FURTHER STUDIES ON THE TRANSMISSION OF POINSETTIA BRANCHING AGENT

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To my Aunt Hilda and Uncle Bodo,

you have showed me what faith in God and perseverance

really means.

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CHAPTER I

INTRODUCTION

Euphorbia pulcherrima Willd. ex. Klotzsch (poinsettia) is the number one potted floral crop in the United States. The United States Department of Agriculture estimated the wholesale value of poinsettias at $170 million for the top 28 floriculture crop producing states in 1991 (USDA, 1992). Poinsettias account for 25% of the total potted floral crop production for the same 28 states.

Poinsettias are native to Southern Mexico and Northern Guatemala and were cultivated by the Aztecs before Christianity came to the Western Hemisphere (Ecke et al., 1990). This plant was first described by Spanish botanist Juan Balme during the seventeenth century. Joel Robert Poinsett, a botanist and United States ambassador to Mexico, introduced the poinsettia to the U.S. in 1825. He distributed the plant to various botanical gardens and to horticultural friends.

By 1909, Albert Ecke was specializing in producing poinsettias for the cut flower market at Eagle Rock Valley, California (Ecke et al., 1990). However, the cultivars he grew were too tall and abscised their leaves when grown as a potted plant for the interiorscape (Shanks, 1981). The height control problem was solved in the

late 1950's by the introduction of growth retardants such as Cycocel (Larson, 1967).

A major increase in poinsettia production took place after the release of Eckespoint C-1 (C-1) in 1968 (Ecke et al., 1990). This cultivar had good leaf retention, good bract color and horizontal bract orientation.

Annette Hegg (AH), a cultivar that had both free-branching and good leaf retention characteristics, was first grown in Norway in 1964. By 1968, all major producers in Europe were growing AH, and it was introduced to the United States and Canada. The introduction of AH increased U.S. poinsettia production. Other cultivars such as Gutbier V-10 and V-14, which had similar characteristics to AH, also helped increase poinsettia production in the U.S. (Shanks, 1981).

Today most commercial poinsettias grown in the United States are free-branching cultivars, which have weak apical dominance and relatively small stem diameters. Free-branching cultivars, including AH, Gutbier V-10 and Gutbier V-14 series, are preferred by growers because they allow production of many cuttings per plant when pinched, and numerous flower-bearing axillary shoots. Less frequently grown are restricted-branching cultivars such as Eckespoint series, which have strong apical dominance, large stem diameters, and relatively few axillary shoots and flowers.

The free-branching trait in Annette Hegg Brilliant Diamond (AHBD) can be induced in the restricted-branching C-1 Red (C-1) poinsettia through grafting (Dole and Wilkins, 1991, 1992). An agent may be present in the leaves or stems of a free-branching poinsettia which is translocated through the graft union to a restricted-branching poinsettia, where it increases basal axillary bud growth. The free-branching

agent moves both acropetally and basipetally through the graft union and can be serially transmitted from plant to plant by grafting (Dole and Wilkins, 1992). CB, (TR) a vegetative or graft-hybrid, was derived from grafting C-1 onto AHBD, resulting in transmission of the branching agent to C-1 (Dole and Wilkins, 1992). Vegetative hybridization is the process of grafting two plants together in order to obtain a unique phenotype from the grafted plant itself. Thus, the presence of the agent in CB makes it different from C-1.

Plant leaves play a role in graft transmission of substances that can alter the characteristics of the plants being grafted. Differences in dry weight and starch accumulation of grafted leaves of *Nicotiana tabacum* L. 'Burley' and 'Flue-Cured' plants were controlled by biochemical or physical factors within an individual leaf (Craft-Brander et al., 1988).

The transmission of the branching agent from CB donors to C-1 receivers may occur because of a source-sink mechanism with the movement of photosynthates from CB to C-1. Translocation of photosynthates from the rootstock to the scion buds has been demonstrated (Beeson and Proebsting, 1988). In addition, heterografting of *Solanum tuberosum* L. 'Sable' scions onto *S. tuberosum* 'Selection F58050' stocks resulted in a slight increase in reducing sugar and a reduction of tuber specific gravity as compared to tubers from autografts of 'F58050' (Tai et al., 1988). This provides evidence that scions (receivers) and stocks (donors) interact with each other and that both may control the performance of tuber traits. Stimart's (1983) research on poinsettia grafts between self-branching (free-branching) and non-branching (restricted-

branching) cultivars suggested that axillary growth is governed by some endogenous factors translocated from the roots across the graft union to the shoots. Interactions between the stocks (donors) of CB and scions (receivers) of C-1 may control movement of photosynthates and branching pattern in C-1.

Changes in the branching pattern of C-1 are retained through a series of vegetative propagations and are considered permanent (Dole and Wilkins, 1992). The percentage of C-1 plants exhibiting the free-branching characteristic increased from 0% for 0, 5, and 10 days of graft contact with AHBD to 100% after 30 days (Dole and Wilkins, 1992). A minimum of 10 days of contact with AHBD plants was required in order to transmit the agent (Dole and Wilkins, 1992).

Anatomical changes during the formation of the graft union between restricted-branching and free-branching poinsettias may induce production of the agent, or allow translocation of the agent through the graft union. The anatomical changes occurring during graft union formation in plants may be involved in vegetative hybridization (Fajnbron, 1953). During graft union formation between CB and C-1 poinsettias, dictyosome activity and callus proliferation may promote the transmission of the branching agent from CB donors to C-1 receivers. In *Sedum telephoides* Michx., dictyosome activity and callus proliferation are pronounced along the graft interface at the 24th day after grafting and both functions are correlated with the initial adhesion (Moore and Walter, 1981).

Wound vessel differentiation may occur during graft union formation in poinsettia and may be required for the production and/or translocation of the branching

agent. In tomato (*Lycopersicon esculentum* Mill. cv. Aisla Craig) autografts, wound vessels differentiate within the callus at the graft union, and are connected into the vascular system of stock and scion by wound vessels differentiating from vascular and cortical parenchyma (Jeffree and Yeoman, 1983). *Picea sitchensis* L. (Bong) xylem elements differentiate directly from vascular cambia of the rootstock and scion and are different from elements arising from parenchymatous callus derived from ray parenchyma (Weatherhead and Barnett, 1986).

The transmission of the branching agent and the flowering hormones between CB donors and C-1 receivers may be correlated. Florigen (flowering hormones) is formed in leaves and transported to shoot meristems and is able to cross graft unions (Lang, 1989). Florigen is readily interchangeable between grafting partners of the same species. Dole and Wilkins (1992) found that the branching agent was also transmissible between AHBD and C-1 poinsettias through the graft union. However, the location of synthesis of the branching agent is not known. The branching agent may be produced in the leaves of poinsettia rootstocks (donors) and translocated through the graft union like florigen.

Branching in C-1 and CB poinsettias sometimes occurs during the flowering process under non-inductive long (LD) photoperiods. A physiological disorder, splitting, may occur in which the vegetative shoot tip becomes reproductive and is transformed into a flower bud (cyanthum) under LD (Zrebiec and Tayama, 1985). Three shoots arise around the bud which normally does not reach anthesis. Splitting occurs more frequently in C-1 poinsettia plants than in CB poinsettia plants under LD

conditions (personal observation).

A complex of growth regulators may control both flowering and expression of free-branching in poinsettias. The flowering and branching control systems in *Lathyrus odonatus* L. and *Pisum sativum* L. appear to involve the same or a very similar hormone(s) (Murfet, 1971; Murfet, 1977). Chailakhyan and Lozhnikova (1985) hypothesized that florigen is a bicomponent complementary complex of two hormones consisting of gibberellins, which participate in formation of the flower stem, and anthesins, substances that affect flower formation. In contrast, poinsettias sprayed with gibberellic acid produce flowers later than nontreated poinsettias (Guttridge, 1963; Evans et al., 1992). Furthermore, substances with gibberellin-like activity were detected in root exudates of poinsettias grown under LD conditions (Criley, 1970). As a result of studies on the regulation of tomato (*Lycopersicon esculentum* Mill.) flowering through reciprocal top-root grafting, Phatak and Wittwer (1965) suggested the presence of a graft transmissible flower stimulator in the leaves of early cultivars and a transmissible flower inhibitor in the leaves of late cultivars.

This research will further characterize the free-branching agent in poinsettia by determining in which organ(s) (stem or leaf) of the plant the agent is contained, if shading the donor stems and leaves prevents the transmission of the branching agent, if new cells or tissues may induce its production and/or translocation within the graft union, and if there is a correlation between flower induction and the branching agent.

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CHAPTER II

POINSETTIA LEAF AND STEM TRANSMISSION OF THE BRANCHING AGENT

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Abstract. Euphorbia pulcherrima Willd. ex. Klotzsch 'Eckespoint C-1 Red' (C-1) a restricted-branching cultivar and 'CB' a free-branching vegetative or graft-hybrid were homo and heterografted. Axillary shoot node numbers and lengths of C-1 receiver plants were partially increased by CB donors with leaves removed (in 1990 and 1990-1991) and with apical and basal parts removed (in 1990-1991 and 1991). In 1991-1992, axillary shoot growth of C-1 receivers was only slightly increased by CB

donor internode chips. However, CB donor node chips did not increase C-1 receiver axillary shoot node numbers and lengths. Shading of CB donors partially increased the axillary shoot node numbers and lengths of C-1 receivers above node 4 in 1991-1992. Both stems and leaves of CB poinsettia donors may transmit the branching agent to C-1 poinsettia receivers. Shading CB donor leaves and stems did not prevent the transmission of the branching agent to C-1 receivers.

Today most commercial poinsettias grown are free-branching cultivars, which have weak apical dominance and relatively small stem diameters. Free-branching cultivars include Annette Hegg, Gutbier V-10 and Gutbier V-14 series and are preferred by growers because they allow production of numerous axillary shoots and cuttings per plant when pinched. Less frequently grown are restricted-branching cultivars, such as the Eckespoint C-1 series, which have strong apical dominance, large stem diameters and few axillary shoots when pinched.

The free-branching trait in 'Annette Hegg Brilliant Diamond' (AHBD) can be induced in the restricted-branching 'Eckespoint C-1 Red' (C-1) poinsettia through grafting (Dole and Wilkins, 1991, 1992). The free-branching agent moves both acropetally and basipetally through the graft union and can be serially transmitted from plant to plant by grafting (Dole and Wilkins, 1992). CB, (TR) a vegetative or graft-hybrid, was derived from grafting C-1 onto AHBD, resulting in transmission of the branching agent to C-1 (Dole and Wilkins, 1992). Vegetative hybridization is the

process of grafting two plants together in order to obtain a unique phenotype from the grafted plant itself. Thus, the presence of the agent in CB makes it different from C-1.

An agent may be present in the leaves or stems of free-branching poinsettias which is translocated through the graft union to a restricted-branching poinsettia's axillary buds. Plant leaves play a role in graft transmission of substances that can alter the characteristics of the plants being grafted. Differences in dry weight and starch accumulation in grafted leaves of *Nicotiana tabacum* L. 'Burley' and 'Flue-Cured' plants were controlled by biochemical or physical factors within an individual leaf (Craft-Brander et al., 1988).

The transmission of the branching agent from CB donors to C-1 receivers may occur concomitantly with the photosynthate movement from CB (the source) to C-1 (the sink). Translocation of photosynthates from the rootstock to the scion buds has been demonstrated (Beeson and Proebsting, 1988). Interaction of photosynthates with scion buds may alter the plant's characteristics. Heterografting of *Solanum tuberosum* L. 'Sable' onto *S. tuberosum* 'Selection F58050' stock resulted in a slight increase in reducing sugar and a reduction of tuber specific gravity as compared to tubers from autografts of 'F58050' (Tai et al., 1988), indicating that materials from both scions (receivers) and stocks (donors) may control the expression of tuber traits. Stimart's (1983) research on poinsettia grafts between self-branching (free-branching) and non-branching (restricted-branching) cultivars suggested that axillary growth is governed by some endogenous factors translocated from the roots across the graft union to the

shoots. Interaction between the stock (donor) of CB and scion (receiver) of C-1 may control movement of photosynthates and the branching agent in C-1.

This research will further characterize the free-branching agent in poinsettia by determining which organs (stems or leaves) of the free-branching poinsettia contain the agent and if shading the donor stems and leaves prevents the transmission of the branching agent.

Materials and Methods

Leaf removal experiments

1990. Cuttings from C-1 and CB poinsettia plants were treated with 1% IBA (Indole-3-Butyric Acid, Hormex Powder #1, Brooker Chemical, North Hollywood, Calif.) and planted in oasis rootcubes growing medium (Smithers-Oasis, Kent, Ohio) on 28 May 1990. After rooting under intermittent mist with a temperature of 21 C, plants were placed in 16.5-cm (1650 cc) pots filled with a commercial peat-based medium (Fafard #2, Springfield, Mass.) on 27 June 1990. Plants were approach grafted (Hartmann et al., 1990) on 22 to 25 Aug. 1990 and placed in a completely randomized design with a 2x2 factorial arrangement of treatments. Factor A was the CB and C-1 cultivars as the donor of the grafted pair (donor) and factor B was the CB and C-1 cultivars as the receiver of the grafted pair (receiver). Donor leaves were removed 30 days after grafting and periodically thereafter. CB and/or C-1 plants (donors and receivers) were pulled apart 40 days after grafting. Six single unit replicates were used for each treatment.

Vegetative growth was maintained by supplementary incandescent and high-intensity-discharge (HID) lamps to provide long (LD) photoperiods (15 h). Light intensity averaged 780 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR during day light hours. Air temperature in the greenhouse was kept at an average 31/21 C day/night. Standard fertilization and pest management practices were used (Ecke et al., 1990).

Poinsettia receiver plants were pinched back to the twelfth node when plants developed at least twelve nodes above the graft union. Data were taken thirty days later on the receiver of each grafted pair. The number of nodes with leaves (including the terminal leaf blade separated from the apical cone) was determined and the length was measured from the base of each axillary shoot to the tip of the apical cone.

1990-1991. Similar materials and methods and the same treatments as in 1990 were used except that plants were propagated on 27 Aug. 1990, planted on 28 Sept. 1990 and approach grafted on 5 to 7 Dec. 1990. However, in this study poinsettia donor leaves were periodically removed beginning 7 days after grafting and CB and/or C-1 plants were pulled apart 30 days after grafting. Six single unit replicates were used for each treatment.

1991. Similar materials and methods as in 1990-1991 were used except that plants were propagated on 24 July 1991, planted on 26 Aug. 1991 and approach grafted on 27 to 30 Sept. 1991. In this year, an additional factor (2x2x2) was used in the treatments; CB and C-1 poinsettia donor leaves were periodically removed (beginning 7 days after grafting) or not removed. Twelve single unit replicates were used for each treatment.

Stem experiments

Stem removal. Materials and methods and factorial arrangement of treatments used were similar and performed simultaneously to the leaf experiments unless otherwise stated. In factor B, the apical and basal parts of poinsettia donor stems were removed 30 days after grafting (1990) or 15 days after grafting (1990-1991 and 1991). Ten single unit replicates were used for each treatment in 1990, 6 in 1990-1991, and 12 in 1991 with no additional factor included. The 1991 trial was done at the Noble Research Center Growth Chambers, Stillwater, Okla., where vegetative growth was maintained by incandescent and fluorescent lamps from 0800 to 2200 HR. Light intensity was maintained at 210 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR from 30 Sept. 1991 to 15 Nov. 1991 and reduced to 125 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR from 16 Nov. 1991 to 16 Dec. 1991 to prevent light blanching of the leaves. Air temperature was kept at 26/18 C day/night.

Node and internode. CB and C-1 poinsettia plants were propagated as in the leaf experiments on 22 Sept. 1991, planted on 26 Oct. 1991 and grafted on 15 to 16 Nov. 1991. A similar factorial arrangement of treatments as in the 1990 leaf experiment was used except that factor B was CB and C-1 node or internode chip donors. A 7 mm node or internode chip from the apical part of one grafted pair stem with a minimum diameter of 6 mm was grafted into the other grafted pair at the same node or internode area. Grafts were not successful, so plants were cut back below the graft union and allowed to regrow. Plant growth was then thinned to one shoot and successfully grafted on 11 Jan. 1992. CB and C-1 poinsettia receiver plants split once in the node experiment and twice in the internode experiment. Splitting is premature

flower initiation under long (LD) photoperiod; the vegetative shoot tip becomes reproductive and three shoots arise around the bud which normally does not reach anthesis. Therefore, to remove the undesired branching (splitting), plants were pinched at the 4th node above the graft union, allowed to regrow and thinned to one shoot. When plants reached twelve nodes, they were pinched again. Nine single unit replicates were used for each treatment in the node experiment and 8 single unit replicates in the internode experiment.

Shade experiment

Similar materials and methods as in the 1990 leaf experiment were used except that CB and C-1 poinsettia plants were propagated on 3 Oct. 1991 and planted on 16 Nov. 1991. Plants were approach grafted on 5 to 20 Apr. 1992 and placed in a completely randomized design with a 2x2x2 factorial arrangement of treatments. Factor A was the CB and C-1 cultivars as the donor of the grafted pair (donor), factor B was placement of donors under shade (95%, black polypropylene fabric, double layer) or no shade and factor C was CB and C-1 cultivars as the receiver of the grafted pair (receiver). Twelve single unit replicates were used for each treatment. Replications were divided into two blocks on one bench, alternating shade and no shade treatments. Black plastic was placed between the shaded donor and the unshaded receiver to isolate one treatment from the other. Light intensity averaged 577 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR (unshaded side) and 15 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR (shaded side) during daylight hours. Supplementary incandescent light during the night (2000-0200

HR) with an intensity of 2.5 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR (unshaded side) and 0.8 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR (shaded side) was used for all treatments to keep the plants vegetative.

Results and Discussion

Leaf removal experiments

Plants from all graft combinations produced similar axillary shoot growth for nodes 10 to 12 in 1990 (Fig. 2.1 and 2.2). However, for CB/CB and CB/C-1 plants axillary shoot node numbers and lengths decreased from node 1 to node 9 in 1990. In contrast, C-1/CB axillary shoot growth increased from node 1 to 10 in 1990 (Fig. 2.1 and 2.2).

CB donors with leaves removed slightly increased the branching of C-1 receivers for nodes 1-9 as compared to C-1/C-1 in 1990 (Fig. 2.1 and 2.2). Leaves were periodically removed from CB donors beginning 30 days after grafting. The agent in CB donor leaves may have been transmitted to C-1 receivers altering its branching pattern within those 30 days. In 1990-1991, leaves were periodically removed from CB donors beginning 7 days after grafting and the C-1 receivers branching pattern was slightly altered (Fig. 2.1 and 2.2). This indicates that the branching agent may have been present in CB donor leaves and was able to be transmitted to C-1 receivers by 7 days after grafting. However, in 1991, the C-1 branching pattern was not altered by CB donors with leaves periodically removed beginning 7 days after grafting (Fig. 2.3 and 2.4). From July to September of 1991,

poinsettia plants were grown and grafted when air temperatures were above the threshold (21/29 C, day/night) for optimum vegetative poinsettia growth (Ecke et al., 1990), which may have adversely affected the CB poinsettia donors capacity to transmit the branching agent to C-1 receivers by 7 days after grafting. High air temperatures are known to reduce the branching capacity of free-branching poinsettias (Dole and Wilkins, 1991). Also in 1991, CB donors with leaves not removed slightly increased C-1 receivers axillary shoot node numbers and lengths at nodes 5-7 (Fig. 2.3 and 2.4). Thus, allowing the leaves to remain on CB donor plants resulted in some transmission of the branching agent despite high air temperatures. The leaf removal experiments results also suggest that the branching agent may not only be present in the leaves of CB poinsettias but also in other parts of the plants. More experiments are needed to determine the specific role of CB donor leaves in the transmission of the branching agent to C-1 receivers.

Stem experiments

Stem removal. As in the leaf removal experiments, plants from all graft combinations showed similar axillary shoot growth from nodes 10 to 12 in 1990, 1990-1991 and 1991 (Fig. 2.5, 2.6, 2.7 and 2.8). However, from nodes 4 to 9 CB/CB and CB/C-1 had a pattern of decreasing axillary shoot node numbers and lengths as compared to C-1/C-1 and C-1/CB in 1990 and 1991 (Fig. 2.5, 2.6, 2.7 and 2.8).

C-1/CB and C-1/C-1 had similar patterns of axillary shoot growth in 1990 (Fig. 2.5 and 2.6). In 1990-1991 however, C-1/CB plants had a pattern of axillary shoot growth similar to CB/C-1 from nodes 5 to 12 (Fig. 2.5 and 2.6). Furthermore, C-1/CB

plants tended to have higher axillary shoot node numbers and greater axillary shoot lengths than C-1/C-1 from nodes 1 to 8 in 1991 (Fig. 2.7 and 2.8).

In 1990, the apical and basal parts of CB donor stems were removed 30 days after grafting and the branching agent was not transmitted from CB donors to C-1 receivers. However, in 1990-1991 and 1991 the apical and basal parts of CB donor stems were removed 15 days after grafting and transmission of the branching agent occured. This suggests the presence of an endogenous growth regulator in the apical and/or basal part of CB donor stems that may have prevented the transmission or the expression of the branching agent after 15 days of grafting. Stems and other parts of CB poinsettia plants may promote transmission of the branching agent.

Nodes and internodes. As in the 1991 leaves and stem removal experiments, C-1/CB and C-1/C-1 plants had a similar pattern of increasing axillary shoot node numbers and lengths from nodes 4 to 10 (Fig. 2.9, 2.10, 2.11 and 2.12). Also, CB/CB and CB/C-1 had a similar pattern of decreasing axillary shoot growth from nodes 3 to 10 (Fig. 2.9, 2.10, 2.11, and 2.12). CB donor node chips did not increase axillary shoot node numbers and lengths of C-1 receivers (Fig. 2.9 and 2.10). Only 20% of the nodes grafted actually developed into shoots. Lack of CB donor chips growth may have limited transmission of the agent from CB donors to C-1 receivers. However, 80% of CB donor internode chip grafts succed and slightly increased C-1 receivers axillary shoot growth above node 6 (Fig. 2.11 and 2.12). The agent present in CB internode chips apparently moved to C-1 receivers increasing C-1 axillary shoot growth. The branching agent in CB internode donors (sink) may have moved against

a source-sink gradient of photosynthates to C-1 receivers (source) via another method of transmission which may have not been as effective as movement by source-sink. The partial transmission of the branching agent from CB internode donors to C-1 receivers also suggested that a source-sink movement of the branching agent may not be the only mechanism of transmission.

The results from the stem experiments and leaf experiments did not demonstrate clear evidence of the specific role of CB donor stems or leaves in the transmission of the branching agent to C-1 receivers (Fig. 2.1 to 2.12). Both stems and leaves of CB poinsettia donors may promote transmission of the branching agent to C-1 receivers.

Shade experiment

CB receivers with donors grown without shade had more axillary shoot nodes than C-1 receivers with donors under shade (Table 2.1, Fig. 2.13). However, CB receivers had higher axillary shoot lengths than C-1 receivers regardless of whether donors were shaded or unshaded (Table 2.1, Fig. 2.14). The difference in axillary shoot nodes between C-1 receivers (donors shaded) and CB receivers (donors unshaded) caused a receiver x shade interaction (Table 2.1, Fig. 2.13).

C-1 and CB receivers with donors under shade had slightly lower axillary shoot node numbers as compared to C-1 and CB receivers with donors unshaded (Fig. 2.13). Photosynthates produced in the unshaded receiver plants may have moved to the shaded donor plants which may have partially reduced photosynthate level of the receiver plants. Reduced photosynthates in receiver plants with shaded donor plants

may have caused the reduction in growth compared to when the donors were unshaded (Fig. 2.13).

Even though there was no significant main effect of CB and C-1 as donors, CB donors (unshaded) partially increased C-1 receiver axillary shoot growth above node 3 as compared to C-1/C-1 (unshaded) (Fig. 2.13 and 2.14). Also, CB donors (shaded) slightly increased C-1 receivers axillary shoot node numbers and lengths above node 4 as compared to C-1/C-1 (shaded) (Fig. 2.13 and 2.14). The lack of significant differences in C-1/CB axillary shoot growth below node 5 precluded the demonstration of a significant main effect of CB as a donor in the transmission of the branching agent. Shading CB donor leaves and stems did not prevent the transmission of the branching agent to C-1 receivers.

