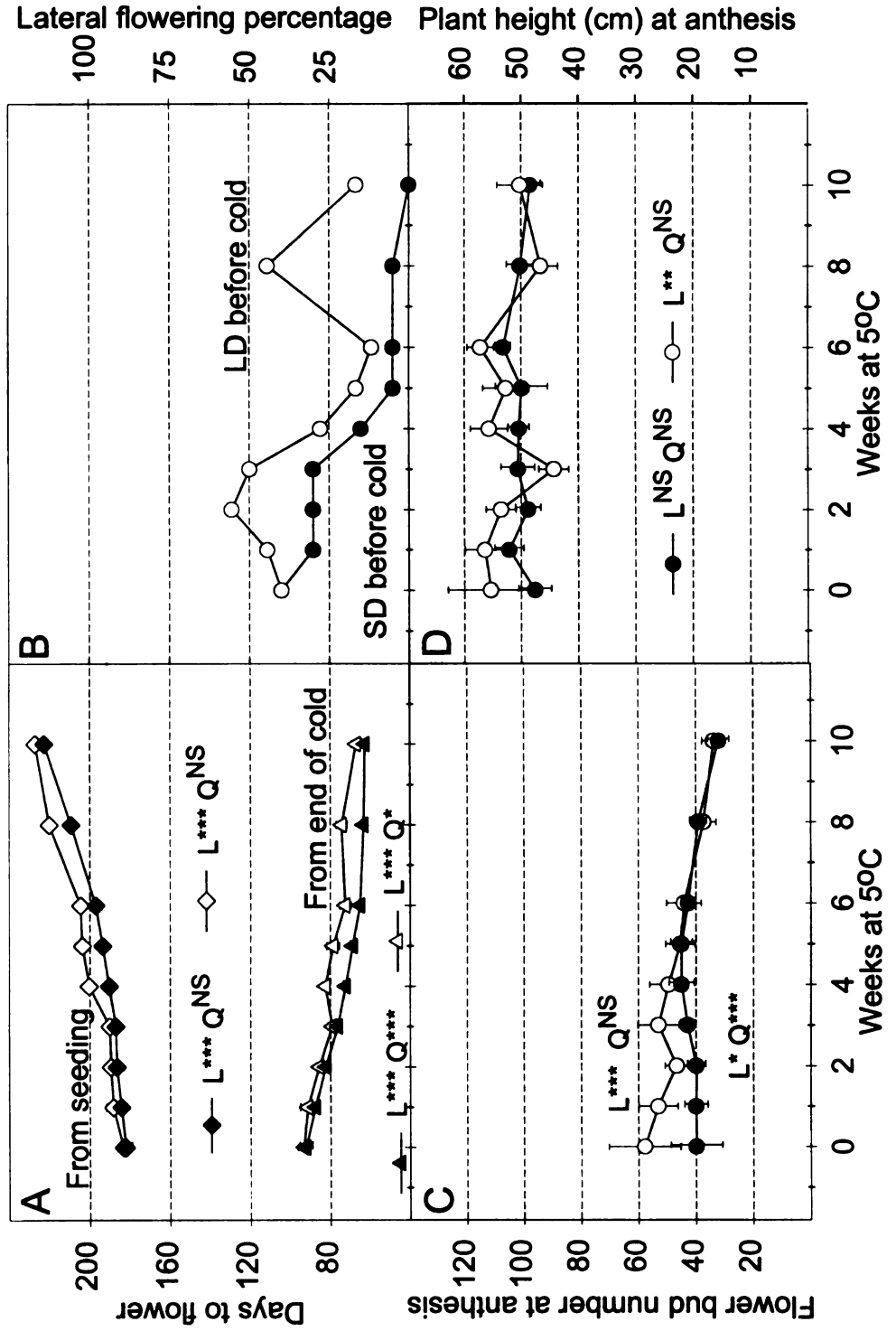

Figure 7. The effects of cold on days to flower (A), lateral flowering percentage (B), flower bud number (C), and plant height (D) of *Rudbeckia fulgida* 'Goldsturm'. Days to flower were calculated from seeding and the end of cold. Black-filled symbols represent SD before cold; unfilled symbols, LD before cold. Error bars are 95% confidence intervals. L = linear; Q = quadratic trends. NS,*,**,*** nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.