If CB donors had been changed to a sink by shading and no transmission of the branching agent to C-1 receivers had occurred, that would have shown that a source-sink relation was necessary for the transmission of the branching agent. However, the amount of shade under which CB donors grew may have caused CB donors to be only partially dependent on C-1 receivers. CB donor leaves grown under shade were green and thus were able to produce photosynthates. We cannot conclude from the results of this experiment that the transmission of the branching agent depends only on a source-sink relationship.

Environmental factors such as light, temperature, inorganic nutrients and water may have affected the transmission of the branching agent between CB donors and

C-1 receivers and thus may have caused the variation in branching between years. Light intensity and duration (photoperiod) during C-1 and CB poinsettia growth may have adversely affected the transmission of the branching agent from CB donors to C-1 receivers. The light intensity in the greenhouse was reduced by a black polypropylene shade used to reduce temperature during the summer and by yellowing of the fiberglass-reinforced plastic glazing. Reduced light intensity in combination with high nitrogen levels is known to increase apical dominance in *Linum usitatissimun* L. var. Redwing (Gregory and Veale, 1957). C-1 receiver plants grafted on CB donors may have had strong apical dominance due to a reduced light intensity in the greenhouse, limiting the transmission or expression of the branching agent.

C-1 receivers and CB donors were grown under long (LD) photoperiods, which favors apical dominance in photoperiodically-sensitive species (Phillips, 1969). Transmission of the branching agent from CB donors to C-1 receivers may have been adversely affected by LD. Also, C-1 apical dominance may have been increased and CB branching capacity reduced by LD, thereby limiting the expression of the branching agent.

Air temperatures were above the threshold (21/29 C day/nigth) for optimum vegetative poinsettia growth during July to September in 1990, 1991 and 1992. High temperatures are known to reduce the branching capacity in free-branching poinsettias (Dole and Wilkins, 1991). The capacity of CB poinsettia donors to transmit the branching agent may have been adversely affected by air temperatures during July to September in 1990, 1991 and 1992.

In the 1990-1991 experiments, C-1 and CB poinsettia donors and receivers had salt toxicity symptoms. Increased soil salinity may have reduced the water availability to the plants and may have reduced the branching pattern of C-1 receivers on CB donors. Apical meristems of C-1, with a strong apical dominance, may have competed for the available water with the axillary buds reducing the water and nutrients available for the axillary shoot growth. Axillary bud growth in *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Pisum sativum* L. has been promoted by water availability and high humidity, indicating that competition for water may play a critical role in apical dominance (McIntyre, 1977).

Both stems and leaves of CB poinsettia donors may promote the transmission of the branching agent to C-1 receivers by influencing the movement of endogenous hormones between CB and C-1. Leaves, buds and the shoot apex may modify apical dominance by influencing the movement of endogenous hormones (Hillman, 1970). An increase in the ratio of endogenous cytokinin in C-1 plants to endogenous auxin may have released C-1 shoots from apical dominance, resulting in emergence of lateral buds (Bidwell, 1991). Furthermore, cytokinins (Sachs and Thimann, 1964; Williams and Stahly, 1968), and auxin-antagonists like 2,3,5-triiodobenzoic acid (Ansen and Hamner, 1953) have increased axillary shoot branching in other plant species.

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Table 2.1. Axillary shoot growth of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with or without shade in 1991-1992. Shade treatment was provided by a 95% black polypropylene fabric (double layer). Black plastic was placed between the shaded donor and the unshaded receiver to isolate one treatment from the other.

		Axillary shoot	
Factors		Number of nodes[z]	Length (mm)[z]
Receiver		**	**
Donor		NS	NS
Shade		**	**
Receiver x Donor		NS	NS
Receiver x Shade		*	*
Donor x Shade		NS	NS
Receiver x Donor x Shade		NS	NS
Receiver	Shade		
C-1	yes	1.8a	19a
CB	yes	4.6ab	73b
C-1	no	3.2ab	28a

CB	no	7.1b	106b

[z]Means followed by the same letter do not differ by LSD, P = 0.05.

[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01 respectively.

Fig. 2.1. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with leaf removal in 1990 and 1991. All leaves were periodically removed from the donor beginning 30 days after grafting in 1990 and beginning 7 days after grafting in 1990-1991. Grafted pairs were pulled apart 40 days after grafting in 1990 and 30 days after grafting in 1990-1991. Means were an average of data from 6 plants.

Fig. 2.2. Axillary shoot length of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with leaf removal in 1990 and 1991. All leaves were periodically removed from the donor beginning 30 days after grafting in 1990 and beginning 7 days after grafting in 1990-1991. Grafted pairs were pulled apart 40 days after grafting in 1990 and 30 days after grafting in 1990-1991. Means were an average of data from 6 plants.

Fig. 2.3. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with or without donor leaf removal in 1991. Donor leaves were periodically removed beginning 7 days after grafting. Plants were pulled apart 30 days after grafting. Means were an average of data from 12 plants.

Fig. 2.4. Axillary shoot length of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with or without leaf removal in 1991. Donor leaves were periodically removed beginning 7 days after grafting. Plants were pulled apart 30 days after grafting. Means were an average of data from 12 plants.

Fig. 2.5. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting and removal of the apical and basal parts of the CB and C-1 donor in 1990 and 1990-1991. The apical and basal parts of the donor stem were removed 30 days after grafting in 1990 and 15 days after grafting in 1990-1991. Means were an average of data from 10 plants in 1990 and 6 plants in 1990-1991.

Fig. 2.6. Axillary shoot length of CB and C-1 poinsettia receivers after grafting and removal of the apical and basal parts of the CB and C-1 donor in 1990 and 1990-1991. The apical and basal parts of the donor stem were removed 30 days after grafting in 1990 and 15 days after grafting in 1990-1991. Means were an average of data from 10 plants in 1990 and 6 plants in 1990-1991.

Fig. 2.7. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting and removal of the apical and basal parts of the CB and C-1 donor, Noble Center Growth Chambers, OSU in 1991. The apical and basal parts of the donor stem were removed 15 days after grafting. Means were an average of data from 12 plants.

Fig. 2.8. Axillary shoot length of CB and C-1 poinsettia receivers after grafting and removal of the apical and basal parts of the CB and C-1 donor, Noble Center Growth Chambers, OSU in 1991. The apical and basal parts of the donor stem were removed 15 days after grafting. Means were an average of data from 12 plants.

Fig. 2.9. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting node chips of the CB and C-1 donor in 1991-1992. The node portion closest to

the apical meristem of one grafted pair was grafted into the other grafted pair at the same node area. Means were an average of data from 9 plants.

Fig. 2.10. Axillary shoot length of CB and C-1 poinsettia receivers after grafting node chips of the CB and C-1 donors in 1991-1992. The node portion closest to the apical meristem of one grafted pair was grafted into the other grafted pair at the same node area. Means were an average of data from 9 plants.

Fig. 2.11. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting internode chips of the CB and C-1 donor in 1991-1992. The internode portion closest to the apical meristem of one grafted pair was grafted into the other grafted pair at the same internode area. Means were an average of data from 8 plants.

Fig. 2.12. Axillary shoot length of CB and C-1 poinsettia receivers after grafting internode chips of the CB and C-1 donor in 1991-1992. The internode portion closest to the apical meristem of one grafted pair was grafted into the other grafted pair at the same internode area. Means were an average of data from 8 plants.

Fig. 2.13. Axillary shoot node number of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with (Yes) or without (No) shade in 1991-1992. Shade treatment was provided by a 95 % black polypropylene fabric (double layer). Black plastic was placed between the shaded donor and the unshaded receiver to isolate one treatment from the other. Means were an average of data from 12 plants.

Fig. 2.14. Axillary shoot length of CB and C-1 poinsettia receivers after grafting on CB and C-1 donors with (Yes) or without (No) shade in 1991-1992. Shade treatment was provided by a 95% black polypropylene fabric (double layer). Black plastic was placed between the shaded donor and the unshaded receiver to isolate one treatment from the other. Means were an average of data from 12 plants.

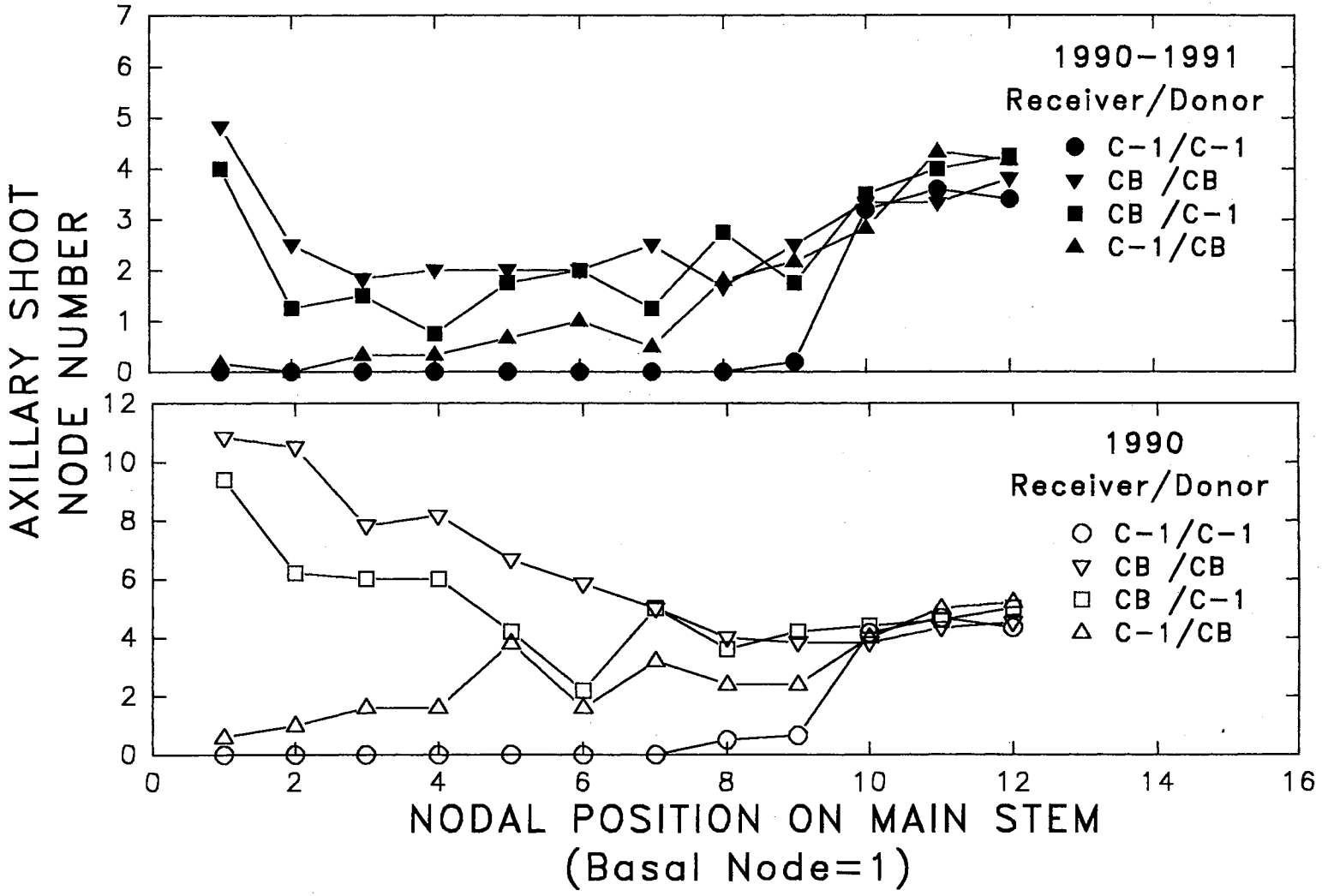

AXILLARY SHOOT
NODE NUMBER
1990–1991
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
1990
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
NODAL POSITION ON MAIN STEM
(Basal Node=1)

Figure 2.1

Figure 2.2

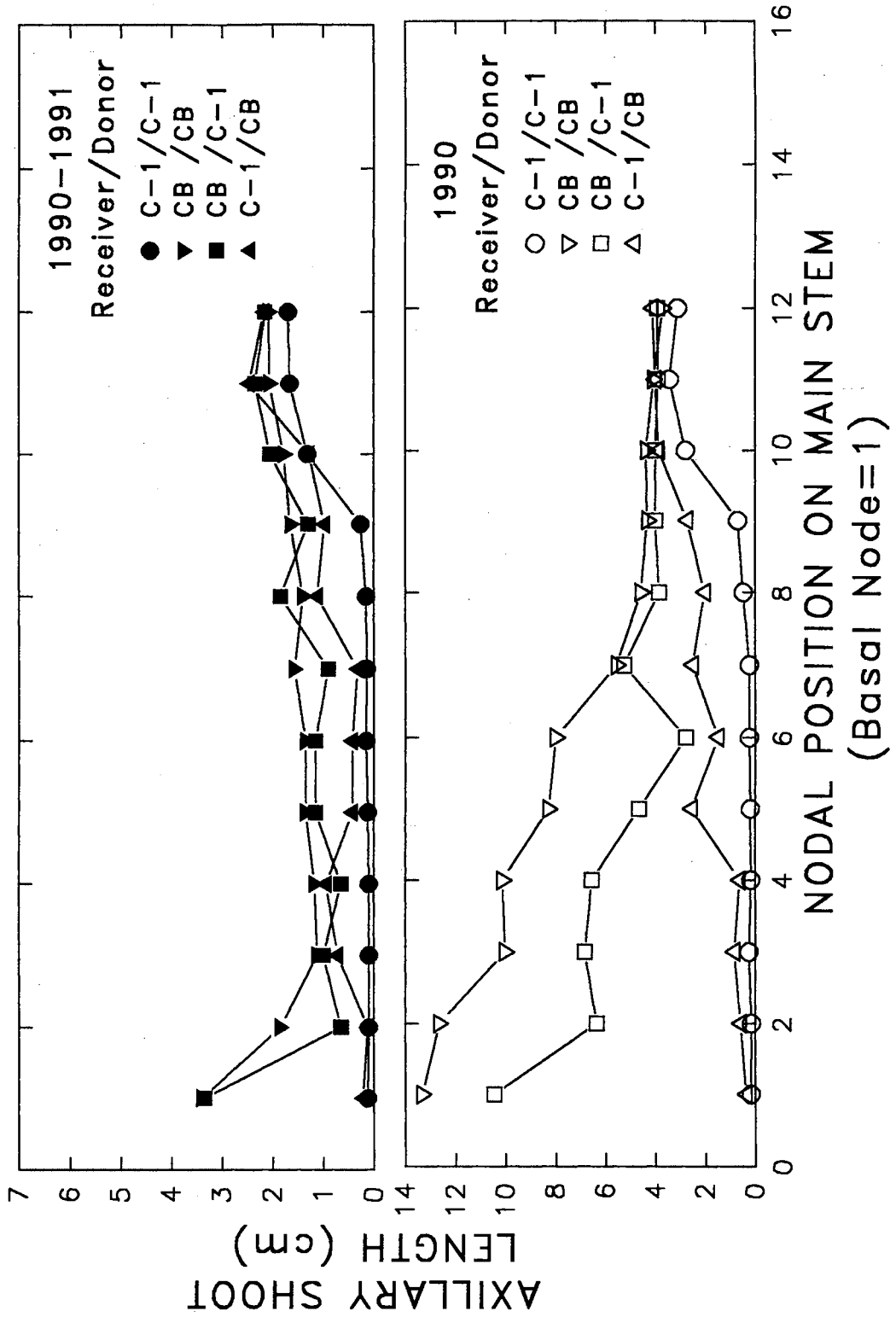

1990–1991
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
1990
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM (Basal Node=1)

Figure 2.3

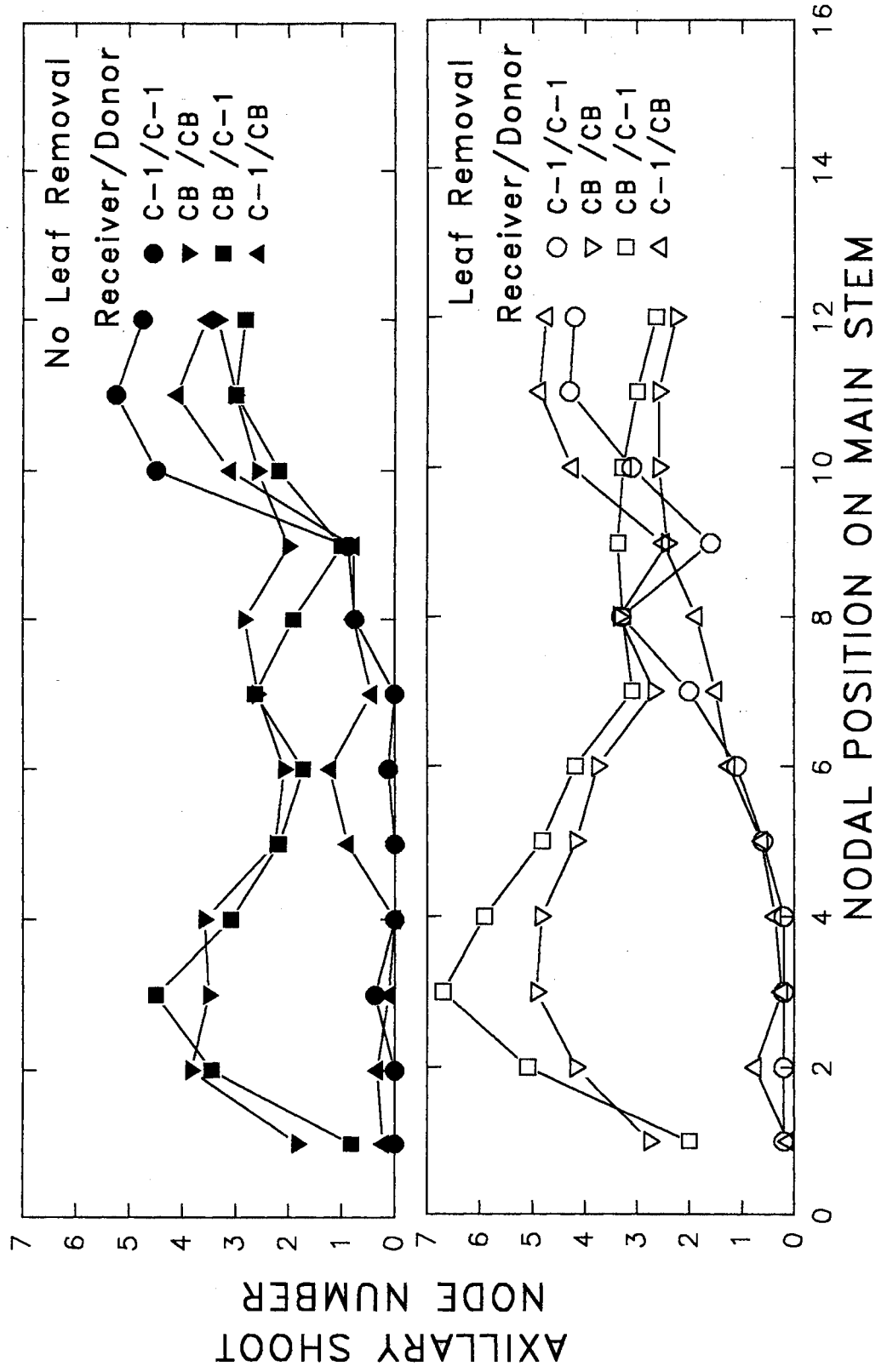
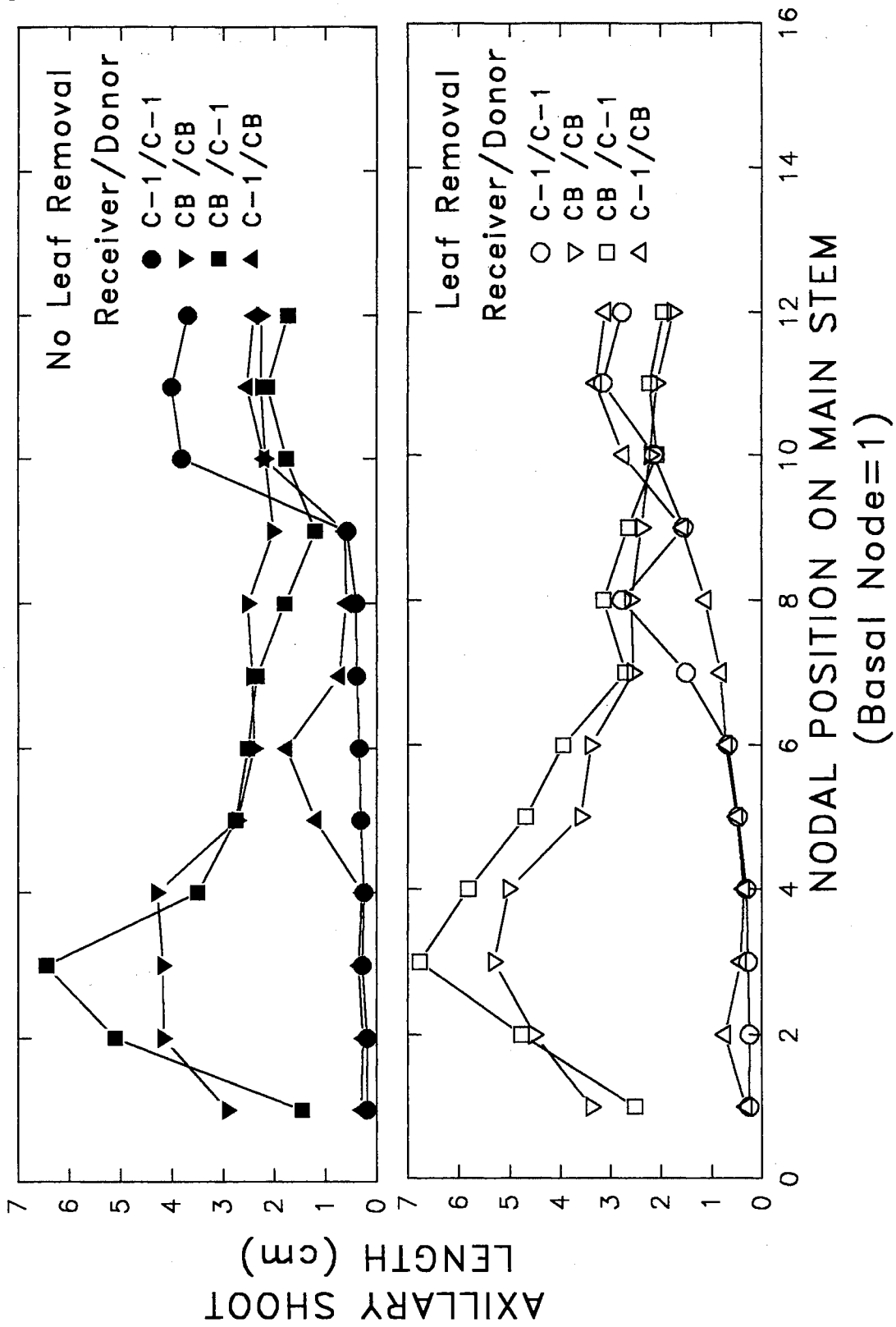

No Leaf Removal
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
Leaf Removal
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
AXILLARY SHOOT NODE NUMBER
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

Figure 2.4


No Leaf Removal
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
Leaf Removal
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM
(Basal Node=1)
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

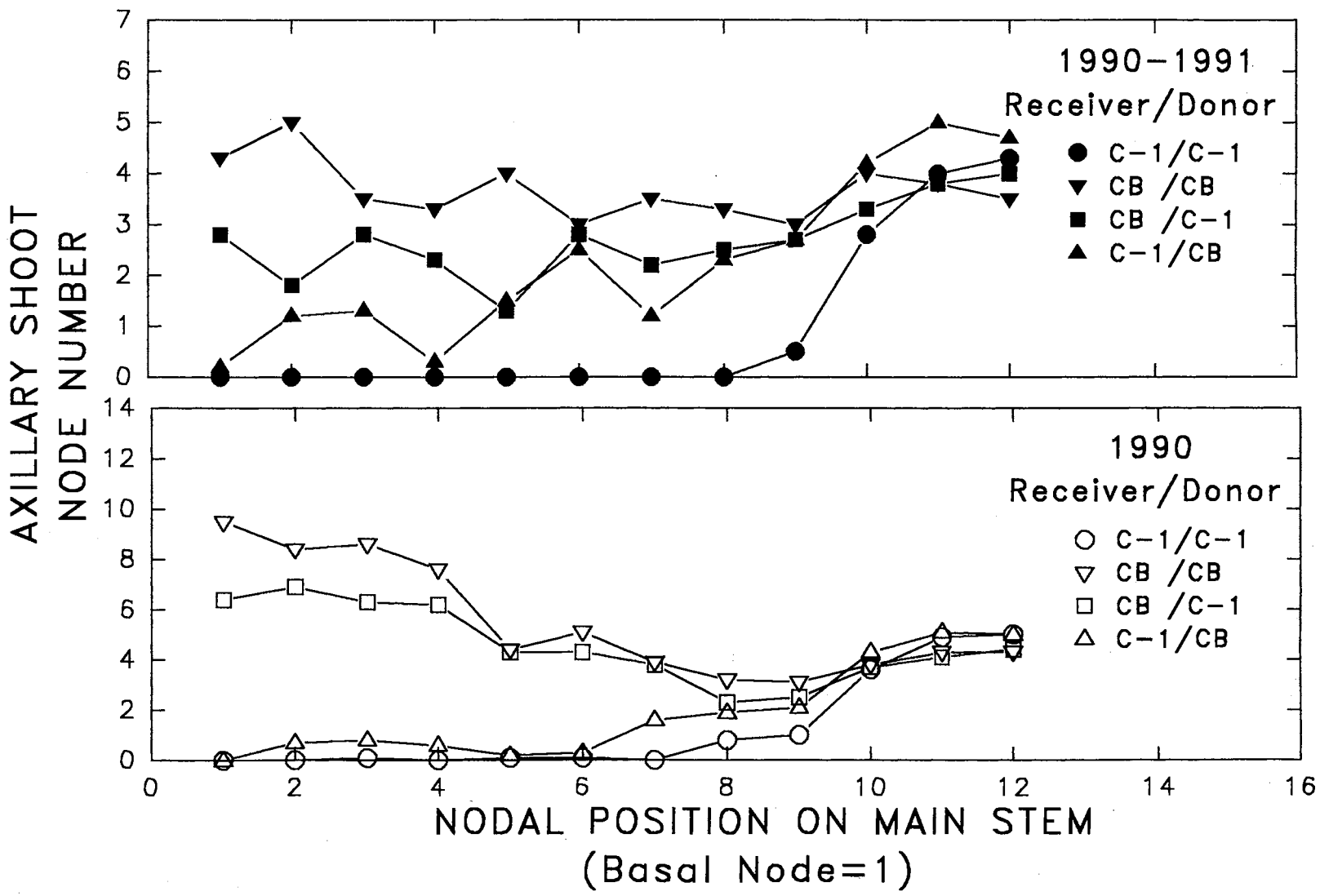

AXILLARY SHOOT
NODE NUMBER
1990–1991
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
1990
Receiver/Donor
C–1/C–1
CB /CB
CB /C–1
C–1/CB
NODAL POSITION ON MAIN STEM
(Basal Node=1)

Figure 2.5

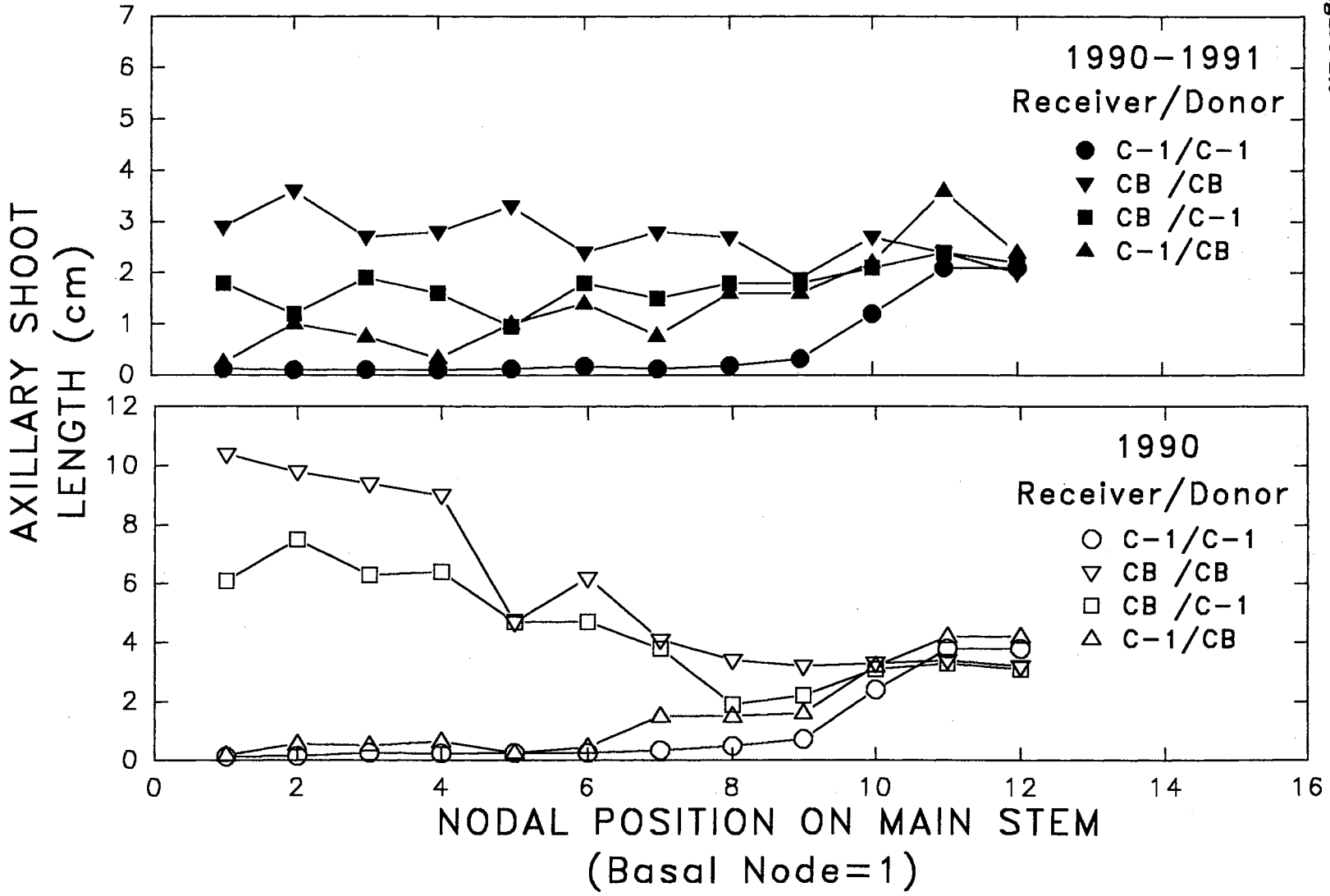

AXILLARY SHOOT LENGTH (cm)
1990–1991
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
1990
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
NODAL POSITION ON MAIN STEM
(Basal Node=1)

Figure 2.6

Figure 2.7

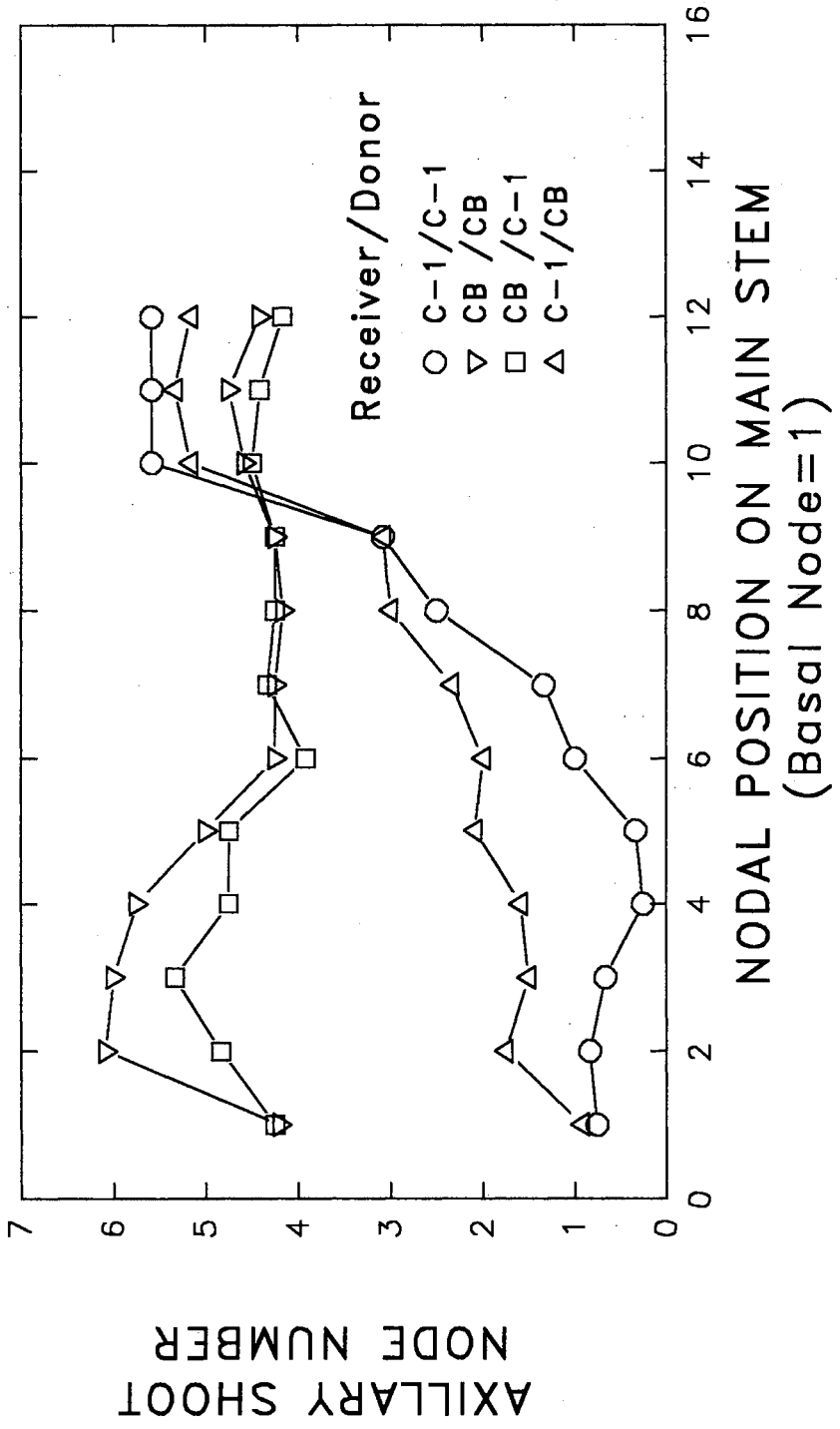

AXILLARY SHOOT
NODE NUMBER
NODAL POSITION ON MAIN STEM
(Basal Node=1)
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

Figure 2.8

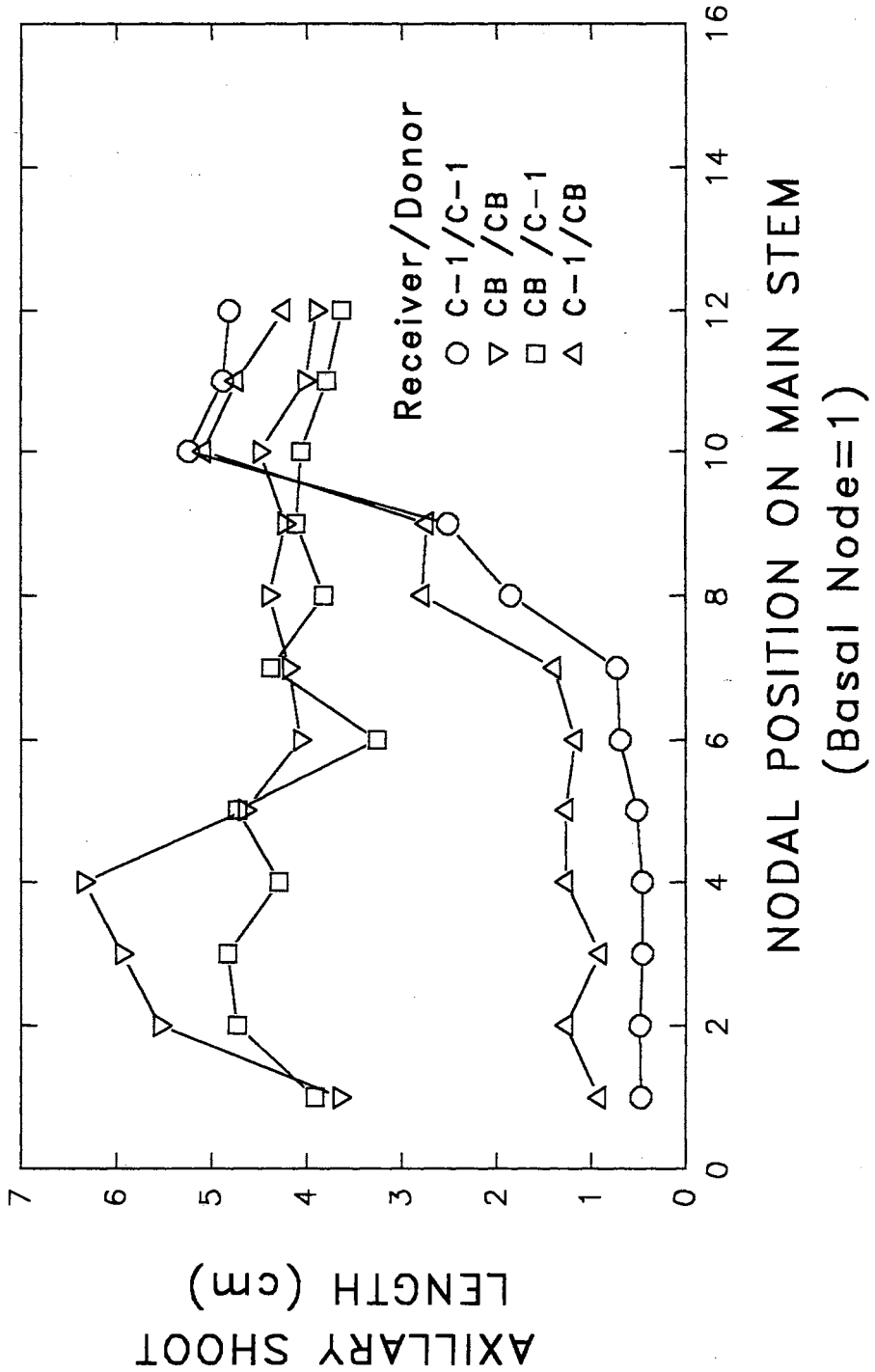

AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM (Basal Node=1)
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

Figure 2.9

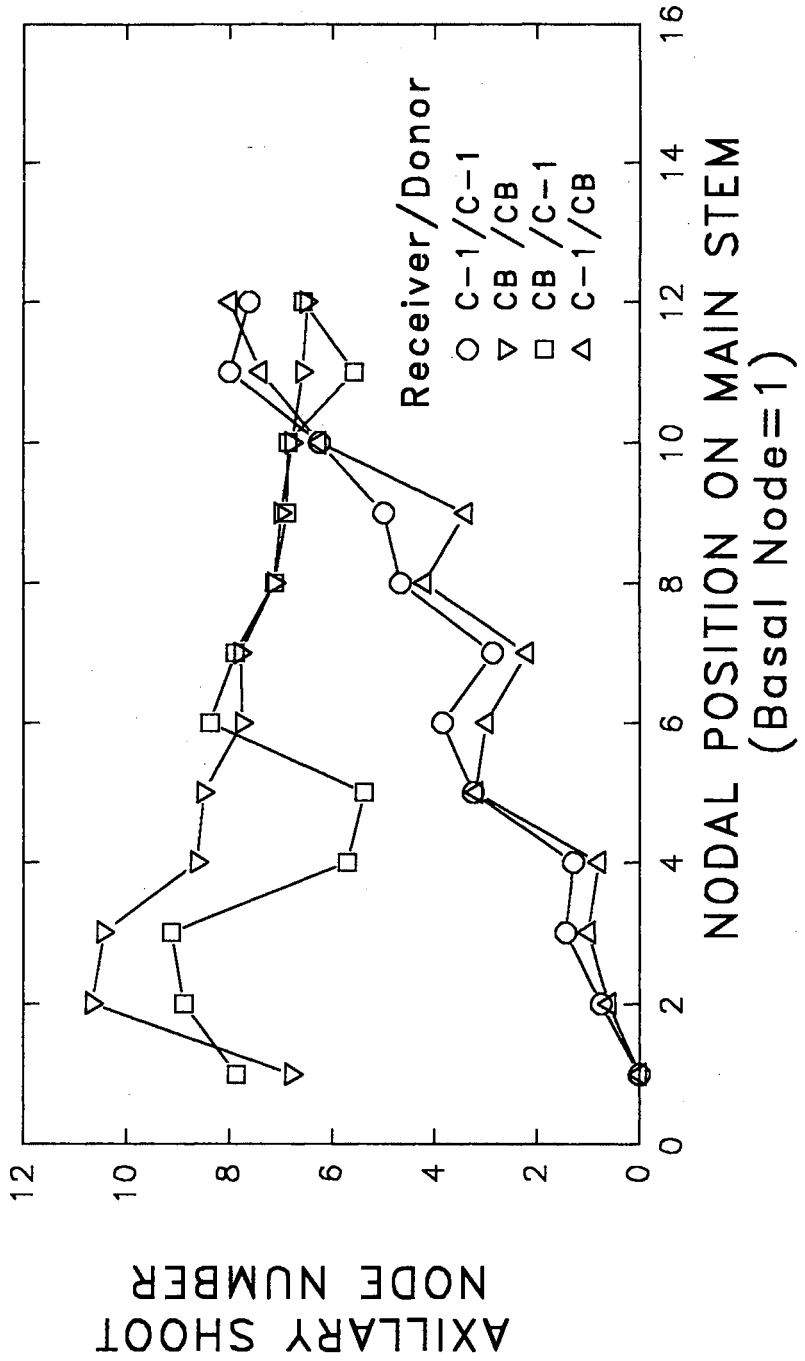

AXILLARY SHOOT
NODE NUMBER
0
2
4
6
8
10
12
NODAL POSITION ON MAIN STEM
(Basal Node=1)
0
2
4
6
8
10
12
14
16
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB

Figure 2.10

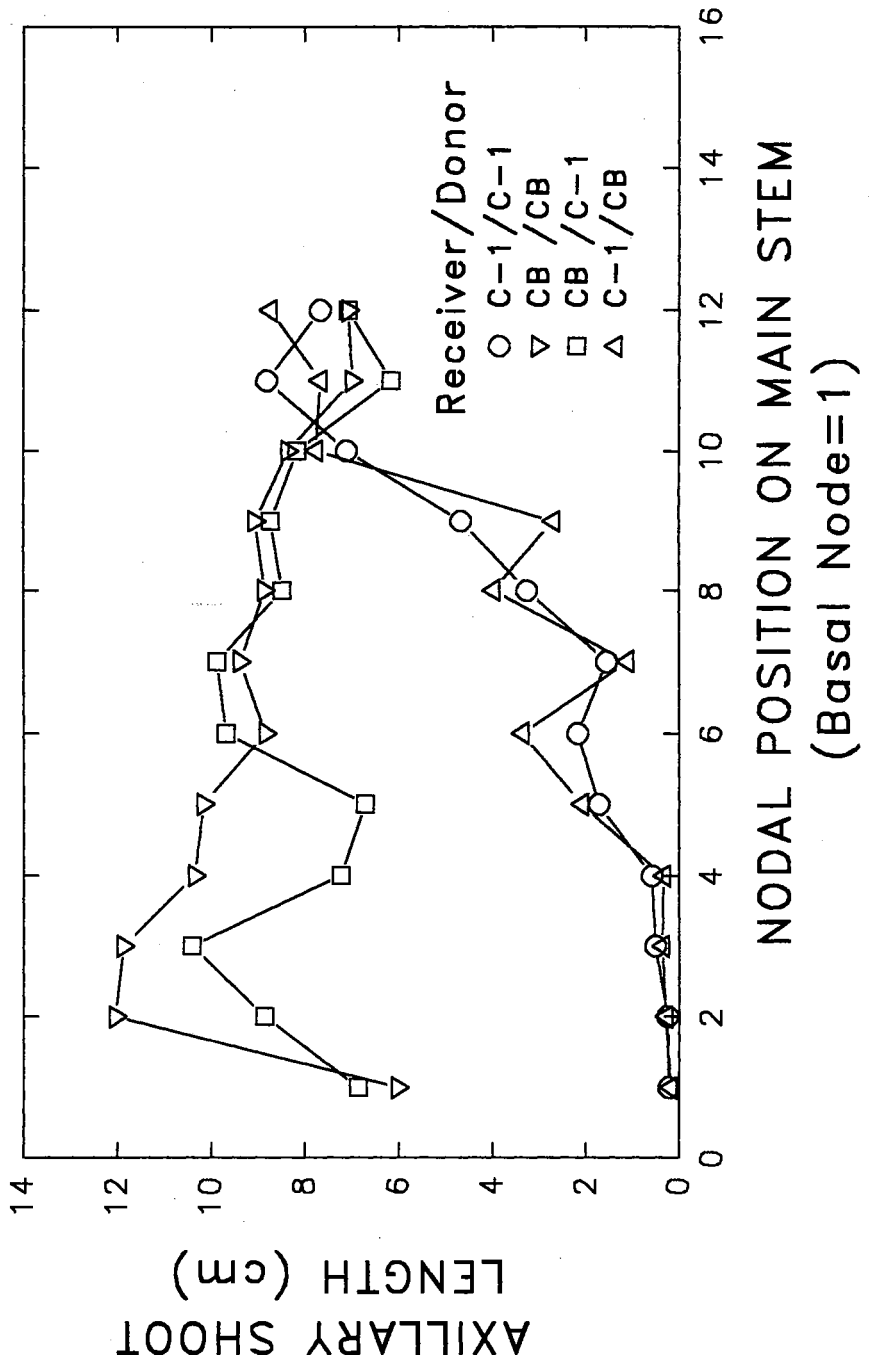

AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM (Basal Node=1)
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
0
2
4
6
8
10
12
14
16

Figure 2.11

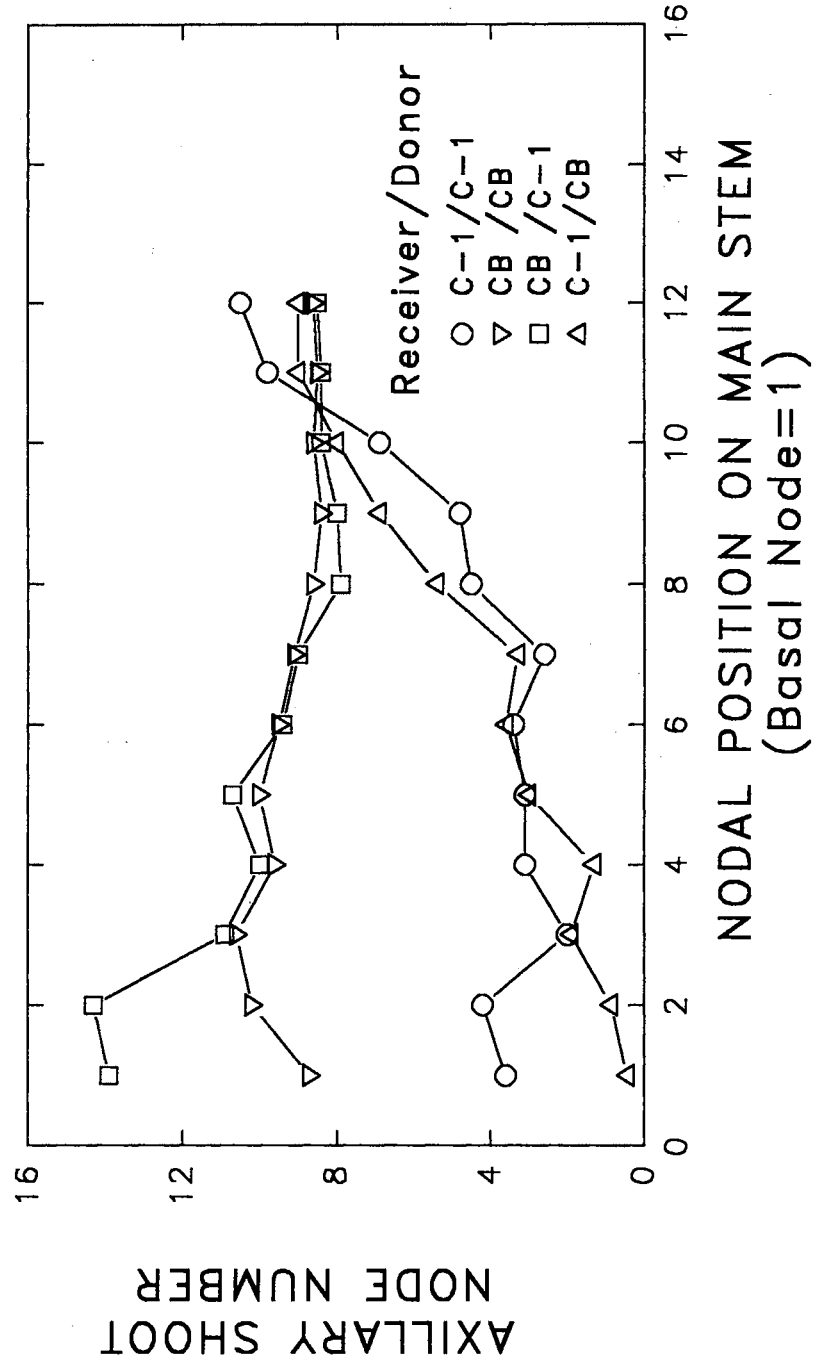

AXILLARY SHOOT
NODE NUMBER
16
12
8
4
0
NODAL POSITION ON MAIN STEM
(Basal Node=1)
0
2
4
6
8
10
12
14
16
Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB

Figure 2.12

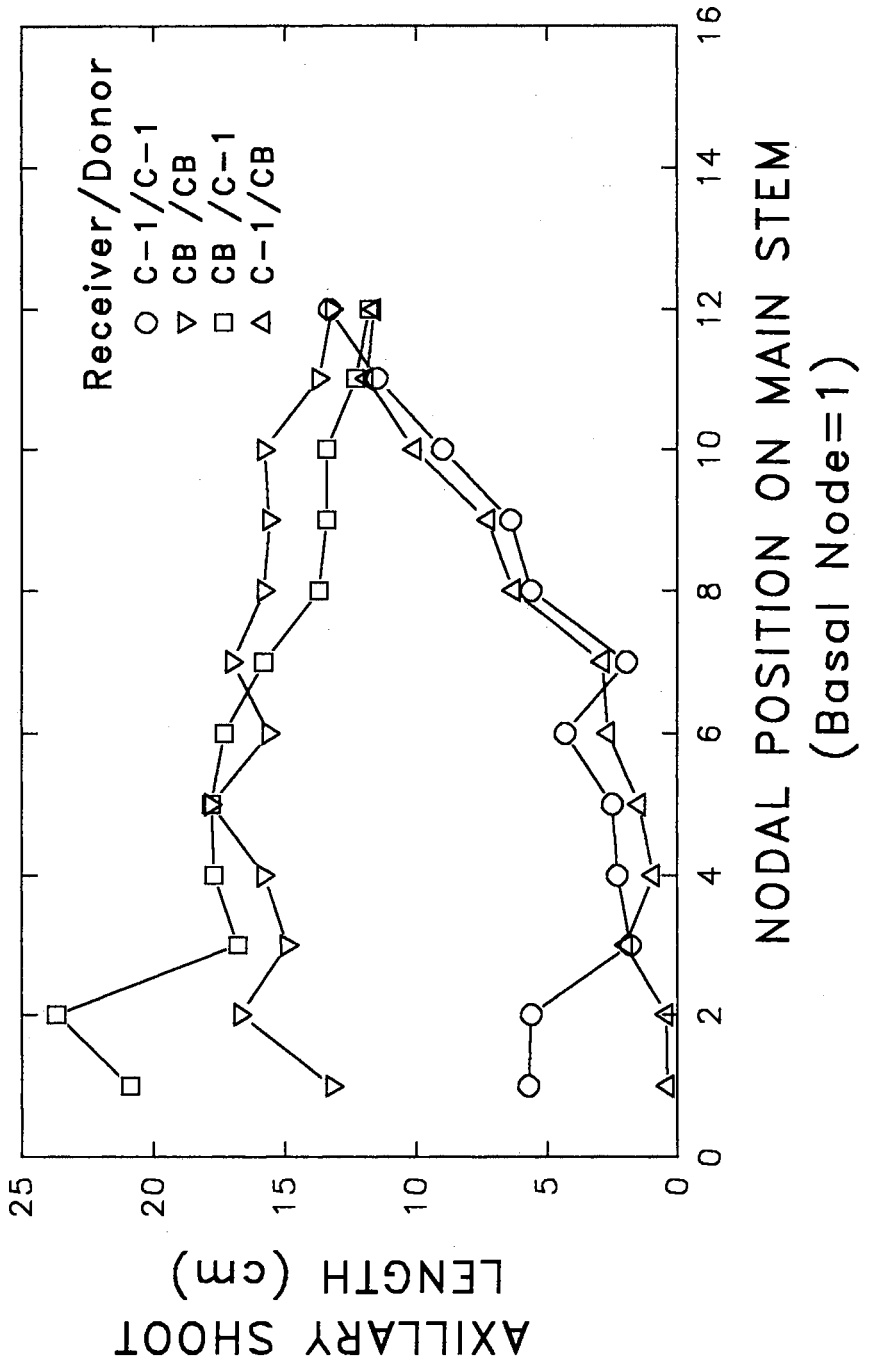

Receiver/Donor
C-1/C-1
CB /CB
CB /C-1
C-1/CB
AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
5
10
15
20
25
0
2
4
6
8
10
12
14
16

Figure 2.13

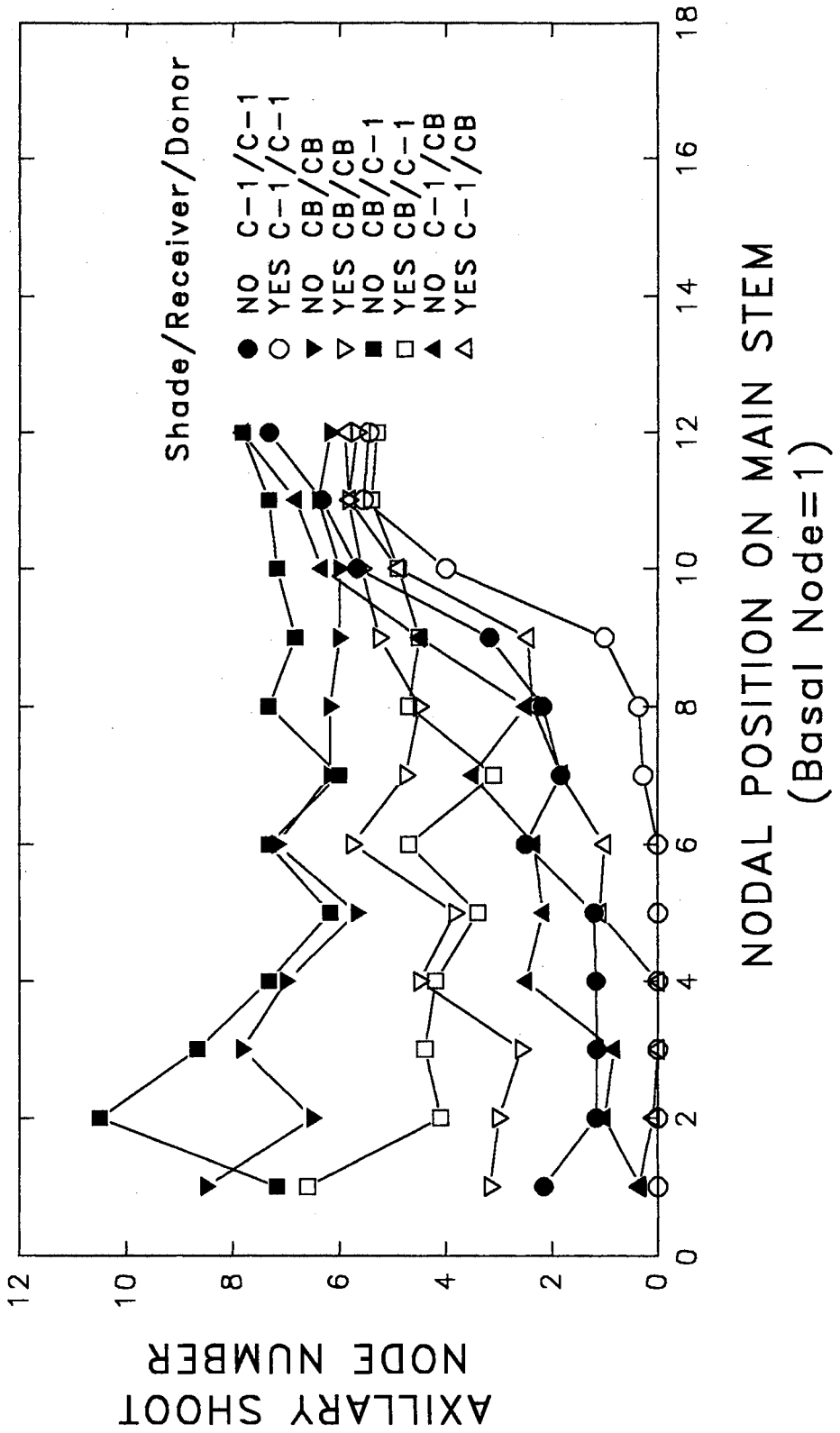
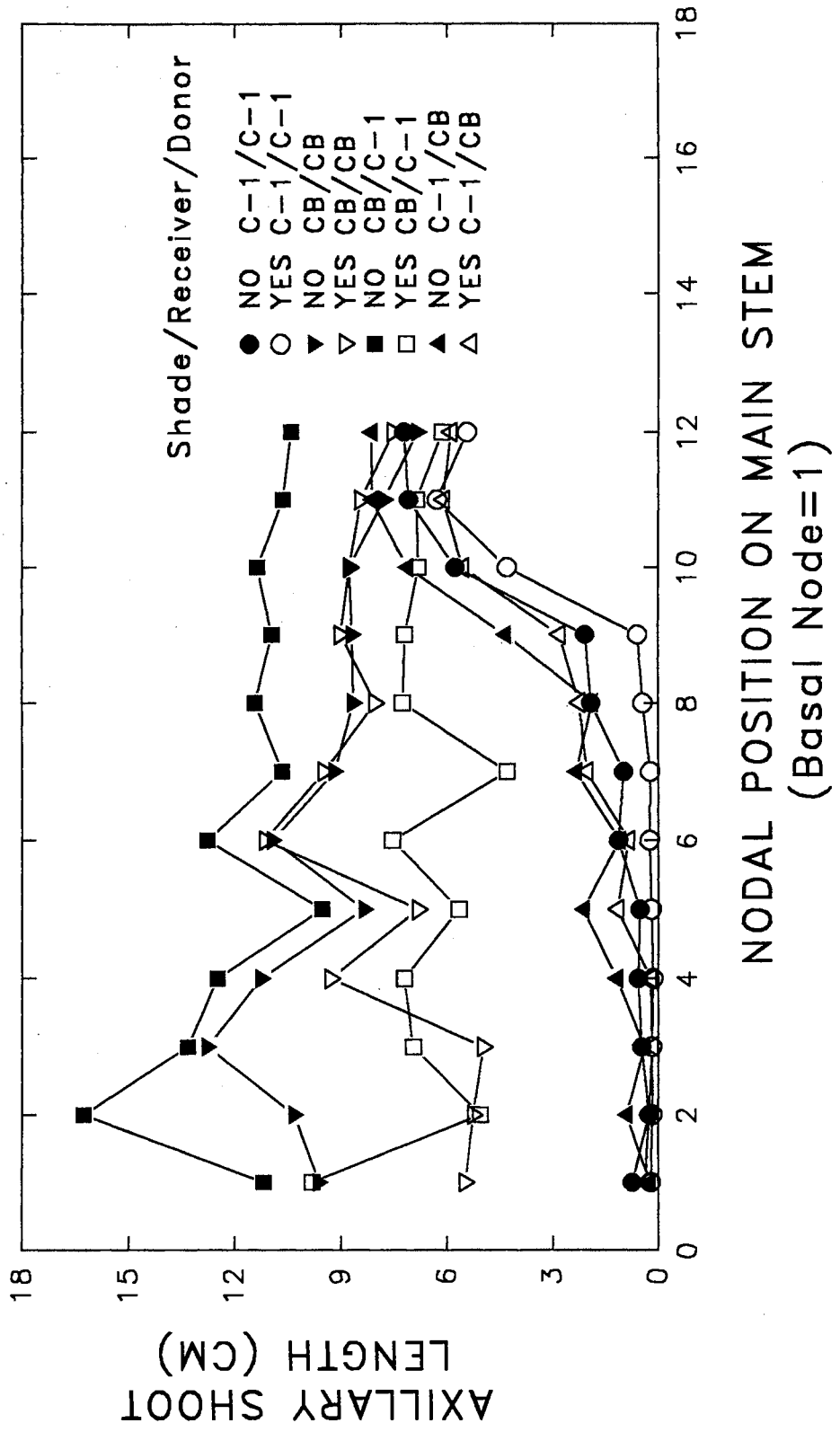

Shade/Receiver/Donor
NO C-1/C-1
YES C-1/C-1
NO CB/CB
YES CB/CB
NO CB/C-1
YES CB/C-1
NO C-1/CB
YES C-1/CB
AXILLARY SHOOT NODE NUMBER
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
2
4
6
8
10
12
14
16
18

Figure 2.14


Shade/Receiver/Donor
NO C-1/C-1
YES C-1/C-1
NO CB/CB
YES CB/CB
NO CB/C-1
YES CB/C-1
NO C-1/CB
YES C-1/CB
AXILLARY SHOOT LENGTH (CM)
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
3
6
9
12
15
18
0
2
4
6
8
10
12
14
16
18

CHAPTER III

CORRELATION OF POINSETTIA GRAFT UNION DEVELOPMENT WITH TRANSMISSION OF THE FREE-BRANCHING AGENT

Gerardo Ruiz-Sifre[1], John M. Dole[1], Paul E. Richardson[2], Brian A. Kahn[1] and Joana Ledford[2]

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Additional index words. *Euphorbia pulcherrima*, apical dominance, axillary branching, free-branching, restricted-branching, isolation layer.

Abstract. *Euphorbia pulcherrima* Willd. ex. Klotzsch cv. 'Eckespoint C-1 Red' (C-1) a restricted-branching poinsettia and 'CB' a free-branching graft-hybrid were approach grafted. Graft unions were removed from poinsettia grafted pairs at 0, 5, 10, 15, 20, 25 or 30 days after grafting for anatomical study and the portion below the graft union was allowed to regrow. By 10 days after grafting, C-1 receivers showed increased branching and C-1 and CB parenchyma cells were actively dividing,

producing new parenchyma cells (callus). Callus connected CB donors and C-1 receivers and may have allowed the transmission of the branching agent by 10 days after grafting. Parenchyma cells differentiated into nodules for the formation of new cambium by 25 days after grafting. CB donors and C-1 receivers were interconnected by new vascular tissue after 25 days of graft formation. CB donors may have controlled the differentiation of vascular tissue of the graft union and further transmission of the branching agent to C-1 receivers.

Grafting a free-branching poinsettia to a restricted-branching poinsettia increases the branching capacity of the restricted-branching poinsettia (Stimart, 1983; Dole and Wilkins, 1991, 1992). A branching agent present in Annette Hegg Brilliant Diamond (AHBD) (a free-branching cultivar) moves acropetally and basipetally through the graft union to Eckespoint C-1 Red (a restricted-branching cultivar) increasing C-1 axillary bud growth (Dole and Wilkins, 1992).

CB (TR), a vegetative or graft-hybrid, was derived from grafting C-1 onto AHBD (Dole and Wilkins, 1992). Vegetative hybridization is the process of grafting two plants together in order to obtain a unique phenotype from the grafted plant itself. Changes in the branching pattern of C-1 were retained through a series of vegetative propagations and are considered permanent (Dole and Wilkins, 1991, 1992). The percentage of C-1 plants exhibiting the free-branching characteristic increased from 0% for 0, 5, and 10 days of graft contact with AHBD to 100% after 30 days (Dole and Wilkins, 1992). A minimum of 10 days were required for AHBD plants to be in

contact with C-1 plants in order to transmit the agent (Dole and Wilkins, 1992).

Anatomical changes during the formation of the graft union between restricted-branching and free-branching poinsettias may induce production of the agent, or allow translocation of the agent through the graft union. The anatomical changes occurring during graft union formation in plants may be involved in vegetative hybridization (Fajnbron, 1953). During graft union formation between CB and C-1 poinsettia, dictyosome activity and callus proliferation may promote the transmission of the branching agent from a CB donor to a C-1 receiver. In *Sedum telephoides* Michx., dictyosome activity and callus proliferation are pronounced along the graft interface at the 24th day after grafting and both functions are correlated with the initial adhesion (Moore and Walter, 1981).

Wound vessel differentiation may occur during graft union formation in poinsettia and may be required for the production and/or translocation of the branching agent. In tomato (*Lycopersicon esculentum* Mill. cv Aisla Craig) autografts, wound vessels differentiate within the callus at the graft union, and are connected into the vascular system of stock and scion by wound vessels differentiating from vascular and cortical parenchyma (Jeffree and Yeoman, 1983). *Picea sitchensis* L. (Bong) xylem elements differentiate directly from vascular cambia of the rootstock and scion and are different from elements arising from parenchymatous callus derived from ray parenchyma (Weatherhead and Barnett, 1986).

The objective of this research was to correlate the development of new cells and tissues during the graft union formation with the movement of the branching agent

a free-branching poinsettia to a restricted-branching poinsettia.

Materials and Methods

Growth and grafting of the plants: Cuttings from C-1 and CB poinsettia plants were treated with 1% IBA (Indole-3-Butyric Acid, Hormex Powder #1, Brooker Chemical, North Hollywood, Calif.) and planted in oasis rootcubes growing medium (Smithers-Oasis, Kent, Ohio) on 19 May 1991. After rooting under intermittent mist at a temperature of 21 C, plants were placed in 16.5-cm (1250 cc) pots filled with commercial peat based medium (Fafard #2, Springfield, Mass.) on 24 June 1991.

Vegetative growth was maintained by supplementary incandescent and high-intensity-discharge (HID) lamps (7.6 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR, 2200 to 0200 HR) to provide long photoperiods (LD) (15 h). Light intensity averaged 780 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR during daylight hours. Air temperature in the greenhouse was maintained at an average 31/21 C day/night. Standard fertilization and pest management practices were used (Ecke et al., 1990).

C-1 and CB plants were approach grafted (Hartmann et al., 1990) from 11 to 14 Aug. 1991 and placed in a completely randomized design. Graft unions for the anatomical study were removed from grafted pairs (donors and receivers) at 0, 5, 10, 15, 20, 25 or 30 days after grafting. The lower portion of C-1 receiver plants below the graft union was allowed to regrow after removal of the graft unions and then thinned to one shoot per plant. CB donor plants were removed at the medium level. Ten single unit replicates were used for each treatment in the anatomical study and 5

were allowed to regrow.

Shoot that regrew from each C-1 receiver plant was pinched at the twelfth node. Thirty days later the number of nodes and the length of the axillary shoots were determined. The number of nodes with leaves (including the terminal leaf blade separated from the apical cone) was determined and the length was measured from the base of each axillary shoot to the tip of the apical cone.

Anatomical study: Ten graft unions were dissected from C-1/CB plants at 0, 5, 10, 15, 20, 25 or 30 days after grafting. The graft union portions were killed and fixed in formalin-propionic acid-alcohol (FPA). They were dehydrated through five increasing concentrations of alcohols, ending in tertiary butanol (TBA) (Johansen, 1940). Specimens were left in each solution (#1-5) for 2 hours. Erythrosin dye in solution #5 provided specimens with a temporary red color which allowed easier orientation during embedding and sectioning. Specimens were put through three consecutive changes of TBA: the first for 2 hours, the second for 24 hours and the third for 24 hours under vacuum.

The material was gradually infiltrated with paraffin (Paraplast, Tissue Embedding Medium, Lancer, St. Louis, MO), by adding shavings of paraffin to stoppered vials containing the specimens in TBA while vials were held at 30 C. After 24 hours, more paraffin was added to the unstoppered vials which were then held at 45 C for 24 hours. After a third addition of paraffin, vials were held under vacuum at 56 C for 24 hours. Vials were then moved to a 60 C oven and melted paraffin was replaced several times with fresh melted paraffin until the odor of TBA was gone.

Specimens were embedded in plastic molds (Polysciences Inc., Warrington, PA).

Embedded graft unions were sectioned at 12 microns with a rotary microtome. Cross or longitudinal serial sections were affixed with Haupt's adhesive and then stained with safranin and fast green (Berlyn and Miksche, 1976). Cover slips were applied with Adams Histoclad mounting medium (Clay Adams, Parsippany, NJ).

Graft union microscope slides were examined through a light microscope (x50, x100, x200 and x450). Anatomical features of the CB and C-1 graft union regions were observed at 0, 5, 10, 15, 20, 25 and 30 days after grafting.

Results and Discussion

A cross section of CB poinsettia at 0 days after grafting shows the tangential cut area that was made in preparation for grafting (Fig. 3.1). CB and C-1 graft union formation began with the secretion of latex fluid onto the wound surface. This latex initially adhered CB and C-1 plants and may have encouraged subsequent cellular interaction through the exchange of plant metabolites between the grafted pair. At 5 days after grafting the formation of necrotic material from the cell contents and cell walls of cut CB donor and C-1 receiver plant tissue was observed (a wound healing response) (Fig. 3.2 and 3.3). This necrotic material consisted of dead cells at the cut surface of approach of both CB and C-1 and was at least a cell layer deep. However, at 5 days after grafting, CB and C-1 plants were not yet adhered and easily separated when the graft union was removed from the grafted pairs.

At both sides of the necrotic material (isolation layer) 5 to 7 layers of

parenchyma cells were formed by enlargement and division of parenchyma cells at 10 days after grafting (Fig. 3.4, 3.5 and 3.6). These parenchyma cells differentiated into ray parenchyma which acted as cambium and produced new parenchyma cells (callus) by 15 days after grafting (Fig. 3.7).

Callus tissue penetrated the thin necrotic layer and filled the space between CB donor and C-1 receiver plants (Fig. 3.7) which became interlocked and provided some mechanical support where the isolation or necrotic layer was broken (Fig. 3.8). Discontinuous cell bridges were found perpendicular to the isolation layer between CB and C-1 plants (where the isolation layer was broken) at 15 days after grafting (Fig. 3.9).

Traces of the isolation layer were not evident within the cortex and vascular tissue of CB and C-1 plants at 20 days after grafting which indicated that the graft union was nearly complete in that area (Fig. 3.10). However, the isolation layer was evident near the pith area indicating that the graft union was still in the process of being formed at 20 days after grafting (Fig. 3.10).

Nodules for the formation of new vascular tissue were formed between CB and C-1 by 25 days after grafting (Fig. 3.11). The newly formed vascular tissue (new xylem and phloem) between CB donor and C-1 receiver plants was evident at 30 days after grafting (Fig. 3.12). Production of new xylem and phloem permitted vascular connection between CB donor and C-1 receiver plants. Furthermore, CB and C-1 graft union formation was almost completed at 30 days after grafting (Fig. 3.13). Traces of the isolation layer were present only near the pith area of grafted pairs (Fig. 3.13).

The results from the anatomical study correlated with the transmission of the branching agent from CB donor to C-1 receiver plants after the graft union was removed and C-1 receiver shoots allowed to regrow. At 0 and 5 days after grafting, C-1 receivers and CB donors did not have axillary shoot growth from node 0 to 9 (Fig. 3.14 and 3.15). At 0 and 5 days after grafting, C-1 receiver and CB donor plants were easily separated when the graft union was removed, suggesting that a connection between the plants had not occurred and the transmission of the branching agent was not possible.

At 10 days after grafting axillary shoot node numbers and node lengths increased significantly from node 1 to 9 in C-1 receiver plants (Fig. 3.14 and 3.15). C-1 receiver and CB donor plants did not separate when the graft union was removed and new parenchyma cells were formed at the C-1 and CB sides of the isolation layer. The new parenchyma cells formed at each side of the isolation layer may have been connected through plasmodesmata across parts of the isolation layer, thereby allowing the transmission of the branching agent between CB donor and C-1 receiver plants. Plasmodesmata connections between stocks (donor) and scions (receiver) has been demonstrated in reciprocal grafts of *Helianthus annuus* L. and *Vicia faba* L. (Kollmann and Glockmann, 1985; Kollmann et al., 1985).

C-1 receiver axillary shoot node numbers and lengths increased from 10 to 25 days after grafting (Table 3.1 and 3.2). Discontinuous cell bridges and callus were formed between CB donor and C-1 receiver plants at 15 days after grafting (Fig. 3.7 and 3.9) and may have enabled symplastic and apoplastic movement of the branching

agent between CB and C-1 plants. Traces of the isolation layer were not evident between the vascular tissue of CB and C-1 grafted pairs at 20 days after grafting (Fig. 3.10) which may have further allowed the transmission of the branching agent. The presence of nodules for the formation of new vascular tissue suggested the possibility of further movement of the branching agent between CB and C-1 through newly formed vascular tissue after 25 days of graft union formation (Fig. 3.11 and 3.12).

According to previous research, between 10 and 15 days of graft union contact between AHBD plants and C-1 plants was required in order to transmit the branching agent (Dole and Wilkins, 1992). However, CB plants needed to be in contact with C-1 plants between 5 and 10 days for the transmission of the agent in the current research. CB and C-1 graft unions developed under high air temperatures during August and September in the current research. High air temperatures may have hastened the development of the graft union and may have allowed the transmission of the agent in a shorter period of time than in the previous research.

The CB side of the grafted pairs may have developed faster than the C-1 side after 10 days of graft union formation (Fig. 3.7 to 3.13). The isolation layer was more broken and more ray parenchyma was present in the CB side than in the C-1 side by 15 days after grafting (Fig. 3.7). More new vascular tissue was evident in the CB side than in the C-1 side by 30 days after grafting (Fig. 3.12). C-1, a restricted-branching poinsettia with a strong apical dominance, may have a higher endogenous concentrations of auxins than of cytokinins. Conversely CB, a free-branching graft-hybrid with a weak apical dominance, may have a higher endogenous concentration of

cytokinins than auxins. Auxins and cytokinins from CB and C-1 poinsettias may have induced cell proliferation and vascularization in the callus of the graft union between CB and C-1 grafted pairs. The dedifferentiation (reversal to a meristematic state of differentiated parenchyma cells) of the *Oleaceae* callus into vascular tissue by direct application of auxin (Wetmore and Rier, 1963) and the cell proliferation and cytodifferentiation (differentiation of parenchyma cells into xylem and phloem) of soybean (*Glycine max* (L.) Merrill) callus by cytokinins (Torrey et al., 1971) have been demonstrated. CB plants with higher cytokinins than auxin may have hastened the vascularization of the callus and connection between CB and C-1 grafted pairs and promoted the transmission of the branching agent between CB and C-1 plants. Increasing levels of cytokinins may have resulted in progressively increased cell proliferation and xylem and phloem differentiation from callus in soybean (Torrey et al., 1971).

High concentrations of endogenous cytokinins in CB donor plants may not only have promoted vascularization during the graft union formation but may also have been transported to C-1 receivers inducing vascularization of C-1 axillary buds and connecting them to the stem. Vascularization and high levels of cytokinin in C-1 axillary shoots may have been necessary to promote their growth. The correlation between vascular connection development and axillary bud outgrowth has been established by histological studies (Gregory and Veale, 1957; Sorokin and Thimann, 1964; Panigrahi and Audus, 1966). Sorokin and Thimann (1964) suggested that bud inhibition may be due to incomplete contact between the bud and the vascular bundles

of the stem, and the effectiveness of kinetin in releasing the inhibition would then be due to its action on xylem differentiation. High concentrations of endogenous auxin in C-1 plants may have prevented the development of vascular connections between C-1 stems and axillary shoots. High auxin content of the stem tissue impedes or prevents the formation of provascular strands in the axillary shoots (Gregory and Veale, 1957).

In summary, the development of new cells and tissues during CB and C-1 graft union formation was correlated with the movement of the branching agent between CB donors and C-1 receivers. C-1 axillary shoot growth was increased by CB donors at 10 days after grafting. New cells and tissues were progressively formed in the graft union between CB and C-1 plants allowing the transmission of the branching agent by 10 days of graft union formation.

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Table 3.1. Axillary shoot growth of C-1 poinsettia receivers grafted on CB donors after removal of the graft unions and regrowth of the receiver plants at 0 to 30 days after grafting in 1991. CB donor plants were removed at the medium level. Means were an average of 5 plants.

	Axillary shoot	
Time after grafting (days)	Number of nodes	Length (mm)
0	1.4	15
5	1.5	14
10	2.4	21
15	2.9	25
20	3.2	28
25	3.8	37
30	2.5	24
Linear	**	*
Quadratic	*	NS

NS,*,**Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Table 3.2. Significance per node on the main stem for axillary shoot node number and length of C-1 poinsettia receivers grafted on CB donors after removal of the graft union and regrowth of the receiver plants at 0 to 30 days after grafting by LSD, in 1991. CB donor plants were removed at the medium level. Means were an average of data from 5 plants.

	Nodes											
Axillary shoot	1	2	3	4	5	6	7	8	9	10	11	12
Node number	*	NS	*	*	NS	**	**	*	**	NS	NS	NS
Length	**	NS	NS	**	NS	*	*	NS	*	NS	NS	NS

NS,*,**Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Fig. 3.1. Cross section of *Euphorbia pulcherrima* 'CB' showing the surface (s) of approach for the graft at 0 days after grafting. x63.

Fig. 3.2. Cross section of *Euphorbia pulcherrima* 'CB' showing the necrosis (n) along the surface (s) of approach for the graft at 5 days after grafting. x63.

Fig. 3.3. Cross section of *Euphorbia pulcherrima* 'Eckespoint C-1 Red' showing the necrosis (n) along the surface (s) of approach for the graft at 5 days after grafting. x63.

Fig. 3.4. Cross section of *Euphorbia pulcherrima* 'CB' showing five to seven layers of parenchyma cells (c) formed by enlargement and division along the isolation (i) layer at 10 days after grafting. x63.

Fig. 3.5. Cross section of *Euphorbia pulcherrima* 'Eckespoint C-1 Red' (C-1) showing five to seven layers of parenchyma cells (c) formed by enlargement and division along the isolation (i) layer at 10 days after grafting. x63.

Fig. 3.6. Longitudinal section of *Euphorbia pulcherrima* 'CB' and 'C-1' poinsettia grafted pair graft union showing five to seven layers of parenchyma cells (c) formed by enlargement and division to either side of the isolation (i) layer at 10 days after grafting. x118.

Fig. 3.7. Longitudinal section of 'CB' and 'C-1' poinsettias grafted pair graft union showing callus tissue (ct) formed by ray (r) parenchyma between the isolation (i) layers at 15 days after grafting. x63.

Fig. 3.8. Longitudinal section of the isolation (i) layer being broken (arrow) between a *Euphorbia pulcherrima* 'CB' and 'C-1' grafted pair at 15 days after grafting. x420.

Fig. 3.9. Longitudinal section showing discontinuous cell bridges (cb) perpendicular to the isolation (i) layer between *Euphorbia pulcherrima* 'CB' and 'C-1' at 15 days after grafting. x63.

Fig. 3.10. Cross section of 'CB' and 'C-1' grafted pair graft union showing that isolation(i) layer was not evident at the cortex (cx) and vascular tissue (vt) at 20 days after grafting. However, in the pith (p) area the isolation (i) layer was evident indicating that the graft union was still in the process of being formed. x63.

Fig. 3.11. Longitudinal section showing nodules (no) for the formation of new vascular tissue were being formed between *Euphorbia pulcherrima* 'CB' and 'C-1' at 25 days after grafting. x118.

Fig. 3.12. Longitudinal section of 'CB' and 'C-1' poinsettia grafted pairs graft union showing a nodule (no) and beside it vascular tissue (vt) being formed between *Euphorbia pulcherrima* 'CB' and 'C-1' at 30 days after grafting. x118.

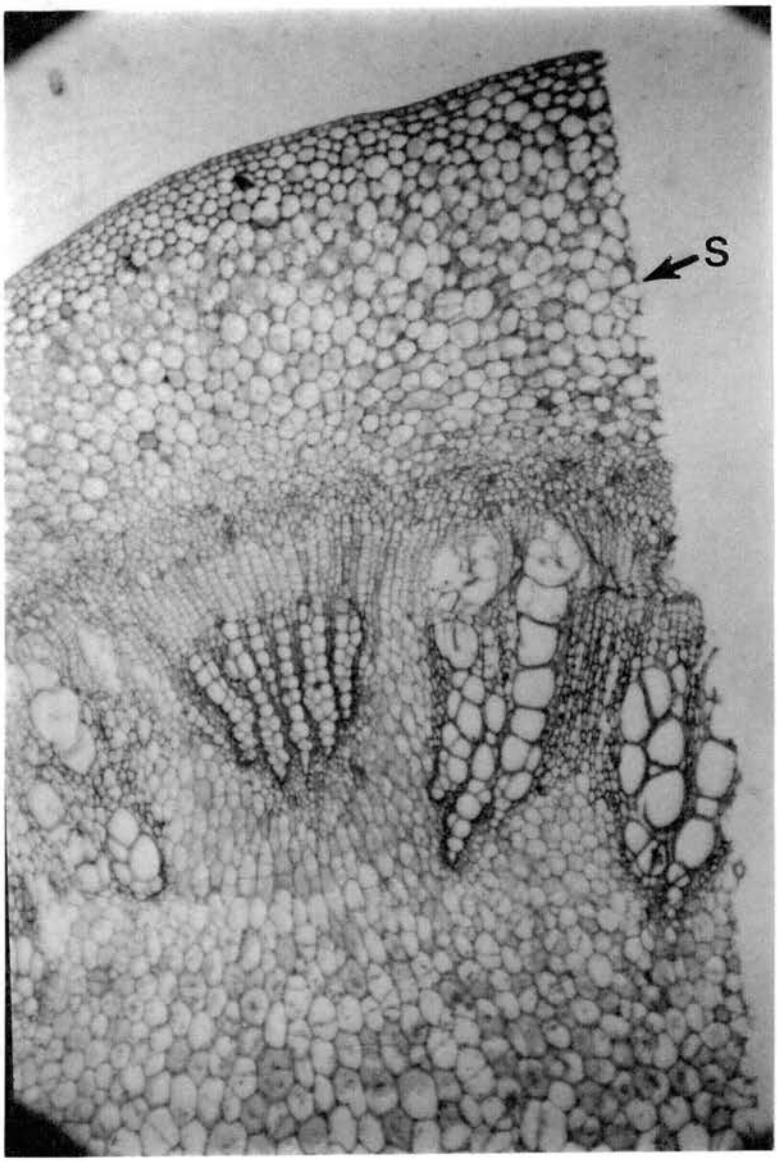
Fig. 3.13. Cross section of the *Euphorbia pulcherrima* 'CB' and 'C-1' healed graft union. Isolation (i) layer was only present near the pith (p) area of the grafted pair. x63.

Fig. 3.14. Axillary shoot node number of C-1 poinsettia receivers on CB donors after cutting back plants to below the graft union at 0 to 30 days after grafting in

1991. CB donor plants were removed at the medium level. Means were an average of data from 5 plants.

Fig. 3.15. Axillary shoot length of C-1 poinsettia receivers on CB donors after cutting back plants to below the graft union at 0 to 30 days after grafting in 1991. CB donor plants were removed at the medium level. Means were an average of data from 5 plants.

Figure 3.1



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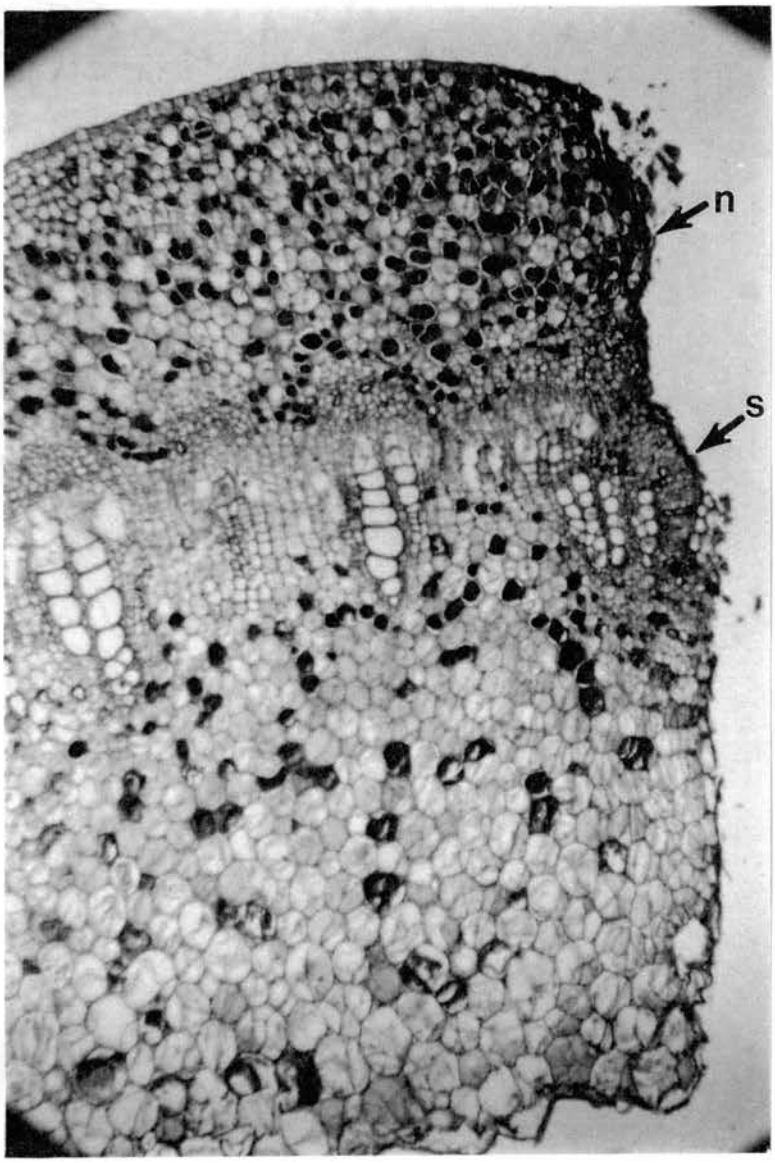

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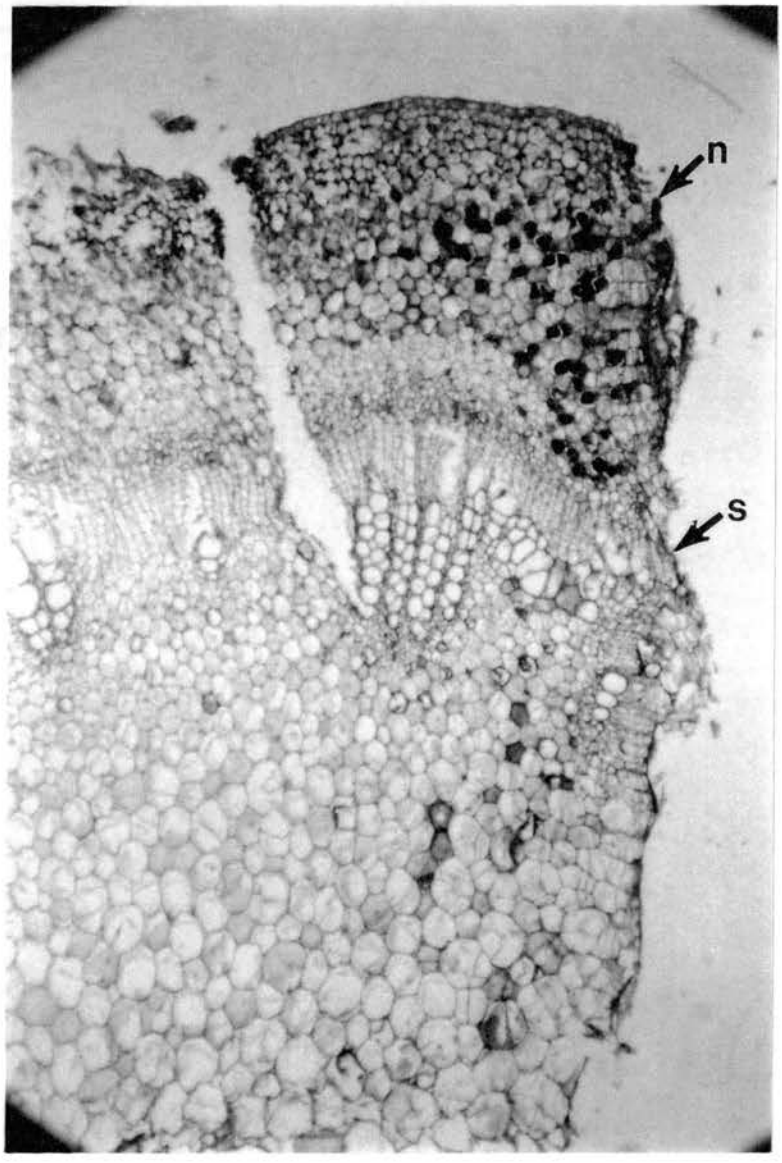

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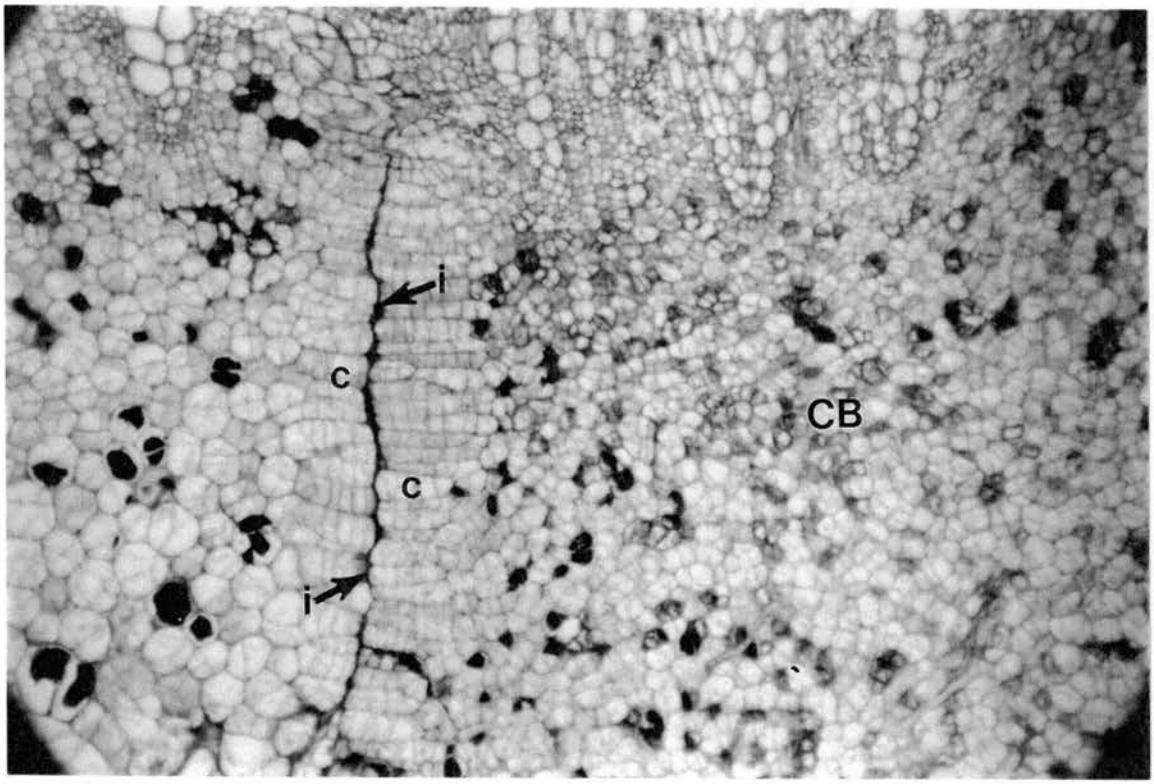

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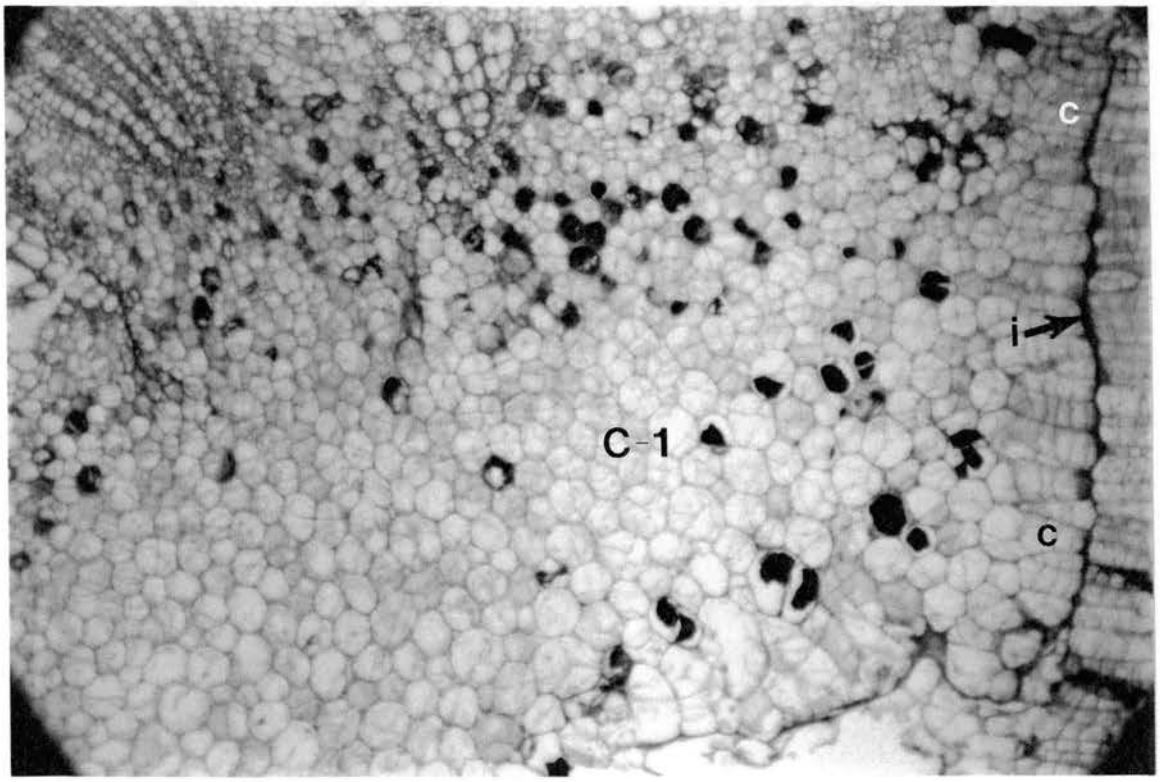

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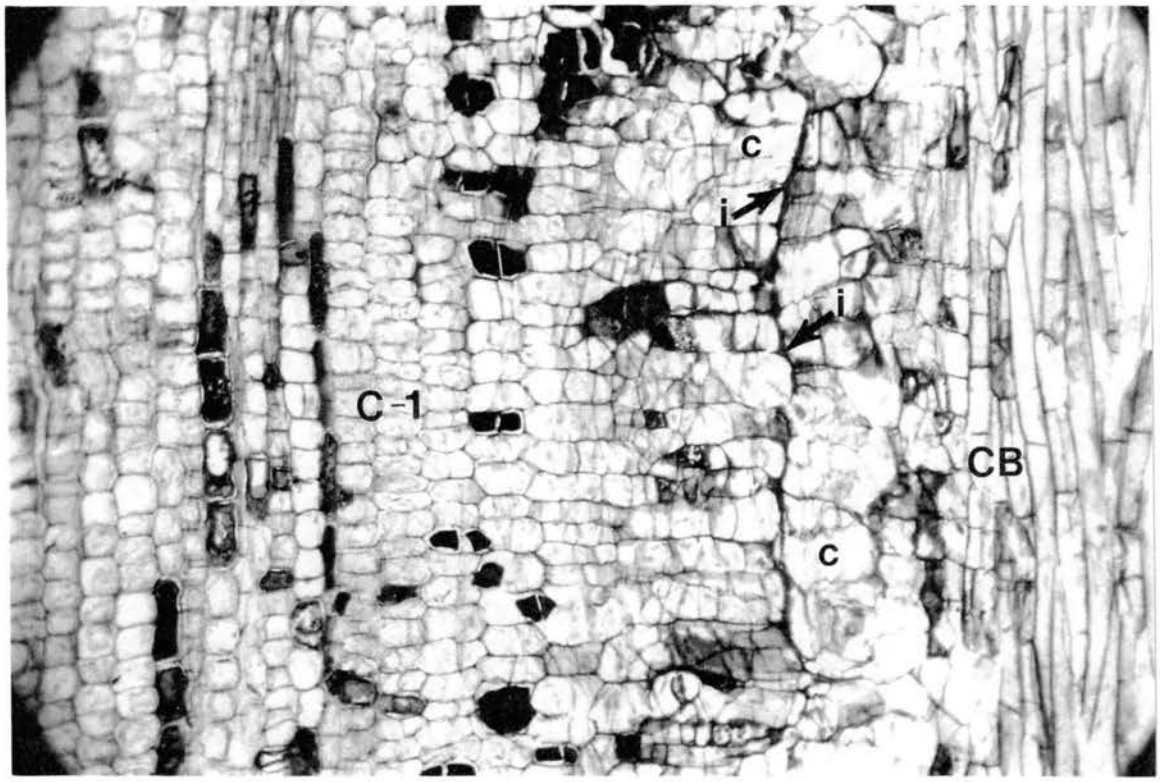
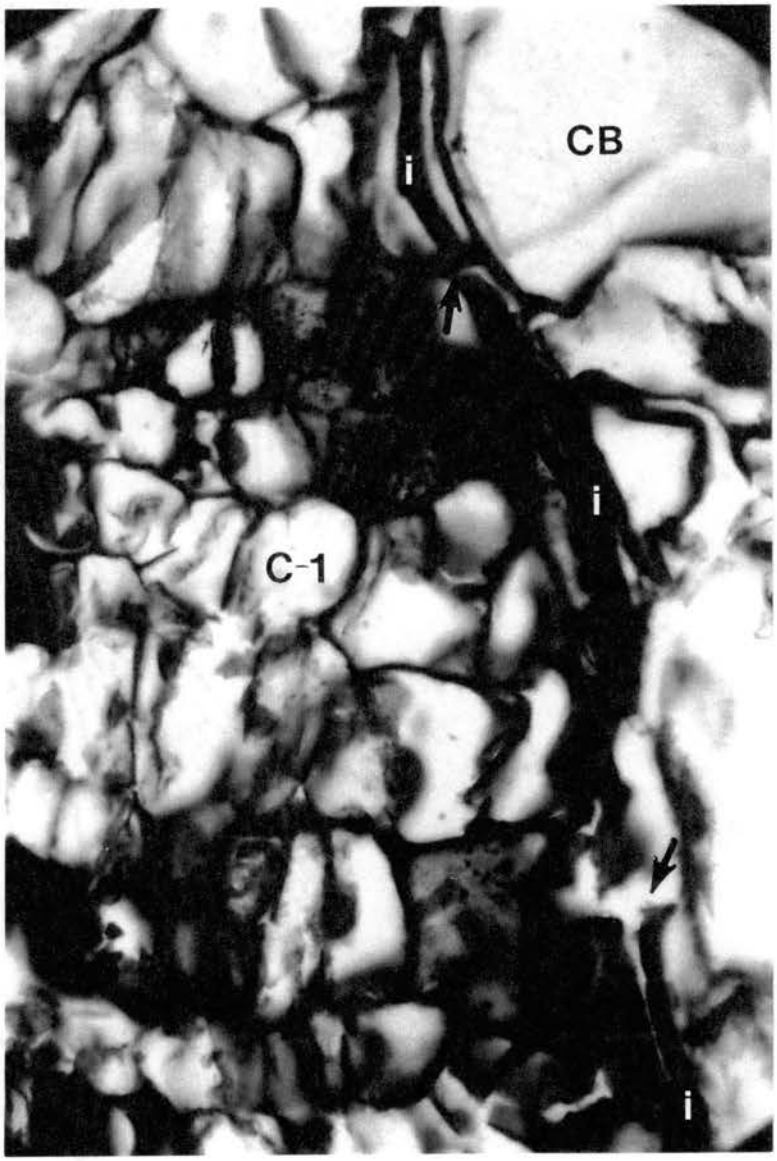


Figure 3.7

Figure 3.8



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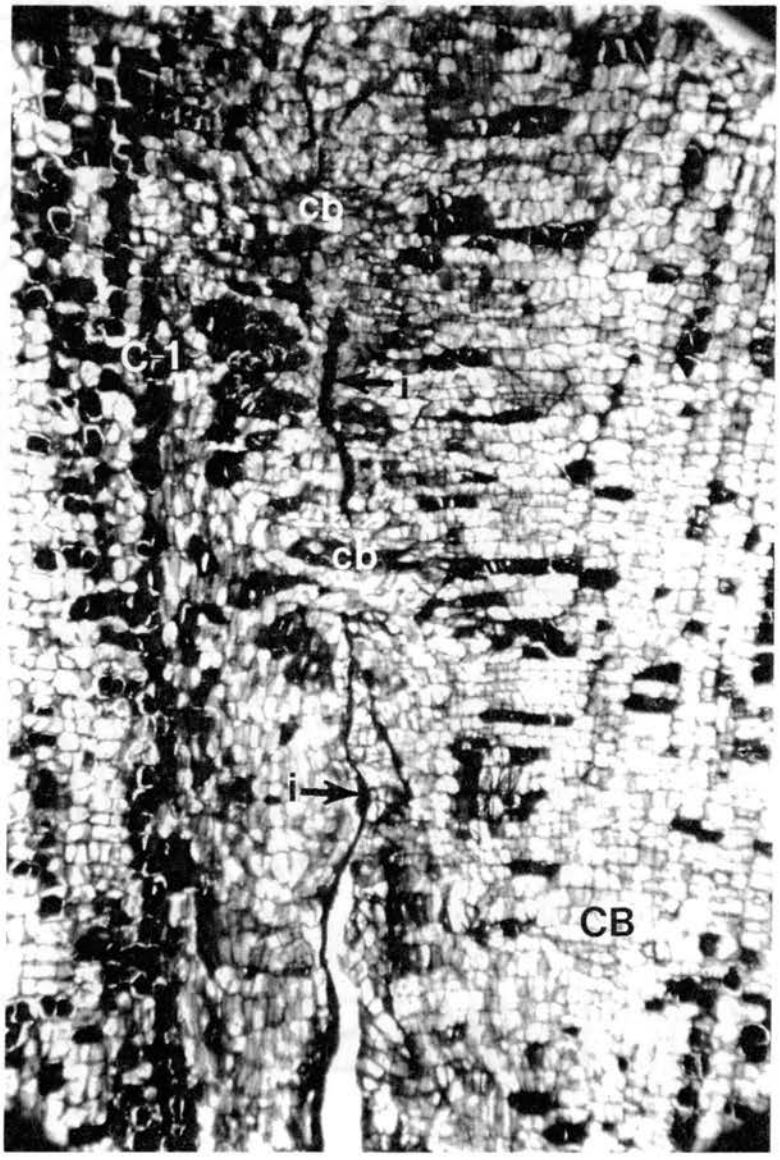

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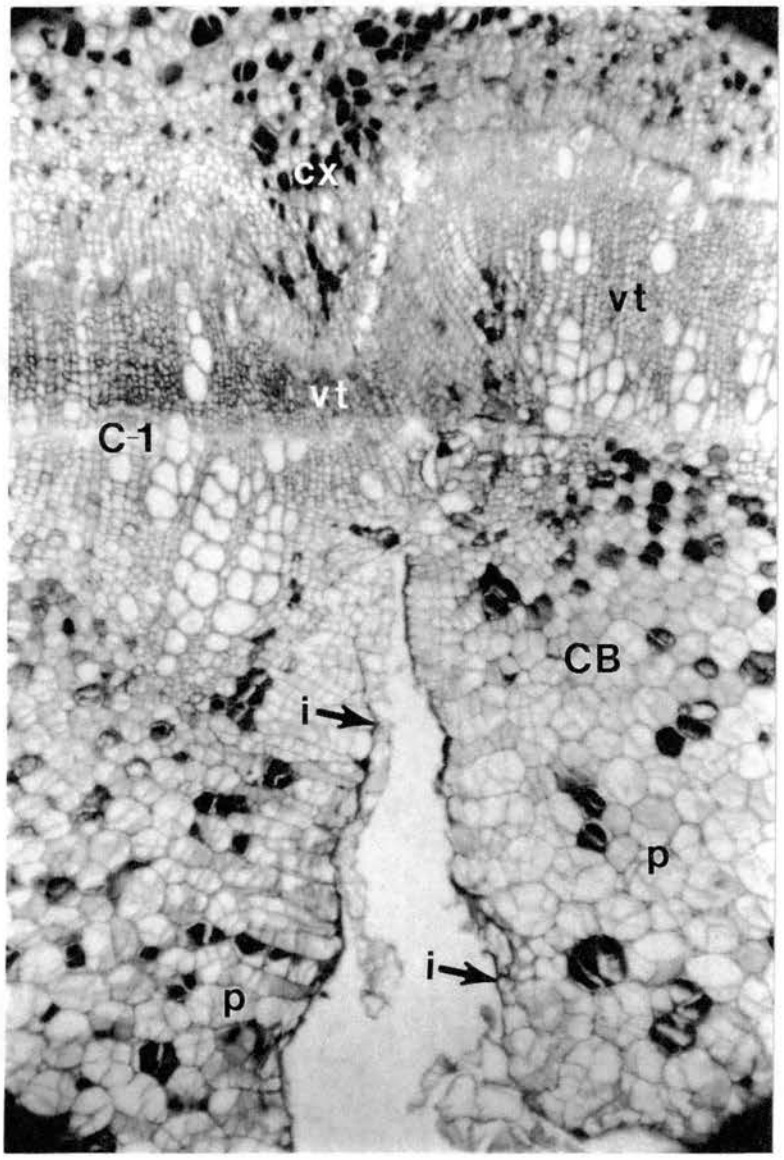

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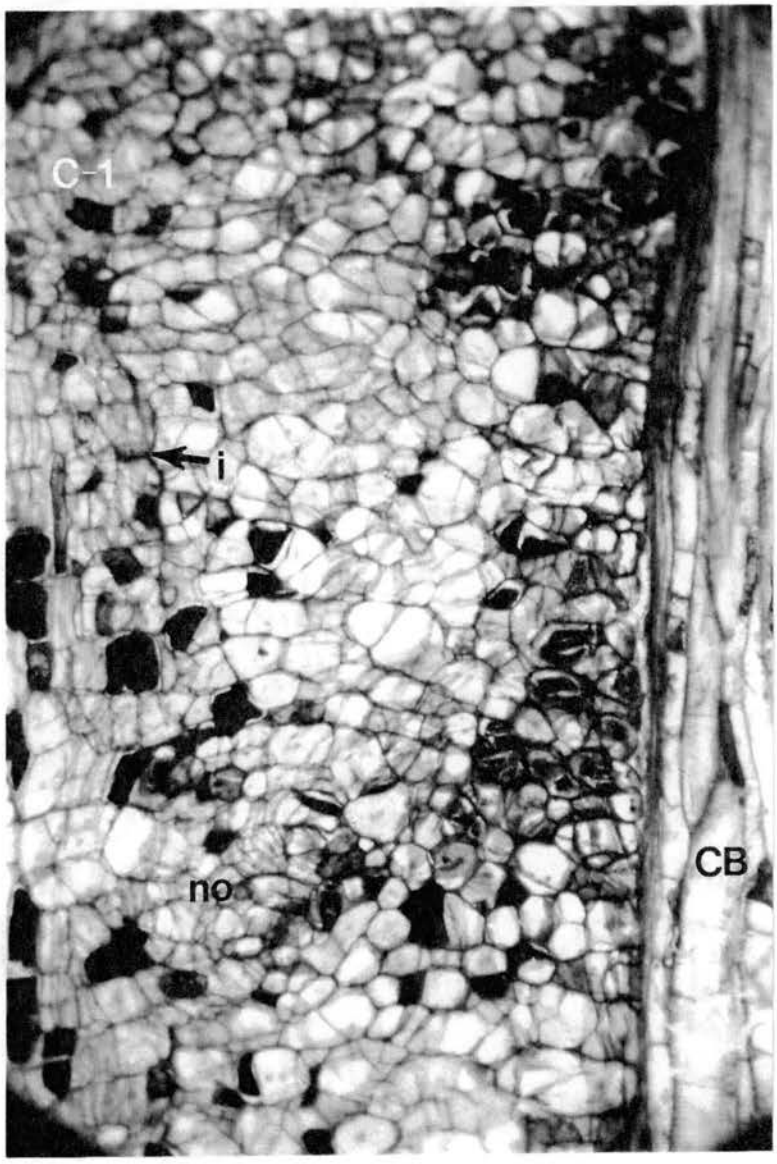

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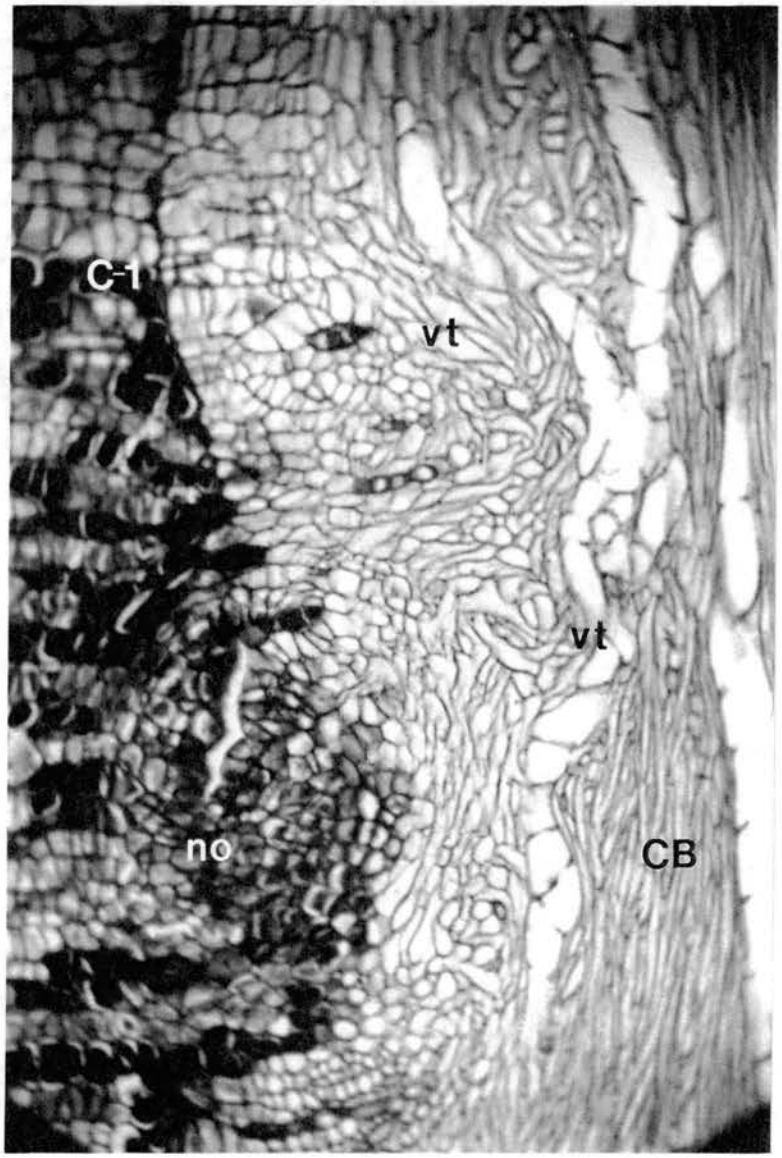

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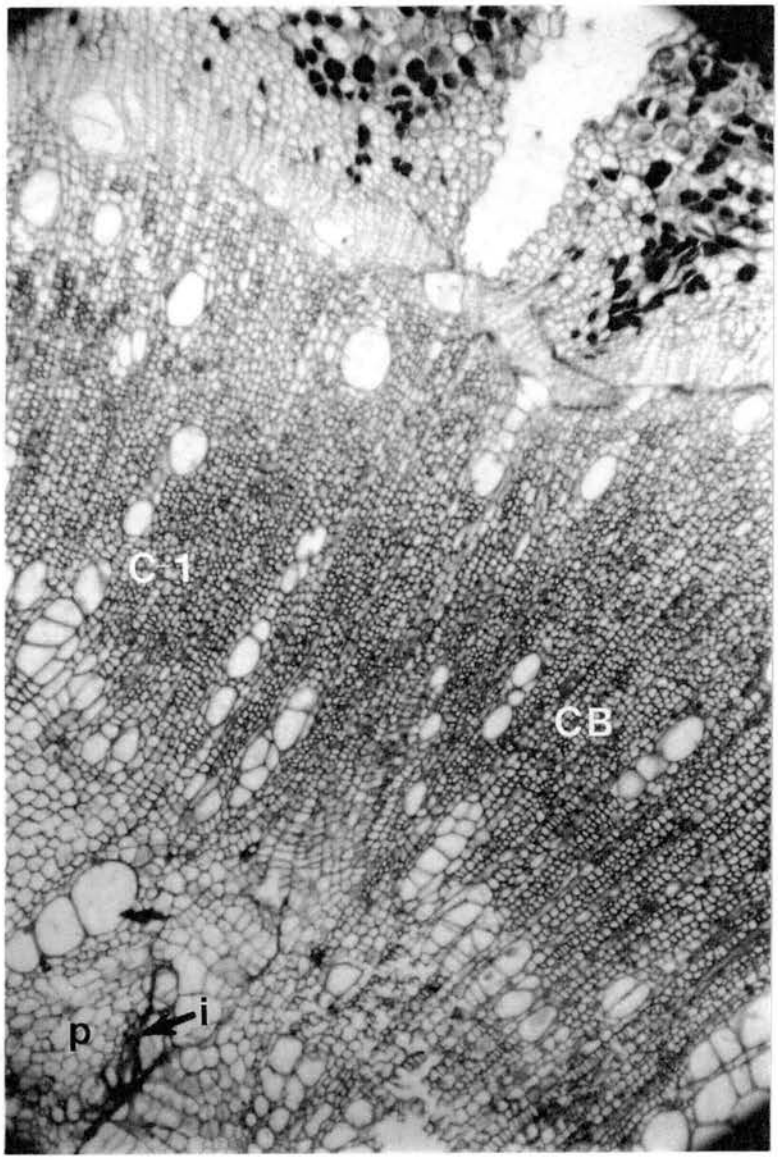

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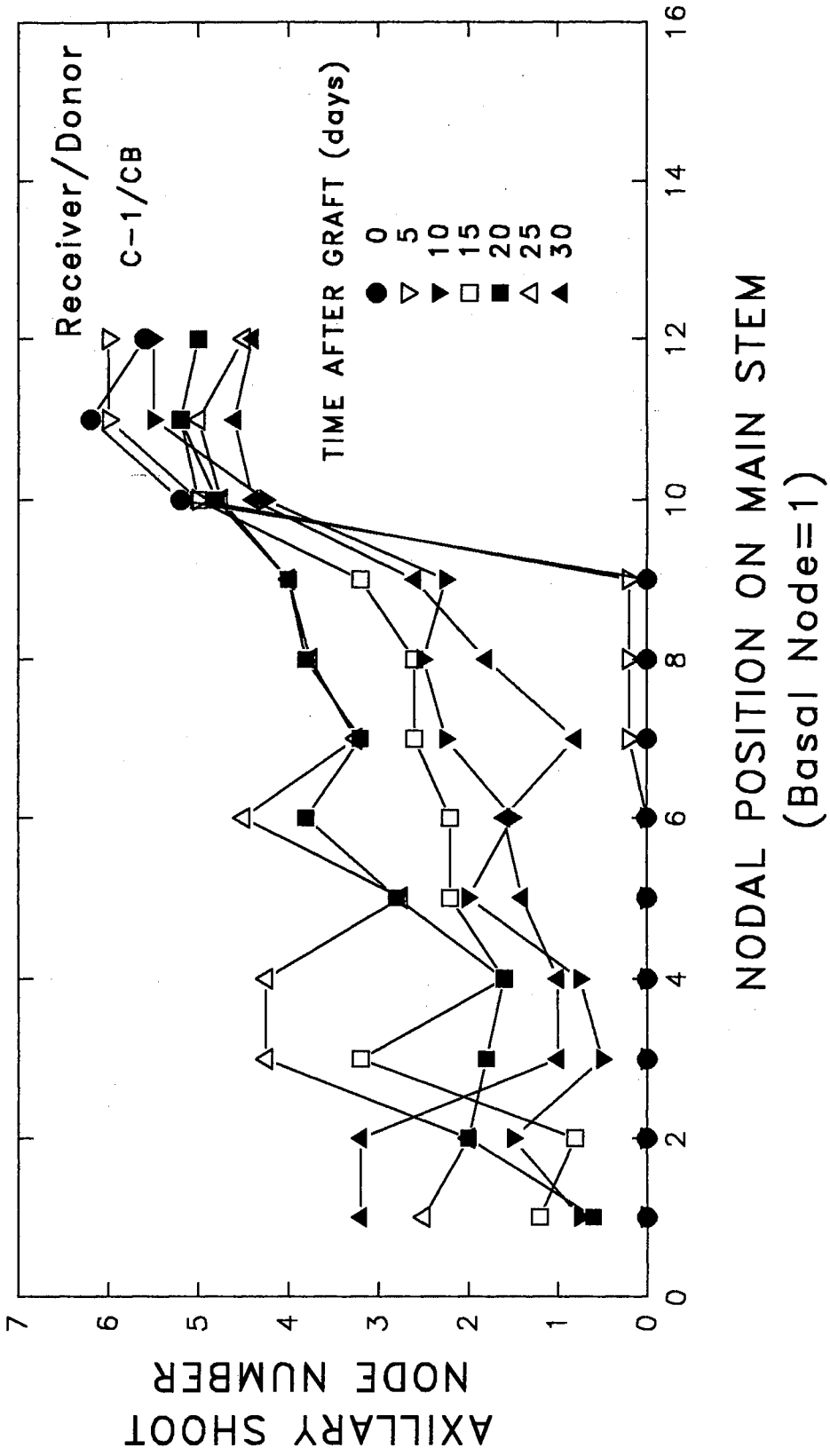
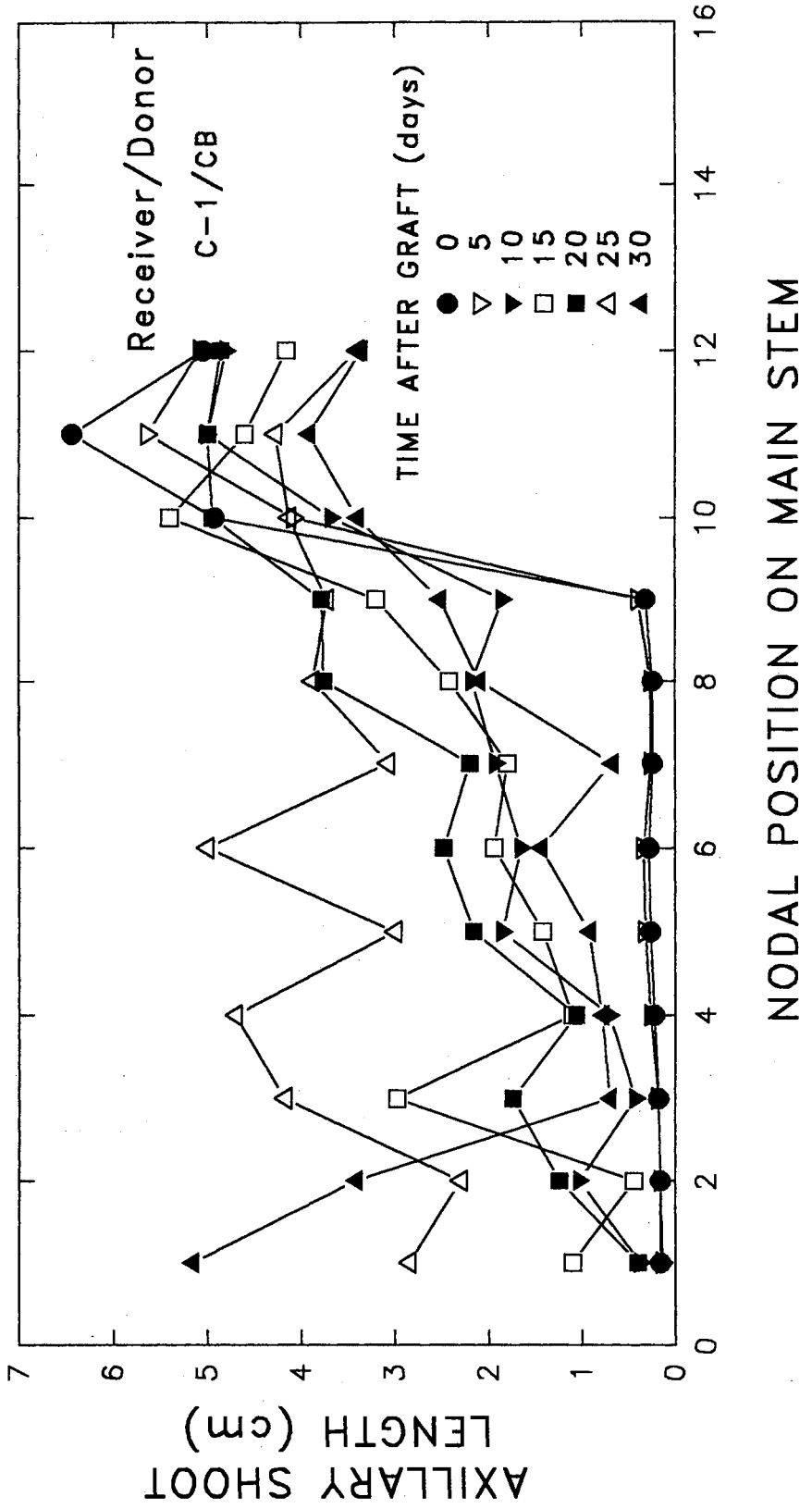

Receiver/Donor
C-1/CB
TIME AFTER GRAFT (days)
0
5
10
15
20
25
30
AXILLARY SHOOT NODE NUMBER
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

Figure 3.15


Receiver/Donor
C-1/CB
TIME AFTER GRAFT (days)
0
5
10
15
20
25
30
AXILLARY SHOOT LENGTH (cm)
NODAL POSITION ON MAIN STEM
(Basal Node=1)
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

CHAPTER IV

FLOWERING AND BRANCHING TRANSMISSION BETWEEN FREE AND RESTRICTED-BRANCHING POINSETTIAS

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Additional index words. *Euphorbia pulcherrima*, apical dominance, axillary branching, free-branching, restricted-branching, photoperiod.

Abstract. *Euphorbia pulcherrima* Willd. ex. Klotzsch cv. 'Eckespoint C-1 Red' (C-1) a restricted-branching cultivar, and 'CB,' a free-branching vegetative or graft-hybrid, were homo and heterografted. C-1 and CB receivers of the grafted pair were kept in long (LD) photoperiods and C-1 and CB donors were exposed to short (SD) photoperiods or LD. C-1/CB (SD) plants for 1990-1991 produced similar axillary shoot node numbers and lengths to CB/CB (SD or LD) when pinched at the tenth node 60 days after grafting. However, in 1991-1992, C-1/CB (SD) did not produce similar axillary shoots as CB/CB but were similar to CB/C-1 (SD) above node 5. The

probability of exhibiting flower induction in C-1/CB (SD) was high (0.5), splitting was low (0.07) and C-1 branching was increased in 1990-1991. In 1991-1992 the C-1/CB (SD) probability of exhibiting flower induction was low (0.04) splitting was high (0.98) and the branching pattern was not altered as in 1990-1991. The potential to branch and flower appear to be correlated, and both are adversely affected by splitting.

Free-branching poinsettias are the predominant cultivars grown in the United States today rather than restricted-branching poinsettias. Free-branching poinsettias are preferred because they produce numerous axillary shoots and cuttings per plant when pinched. In contrast, restricted-branching poinsettias produce fewer axillary shoots and cuttings per plant when pinched.

The branching capacity of restricted-branching poinsettias may be increased by grafting them to free-branching cultivars (Stimart, 1983; Dole and Wilkins, 1991, 1992). A branching agent present in Annette Hegg Brilliant Diamond (AHBD) (a free-branching cultivar) moves acropetally and basipetally through the graft union to 'Eckespoint C-1 Red' (C-1) (a restricted-branching cultivar) increasing C-1 axillary bud growth (Dole and Wilkins, 1992). Changes in the branching pattern of C-1 are retained through a series of vegetative propagations and are considered permanent (Dole and Wilkins, 1991, 1992). Thus, CB (TR), a vegetative or graft-hybrid, was derived from grafting C-1 onto AHBD (Dole and Wilkins, 1992). Vegetative hybridization is the process of grafting two plants together in order to obtain a unique phenotype from the grafted plant itself.

Florigen (flowering hormones) is formed in leaves and transported to shoot meristems and is able to cross graft unions (Lang, 1989). Florigen is readily interchangeable between grafting partners of the same species. Dole and Wilkins (1992) found that the branching agent was also transmittable between AHBD and C-1 poinsettias through the graft union. However, the location of synthesis of the branching agent is not known. The branching agent may be produced in the leaves of poinsettia rootstocks (donor) and translocated through the graft union like the florigen.

Branching in C-1 and CB poinsettias sometimes occurs during the flowering process under non-inductive long (LD) photoperiods. A physiological disorder, splitting, may occur in which the vegetative shoot tip becomes reproductive and is transformed into a flower bud (cyanthum) under LD (Zrebiec and Tayama, 1985). Three shoots arise around the bud which normally does not reach anthesis. Splitting occurs more frequently in C-1 poinsettia plants than in CB poinsettia plants under LD conditions (personal observation).

A complex of growth regulators may control both flowering and expression of free-branching in poinsettias. The flowering and branching control systems in *Lathyrus odonatus* L. and *Pisum sativum* L. appear to involve the same or a very similar hormone(s) (Murfet, 1971; Murfet, 1977). Chailakhyan and Lozhnikova (1985) hypothesized that florigen is a bicomponent complementary complex of two hormones, consisting of gibberellins, which participate in formation of the flower stem, and anthesins, which affect flower formation. In contrast, poinsettias sprayed with gibberellic acid produce flowers later than nontreated poinsettias (Guttridge, 1963;

Evans et al., 1992b). Furthermore, substances with gibberellin-like activity were detected in root exudates of poinsettias grown under LD conditions (Criley, 1970). As a result of studies on the regulation of tomato (*Lycopersicon esculentum* Mill.) flowering through reciprocal top-root grafting, Phatak and Wittwer (1965) suggested the presence of a graft transmissible flower stimulator in the leaves of early cultivars and a transmissible flower inhibitor in the leaves of late cultivars.

This research will further characterize the free-branching agent in poinsettia by determining if there is a correlation between flower induction and the free-branching agent.

Materials and Methods

1990-1991. Cuttings from C-1 and CB poinsettia plants were treated with 1% IBA (Indole-3-Butyric Acid, Hormex Powder #1, Brooker Chemical, North Hollywood, Calif.) and planted in oasis rootcubes growing medium (Smithers-Oasis, Kent, Ohio) on 3 Oct. 1990. After rooting under intermittent mist with a temperature of 21 C, plants were placed in 16.5-cm (1250 cc) pots filled with a commercial peat-based medium (Fafard #2, Springfield, MA) on 16 Nov. 1990. Plants were approach grafted (Hartmann et al., 1990) on 6 and 7 Feb. 1991, and placed in a completely randomized design with a 2x2x2 factorial arrangement of treatments. Factor A was CB and C-1 cultivars as the donor of the grafted pair (donor); factor B was placement of the donor under short (SD) or long (LD) photoperiods and factor C was CB and C-1 cultivars as the receiver of the grafted pair (receiver). All receivers were kept in

LD. Six single unit replicates were used for each treatment.

Vegetative growth was maintained by supplementary incandescent and high-intensity-discharge (HID) lamps (7.6 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR, 2200-0200 HR) to provide LD (15 h). SD (9h) were provided by black cloth (1700-0800 HR) to induce the reproductive stage on one of the grafted pairs from 7 Feb. 1991 to 8 May 1991. Average natural light intensity was 477 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR during daylight hours. Standard fertilization and pest management practices were used (Ecke et al., 1990).

Poinsettia receivers were pinched at the tenth node above the graft union 60 days after grafting. Data were taken thirty days later on the receiver of each grafted pair. The number of nodes with leaves (including the terminal leaf blade separated from the apical cone) was determined on axillary shoots and the length was measured from the base of each axillary shoot to the tip of the apical cone.

1991-1992. Similar propagation and planting procedures as in year 1990-1991 were used except that C-1 and CB poinsettia plants were propagated on 7 Oct. 1991 and planted on 18 Nov. 1991. C-1 and CB plants split once so they were pinched and allowed to regrow. Plant growth was then thinned to one shoot and approach grafted from 25 to 28 Mar. 1992. Grafted plants were placed in a completely randomized design with a 2x2x2 factorial arrangement of treatments. Factor A was the CB and C-1 cultivars as the donor of the grafted pair (donor), factor B was placement of the donor under SD or LD and factor C was CB and C-1 cultivars as the receiver of the grafted pair (receiver). All receivers were kept in LD. Twelve single unit replicates were used for each treatment. Replications were divided into two blocks on one

bench, alternating LD and SD treatments.

Vegetative growth was maintained by supplementary incandescent and HID lamps (2200-0200 HR) to provide LD. Light intensity during the light break (2200-0200 HR) at night was 7.6 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR for block 1 and 3.9 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR for block 2. SD (10h) were provided by black cloth (2000-1000 HR) to induce reproduction on one member of the grafted pair from 31 Mar. 1992 to 28 July 1992. Temperature under the black cloth was maintained at an average of 25/22 C day/night by using fans blowing under the black cloth from 16 Apr. 1992 to 28 July 1992. Average natural light intensity was 477 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PAR during daylight hours. Standard fertilization and pest management practices were used (Ecke et al., 1990).

Splitting and flower induction in the apical meristems of C-1 and CB poinsettia receiver main stems were determined before pinching. Bract and apical leaf color was measured with a colorimeter (Minolta Corporation, Ramsey, N.J.) by taking a representative color quality sample of 3 bracts or apical leaves per treatment. Poinsettia plants were then pinched at the eleventh node 90 days after grafting. Data were taken thirty days later on the axillary shoots of the receiver of each grafted pair.

Results and Discussion

Poinsettia C-1 receivers grafted on CB donors (C-1/CB) had more axillary shoot nodes and greater shoot lengths from nodes 2 to 6 and nodes 1 to 8 than C-1/C-1 plants regardless of the donor photoperiod in 1990-1991 and 1991-1992

respectively (Fig. 4.1, 4.2, 4.3 and 4.4). Also, C-1/CB with the donor under SD had similar axillary shoot node numbers and lengths as CB receivers on CB or C-1 donors under SD or LD in 1990-1991 (Table 4.1, Fig. 4.1 and 4.2). However, in 1991-1992 C-1/CB (SD) had axillary shoot node numbers and lengths only similar to CB/C-1 (SD) above node 5 (Table 4.2, Fig. 4.3 and 4.4).

CB donors induced more axillary shoot growth in C-1 and CB receivers than C-1 donors regardless of photoperiod in 1991-1992 (Table 4.3). When C-1 was the receiver, the daylength exposure of the donor had no significant effect on axillary shoot growth (Table 4.3, Fig. 4.3 and 4.4). However, when CB was the receiver, donors kept under SD induced less axillary shoot growth than donors kept under LD in 1991-1992 (Table 4.3, Fig. 4.3 and 4.4). The increase in axillary shoot growth of CB receivers when donors were under LD caused a receiver x day length interaction in 1991-1992 (Table 4.3, Fig. 4.3 and 4.4). In 1990-1991 no receiver x day length interactions were evident because axillary shoot growth of CB receivers was similar regardless of the day length of the donor (Table 4.1, Fig. 4.1 and 4.2). Poinsettia donors were exposed to SD or LD for 120 days in 1991-1992 and for only 90 days in 1990-1991. The longer period of time under altered day length of CB and C-1 donors in 1991-1992 may have allowed CB receivers to show more axillary shoot growth when donors were under LD than in SD as compared to 1990-1991.

CB receivers had a higher probability of exhibiting flower induction than C-1 receivers in 1991-1992 (Table 4.4). Also, CB receivers with C-1 donors under SD had the most positive tristimulus 'a' value in 1991-1992 (Table 4.5). The more positive

tristimulus 'a' value in the bracts of the receivers with donors under SD means the bracts were more red than green. Also, CB receivers with donors under LD had relatively negative tristimulus 'a' values (that is, they were more green than red) in 1991-1992 (Table 4.5). Furthermore, CB receivers showed the lowest probability of exhibiting splitting during 1991-1992 (Table 4.6). In contrast, C-1 receivers with donors under SD had the lowest probability of exhibiting flower induction in 1991-1992 (Table 4.4). Also, C-1 receivers had similar tristimulus 'a' values regardless of the donor photoperiod in 1991-1992 (Tables 4.5). However, in 1990-1991 C-1 receivers with donors under SD had a slightly higher probability (nonsignificant at P = 0.05) of exhibiting flower induction than CB receivers (Table 4.4).

C-1/CB with the donors under SD had a low (0.07) probability of exhibiting splitting, a high (0.5) probability of exhibiting flower induction (C-1 receiver) and the axillary shoot growth was similar to CB receivers with donors under SD and LD in 1990-1991 (Tables 4.4 and 4.6, Fig. 4.1 and 4.2). However, in 1991-1992 C-1/CB with the donors under SD had a high (0.98) probability of exhibiting splitting which may have caused a low (0.04) probability of exhibiting flower induction and lower axillary shoot growth as compared to 1990-1991 (Tables 4.4 and 4.6, Fig. 4.1, 4.2, 4.3 and 4.4). CB and C-1 poinsettia donors were under altered day length for 120 days in 1991-1992 and for 90 days in 1990-1991. The longer period of growth in 1991-1992 increased the probability of splitting over the previous year (Table 4.6). The more mature the poinsettia shoots, the higher the probability of exhibiting splitting under LD conditions (Evans et al., 1992a). In C-1 poinsettia receivers with CB donors under

SD, the higher the probability of splitting, the lower the probability of showing flowering induction and the lower the branching transmission between the CB donors and the C-1 receivers (Tables 4.1, 4.2, 4.3, 4.4 and 4.6, Fig. 4.1, 4.2, 4.3 and 4.4).

The branching pattern of C-1 receivers was significantly altered by grafting onto CB donors under SD (Tables 4.1, 4.2, and 4.3, Fig. 4.1, 4.2, 4.3 and 4.4). C-1 apical dominance was reduced, increasing axillary shoot growth in the lower nodes close to the graft union (basal nodes) in 1990-1991 (Table 4.2, Fig. 4.1 and 4.2). After grafting to CB, C-1 had a branching pattern similar to CB, which had long axillary shoots with many nodes (Table 4.1, Fig. 4.1 and 4.2). CB donors under SD flowered and transmitted the flower induction to C-1 receivers under LD. Flowering of C-1 receivers under LD may have induced a strong source-sink relationship with CB donors under SD. C-1 was under LD, provided by a light break during the night (2200-0200 HR). This light break provided illumination for a prolonged period of time (natural day light + light break at night) and may have promoted a stronger sink in C-1. The effect of light quantity and quality on the developing rose bud in promoting sink strength has been described (Mor and Halevy, 1980; Mor et al., 1980; Zieslin and Halevy, 1975). Assimilates and the flowering and branching agents in CB donors under SD (the source) may have moved to C-1 receiver (the sink) under LD and changed C-1 branching and flowering patterns.

The probability of CB and C-1 receivers with donors under SD exhibiting flower induction was higher in block 1 than in block 2 in 1991-1992 (Table 4). In block 1, receivers were exposed to a higher light intensity during the light break

(2200-0200 HR) at night (7.6 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) than in block 2 (3.9 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). The light intensity in block 1 may have caused a stronger sink activity in the receivers and a higher rate of flower induction than in block 2.

Developing leaves and flowers have high cytokinin activity and are strong sinks (Salisbury and Ross, 1978). Cytokinins are known to enhance sink activity in grape vines (*Vitis vinifera* L.) (Shindy et al., 1973) and are implicated as inducers of direct transport of nutrients in plants (Mothes and Englebrecht, 1961). Flowering CB plants grafted onto C-1 plants induced flowering in C-1, and may have increased the cytokinin concentration in the C-1 apical meristems. Cycocel showed a promoting effect in poinsettia 'Paul Milkkelsen' flower initiation under LD (16 h) and marginal photoperiods (Criley, 1970). Zieslin and Halevy (1976) observed that total cytokinin activity was higher in the leaf extracts from flowering than from non-flowering shoots of *Rosa hybrida* 'Baccara'. Furthermore, high flower production was correlated with high levels of cytokinin in the shoot tip of 'Golden Time' rose (Van Staden et al., 1981). Cytokinins have also been observed to promote flower formation in several plant species (Salisbury and Ross, 1978) and inflorescence development in *Bougainvillea* 'San Diego Red' (Tse et al.,1974). A combination of a cytokinin (BA) and GA_3 induced flowering in one SD variety of *Chrysanthemum x morifolium* Ramat (Salisbury and Ross, 1978) and BA with GA_4 and GA_7 (Promalin) applied to C-1 and V-14 Glory poinsettias a few weeks after flower initiation changed cyathia structure (Shanks, 1981).

A high concentration of cytokinins in the apical meristems of C-1 receiver

plants grafted onto CB donor plants may release the shoots from apical dominance, resulting in development of lateral buds. The apical dominance release has been reported in both monocots and dicots and was due to an increased ratio of cytokinins to auxins (Bidwell, 1991). Kinetin (6-furfurylamino purine) applied directly to the poinsettia bud at 1 ppm was effective in inducing axillary shoot stimulation (Milbocker, 1972). Cytokinins and the flowering hormones may have moved with the assimilate stream from CB through the graft union to C-1, increasing their concentrations in C-1.

Cytokinin and the flowering hormones may act together in promoting flowering and branching in C-1 poinsettia but their action may be affected by branching and flowering inhibitors. Auxin, more often than not, inhibits flowering (Salisbury and Ross, 1978). Auxin in high concentration is responsible for apical dominance, inhibiting axillary shoot growth. Thus, a balance of auxin and cytokinin in C-1 meristems may determine if C-1 will flower and branch (high cytokinin concentration) or not flower and not branch (high auxin concentration).

Inhibitory effects on flower induction are often due to influences on the translocation of assimilates (Salisbury and Ross, 1978). For example, a long-day leaf growing between an induced short day leaf and the bud may export assimilates directly to the bud and block movement of assimilates and the flowering hormones from the induced leaf to the bud (Salisbury and Ross, 1978). Assimilates, cytokinins and the flowering hormones moving from CB donor (SD) to C-1 receiver (LD) axillary buds may have been blocked by a LD leaf close to the axillary bud.

Cytokinins and the flowering hormones may have partially reached C-1 receiver axillary buds and flowering and branching was reduced as in 1991-1992 (Fig. 4.3 and 4.4).

This research suggests that flower induction and transmission of the branching agent between C-1 receivers and CB donors were correlated and that both processes were adversely affected by splitting. Thus, the lower the probability of splitting in C-1 poinsettias with CB donors under SD, the higher the probability of showing flower induction and the higher the branching transmission between the CB donors and the C-1 receivers.

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Table 4.1. Photoperiod effect on axillary shoot growth of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1990-1991. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD.

			Axillary shoot	
Factors			Number of nodes[z]	Length (mm)[z]
Receiver			**	**
Donor			*	NS
Day Length			NS	*
Receiver x Donor			*	*
Receiver x Day Length			NS	NS
Donor x Day Length			NS	NS
Receiver x Donor x Day Length			NS	NS
Receiver	Donor	Day Length		
C-1	C-1	SD	4.4	49
CB	CB	SD	6.8	76
CB	C-1	SD	7.1	91
C-1	CB	SD	7.1	86

C-1	C-1	LD	3.9	34
CB	CB	LD	7.6	75
CB	C-1	LD	7.4	80
C-1	CB	LD	5.5	48
Receiver	Donor			
C-1	C-1		4.2c	42c
CB	CB		7.2a	75ab
CB	C-1		7.3a	86a
C-1	CB		6.4b	67b
Day Length				
Long day			6.1	59
Short day			6.4	76

[z]Means followed by the same letter do not differ by LSD, P = 0.05.

[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01 respectively.

Table 4.2. Significance per node for axillary shoot node number and length of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1990-1991 and 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Means were an average of 6 plants in 1990-1991 and 12 plants in 1991-1992.

	Nodes										
Axillary shoot	1	2	3	4	5	6	7	8	9	10	11
					1990-1991						
Node number	*	*	*	NS	NS	**	NS	NS	*	NS	--
Length	*	*	**	NS	NS	**	NS	NS	NS	NS	--
					1991-1992[z]						
Node number	NS	NS	NS	**	**	NS	NS	NS	NS	NS	NS
Length	NS	NS	NS	*	**	NS	NS	NS	*	**	**

[z]Receiver x donor x nodes was significant by LSD, P = 0.01.

[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Table 4.3. Photoperiod effect on axillary shoot growth of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD.

			Axillary shoot	
Factors			Number of nodes[z]	Length (mm)[z]
Receiver			**	**
Donor			**	**
Day Length			*	NS
Receiver x Donor			NS	NS
Receiver x Day Length			**	**
Donor x Day Length			NS	NS
Receiver x Donor x Day Length			NS	NS
Receiver	Donor	Day Length		
C-1	C-1	SD	4.2	73
CB	CB	SD	11.1	231
CB	C-1	SD	9.0	172
C-1	CB	SD	8.3	134

C-1	C-1	LD	5.5	73
CB	CB	LD	14.2	292
CB	C-1	LD	13.4	254
C-1	CB	LD	7.5	109
Receiver				
C-1			6.3	100
CB			11.3	225
Donor				
C-1			7.5	135
CB			10.1	189
Day Length	Receiver			
Long day	C-1		6.5c	91c
Long day	CB		13.8a	273a
Short day	C-1		6.2c	104c
Short day	CB		10.1b	201b

[z]Means followed by the same letter do not differ by LSD, P = 0.01.

[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01 respectively.

Table 4.4. Probability of CB and C-1 poinsettia receivers exhibiting flower induction after altering day length on CB and C-1 donors in 1990-1991 and 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Only those receivers whose donors were under SD exhibited flower induction. Means were an average of data from 6 plants in 1990-1991 and 12 plants for 1991-1992.

Factor	1990-1991[z]	1991-1992
Receiver	NS	**
Donor	NS	NS
Block	--	**
Receiver x Donor	NS	NS
Receiver	Block 1	
C-1	0.50	0.08
CB	0.17	0.75
Receiver	Block 2	
C-1	--	0.01
CB	--	0.08

[z]Only one block was used in 1990-1991.

[NS, **]Nonsignificant or significant at P = 0.01 respectively.

Table 4.5. Bracts or apical leaves color (tristimulus 'a' value) on CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD.

Factors			Tristimulus 'a' Value
Receiver			*
Donor			*
Day Length			NS
Receiver x Donor			*
Receiver x Day Length			*
Donor x Day Length			*
Receiver x Donor x Day Length			*
Receiver	Donor	Day Length	
C-1	C-1	SD	-12.5bc
CB	CB	SD	-11.1c
CB	C-1	SD	2.5d
C-1	CB	SD	-11.7c
C-1	C-1	LD	-12.7abc
CB	CB	LD	-14.8ab
CB	C-1	LD	-15.5a

C-1	CB	LD	-11.7bc

[z]Means followed by the same letter do not differ by LSD, P = 0.05.

[NS,*]Nonsignificant or significant at P = 0.05 respectively.

Table 4.6. Probability of CB and C-1 poinsettia receivers exhibiting splitting after altering day length on CB and C-1 donors in 1990-1991 and 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Means were an average of data from 6 plants in 1990-1991 and 12 plants for 1991-1992.

Factors			1990-1991	1991-1992
Receiver			NS	**
Donor			NS	NS
Day Length			NS	NS
Receiver x Donor			*	NS
Receiver x Day Length			NS	**
Donor x Day Length			**	NS
Receiver x Donor x Day Length			NS	NS
Receiver	Donor	Day Length		
C-1	C-1	SD	0.32	0.98
CB	CB	SD	0.07	0.73
CB	C-1	SD	0.07	0.73
C-1	CB	SD	0.07	0.98
C-1	C-1	LD	0.85	0.98
CB	CB	LD	0.07	0.73

CB	C-1	LD	0.48	0.73
C-1	CB	LD	0.07	0.98

[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01 respectively.

Fig. 4.1. Axillary shoot node number of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1990-1991. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Means were an average of 6 plants.

Fig. 4.2. Axillary shoot length of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1990-1991. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Means were an average of 6 plants.

Fig. 4.3. Axillary shoot node number of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donors were kept either in short (SD) photoperiods or LD. Means were an average of 12 plants.

Fig. 4.4. Axillary shoot length of CB and C-1 poinsettia receivers after altering day length on CB and C-1 donors in 1991-1992. CB and C-1 receivers were kept in long (LD) photoperiods and CB and C-1 donor were kept either in short (SD) photoperiods or LD. Means were an average of 12 plants.

Figure 4.1

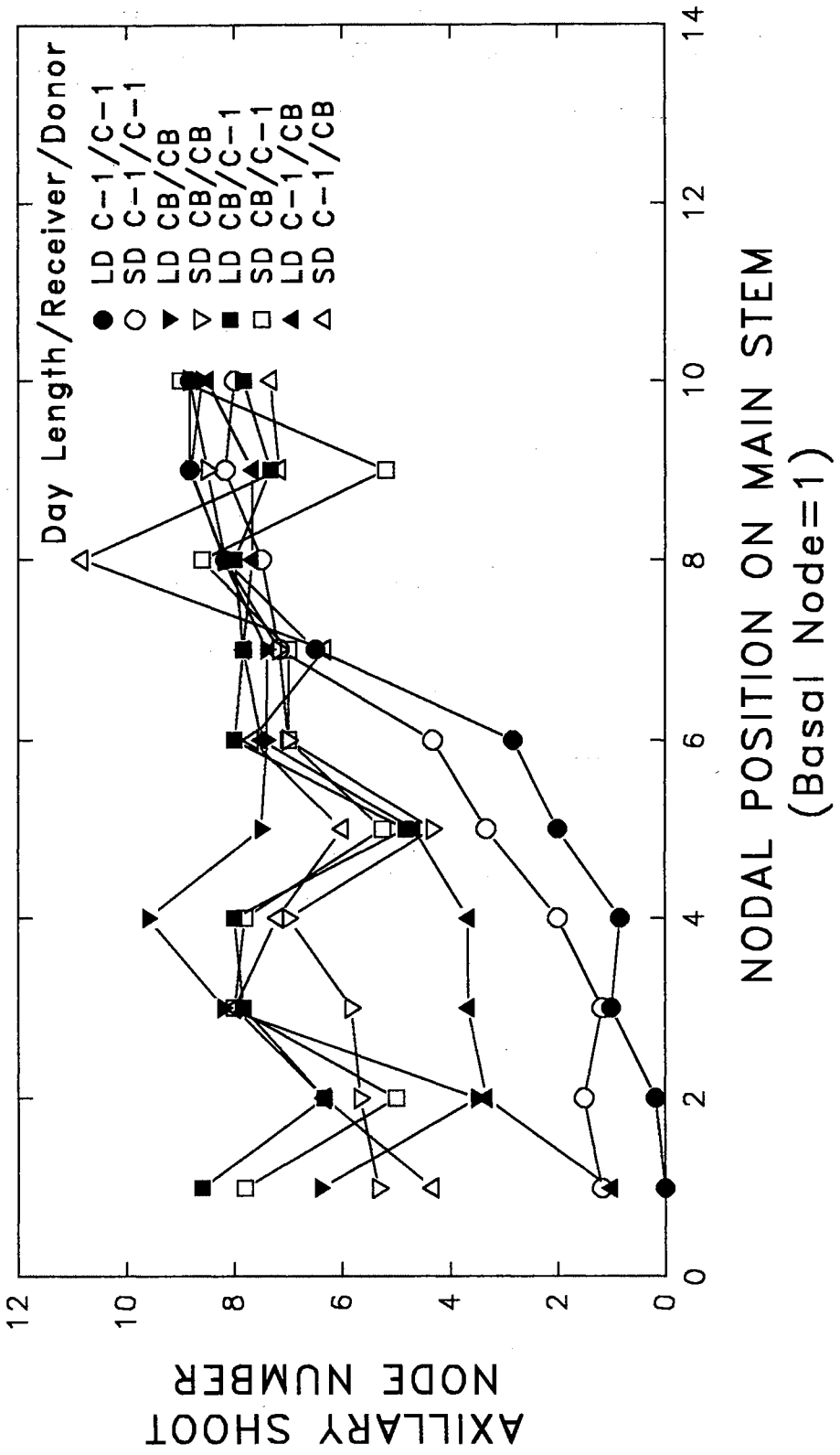

Day Length/Receiver/Donor
LD C-1/C-1
SD C-1/C-1
LD CB/CB
SD CB/CB
LD CB/C-1
SD CB/C-1
LD C-1/CB
SD C-1/CB
AXILLARY SHOOT NODE NUMBER
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
2
4
6
8
10
12
14

Figure 4.2

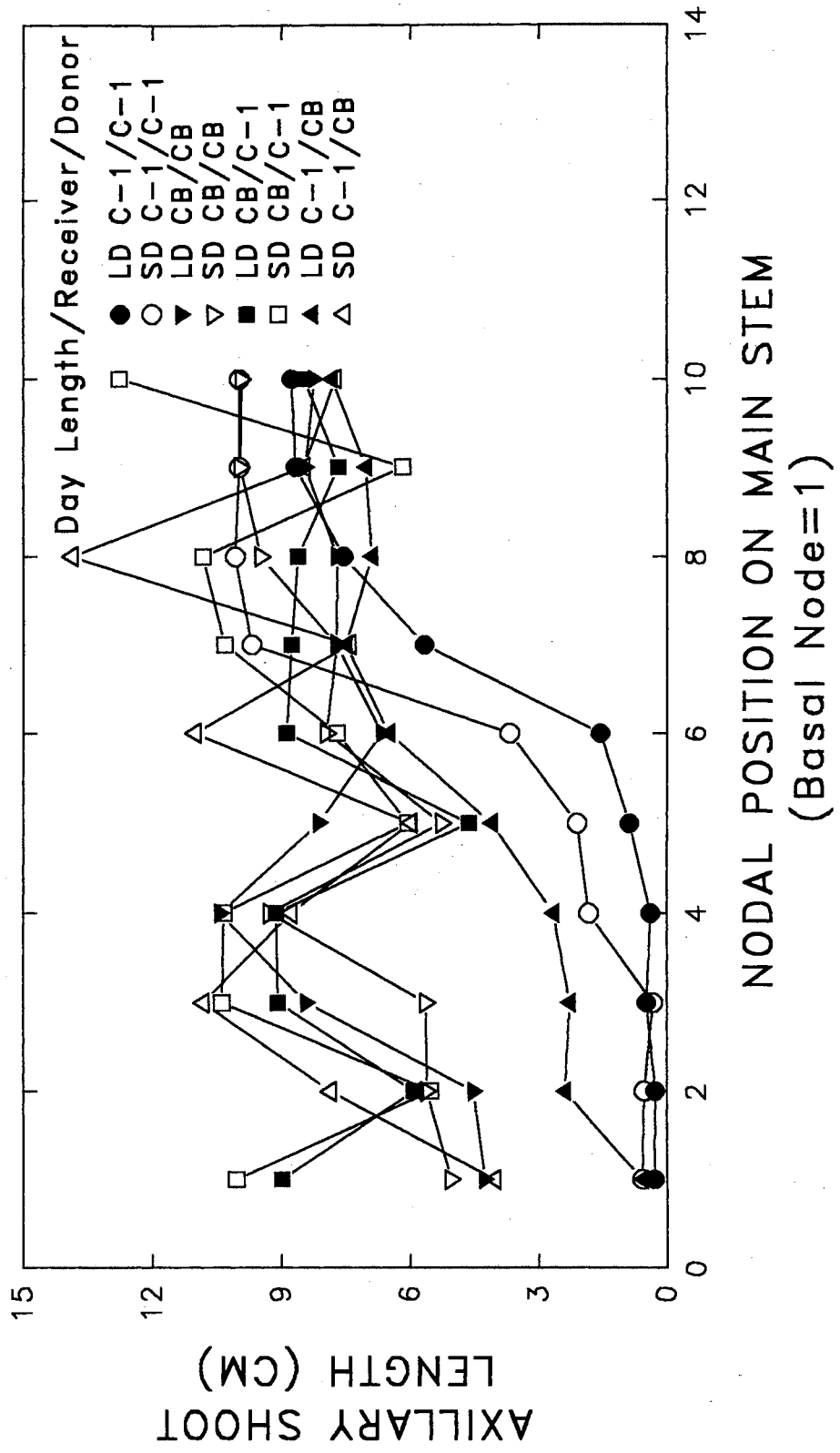

Day Length/Receiver/Donor
LD C-1/C-1
SD C-1/C-1
LD CB/CB
SD CB/CB
LD CB/C-1
SD CB/C-1
LD C-1/CB
SD C-1/CB
AXILLARY SHOOT LENGTH (CM)
0
3
6
9
12
15
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
2
4
6
8
10
12
14

Figure 4.3

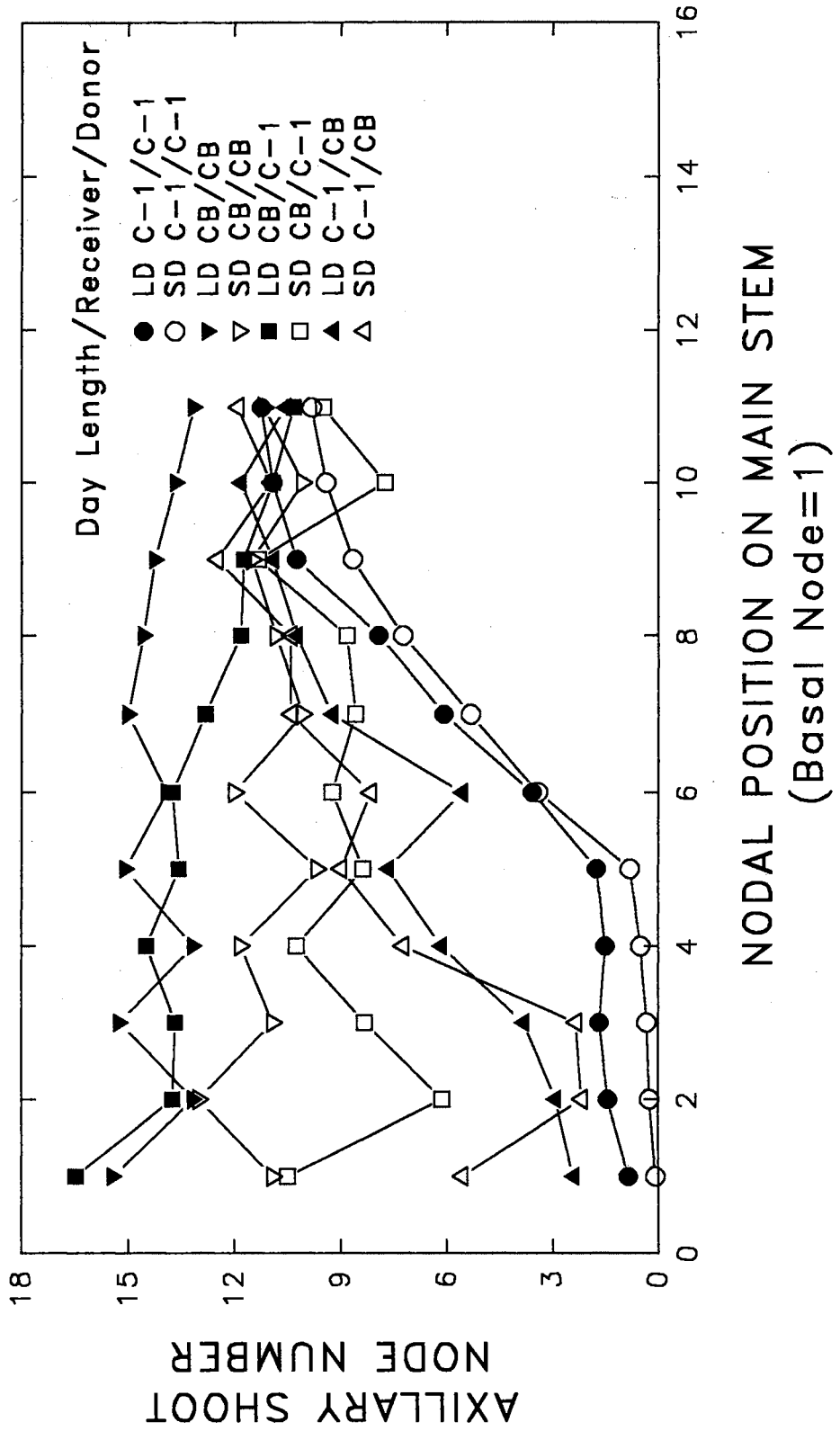
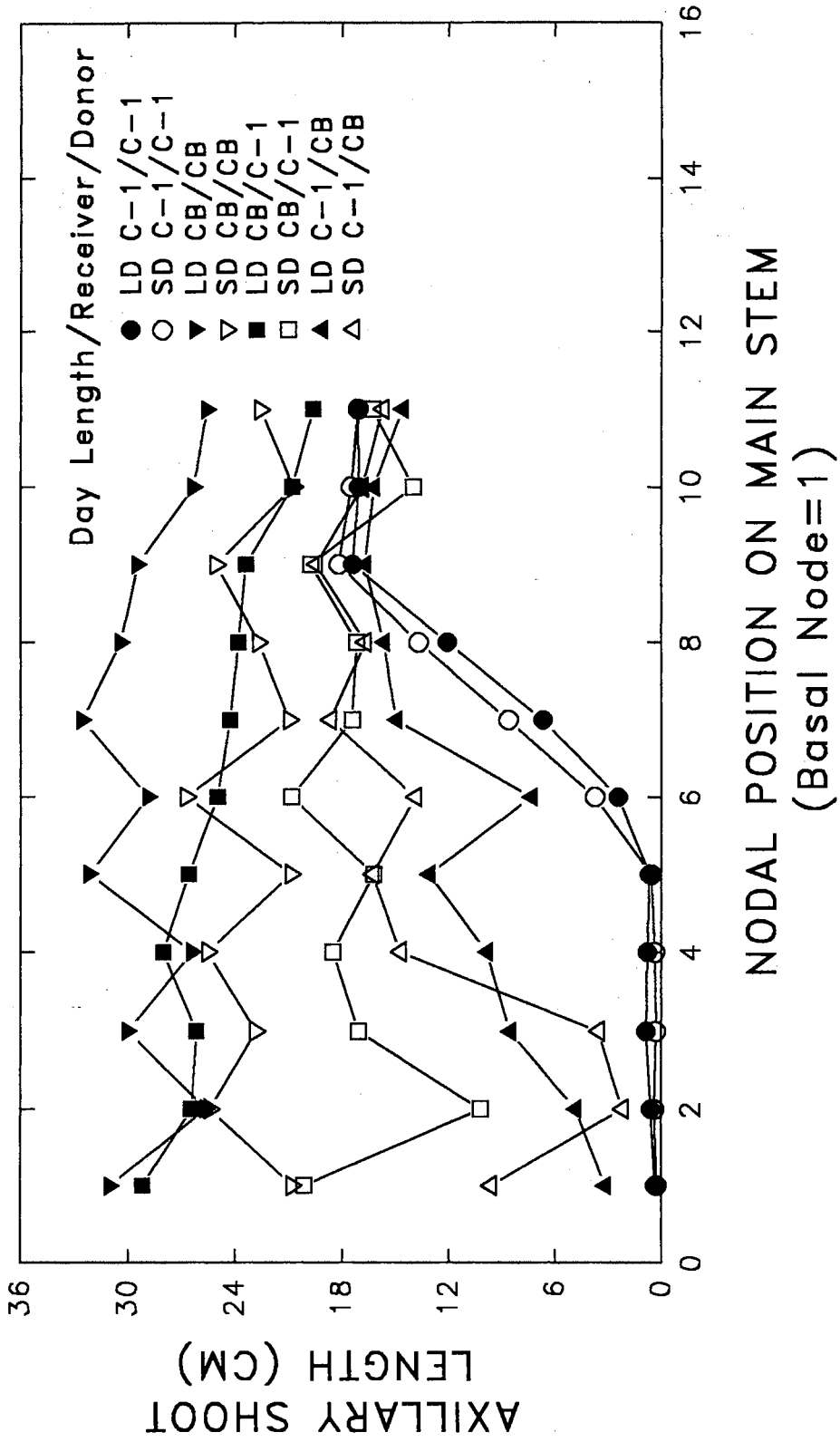

Day Length/Receiver/Donor
LD C-1/C-1
SD C-1/C-1
LD CB/CB
SD CB/CB
LD CB/C-1
SD CB/C-1
LD C-1/CB
SD C-1/CB
AXILLARY SHOOT NODE NUMBER
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
3
6
9
12
15
18
0
2
4
6
8
10
12
14
16

Figure 4.4


Day Length/Receiver/Donor
LD C-1/C-1
SD C-1/C-1
LD CB/CB
SD CB/CB
LD CB/C-1
SD CB/C-1
LD C-1/CB
SD C-1/CB
AXILLARY SHOOT LENGTH (CM)
0
6
12
18
24
30
36
NODAL POSITION ON MAIN STEM (Basal Node=1)
0
2
4
6
8
10
12
14
16

CHAPTER V

SUMMARY

Euphorbia pulcherrima Willd. ex. Klotzsch (poinsettia) is the number one potted floral crop in the United States. Today most commercial poinsettias grown are free-branching cultivars, which have weak apical dominance and relatively small stem diameters. Free-branching cultivars including Annettte Hegg, Gutbier V-10 and Gutbier V-14 are preferred by growers because they produce numerous axillary shoots and flowers when pinched. Less frequently grown are restricted-branching cultivars such as the Eckespoint series, which have strong apical dominance, large stem diameters, and relatively few axillary shoots and flowers when pinched.

The free-branching trait in 'Annette Hegg Brilliant Diamond' (AHBD) can be induced in the restricted-branching 'Eckespoint C-1 Red' (C-1) poinsettia through grafting (Dole and Wilkins, 1991, 1992). A free-branching agent moves both acropetally and basipetally through the graft union and can be serially transmitted from plant to plant by grafting (Dole and Wilkins, 1992). CB (TR), a vegetative or graft-hybrid was derived from grafting C-1 onto AHBD (Dole and Wilkins, 1992). Thus, the presence of the agent in CB makes it different from C-1. The work herein

reported further characterized the free-branching agent in poinsettia by determining in which organ(s) (stem or leaf) of the plant the agent is contained, if shading the donor stems and leaves prevents the transmission of the branching agent, if new cells or tissues may induce its production and/or translocation within the graft union, and if there is a correlation between flower induction and the transmission of the branching agent.

The role of CB donor stems and leaves in the transmission of the branching agent to C-1 receivers was investigated. 'C-1' a restricted-branching cultivar and 'CB' a free-branching vegetative or graft-hybrid were homo and heterografted. Axillary shoot node numbers and lengths of C-1 receiver plants were partially increased by CB donors with leaves removed (in 1990 and 1990-1991) and with apical and basal parts removed (in 1990-1991 and 1991). In 1991-1992, axillary shoot growth of C-1 receivers was only slightly increased by CB donor internode chips. However, CB donor node chips did not increase C-1 receiver axillary shoot node numbers and lengths. The branching agent in CB internode donors (sink) may have moved against a source-sink gradient to C-1 receivers (source) via another method of transmission which may not have been as effective as movement by source-sink. The partial transmission of the branching agent from CB internode donors to C-1 receivers also suggested that a source-sink movement of the branching agent may not be the only mechanism of transmission. Shading of CB donors partially increased axillary shoot node numbers and lengths of C-1 receivers above node 4 in 1991-1992. A source-sink mechanism in the transmission of the branching agent was not proved because shading

CB donor leaves and stems did not prevent the transmission of the branching agent to C-1 receivers.

The results from the stem experiments and leaf experiments did not show clear evidence of the specific role of CB donor stems or leaves in the transmission of the branching agent to C-1 receivers. Both stems and leaves of CB poinsettia donors may promote the transmission of the branching agent to C-1 receivers.

The development of new cells and tissues during CB and C-1 graft union formation was correlated with the movement of the branching agent between CB donors and C-1 receivers. CB donor plants and C-1 receiver plants were approach grafted. Graft unions were removed from poinsettia grafted pairs at 0, 5, 10, 15, 20, 25 or 30 days after grafting for anatomical study and the portion below the graft union was allowed to regrow. C-1 receiver branching was increased by 10 days after grafting to CB donors. New cells and tissues were progressively formed in the graft union between CB and C-1 plants which may have allowed the transmission of the branching agent to occur between 5 and 10 days after grafting.

By 10 days after grafting, C-1 and CB parenchyma cells were actively dividing, producing new parenchyma cells (callus). Callus connected CB donors and C-1 receivers by 10 days after grafting. Parenchyma cells differentiated into nodules for the formation of new cambium by 25 days after grafting. CB donors and C-1 receivers were interconnected by new vascular tissue after 25 days of graft formation. CB donors may have controlled the differentiation of vascular tissue of the graft union and allowed the transmission of the branching agent to C-1 receivers.

Flower induction and the transmission of branching between C-1 receivers and CB donors were correlated. C-1 and CB poinsettia plants were homo and heterografted. C-1 and CB receivers of the grafted pair were kept in long (LD) photoperiods and C-1 and CB donors were exposed to short (SD) photoperiods or LD. C-1 branching characteristics were altered when grafted onto CB donors (C-1/CB) kept under SD, making C-1 similar to CB in 1990-1991. C-1/CB (SD) produced similar axillary shoot node numbers and lengths to CB/CB (SD or LD) when pinched at the tenth node 60 days after grafting. In 1991-1992, C-1/CB (SD) did not produce similar axillary shoots as CB/CB but were similar to CB/C-1 (SD) above node 5. Assimilates and the flowering and branching agents in the CB donor under SD (the source) may have moved to the C-1 receiver (the sink) under LD and changed C-1 branching and flowering patterns through a source-sink mechanism.

The probability of exhibiting flower induction in C-1/CB (SD) was high (0.50), splitting was low (0.07) and C-1 branching was increased in 1990-1991. However, in 1991-1992 C-1/CB (SD) probability of exhibiting flower induction was low (0.04), splitting was high (0.98) and the branching pattern was not altered as in 1990-1991. The potential to branch and flower appear to be correlated, and both are adversely affected by splitting.

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