DEVELOPMENTAL AND SPATIAL CHARACTERIZATION OF FLOWERING IN *HYDRANGEA MACROPHYLLA* (THUNB.) SER.

by

WARNER OROZCO-OBANDO

(Under the Direction of HAZEL Y. WETZSTEIN)

ABSTRACT

Hydrangea macrophylla cultivars widely differ in their relative abundance and duration of flower production. However, the reasons for this variation are not well understood. This study consisted of 3 experiments to determine where and when floral induction occurred on commercial cultivars and in the newer re-flowering hydrangeas. It also determined the patterns of floral development of the induced buds and the effect of different pruning times on growth and development of the plants. In evaluations of dormant shoots in 18 cultivars, flower development was very consistent in terminal buds, and occurred in 100% of the terminal buds for all of the cultivars with the exception of 'Ayesha' (33%). In contrast, lateral buds showed a wide variation in flower development among different genotypes; and the percentage of induction range from 0 to 100%. Flower development was more advanced in terminal than in lateral buds. In the second experiment examining shoots throughout an annual cycle, cultivars had floral primordia initiated in lateral buds at the first sampling period prior to receiving cold or short days. The degree of induction and development varied according to the cultivar and evaluation (harvest) period. Although, differences were found among some cultivars and evaluation

periods, most of the cultivars reached their maximum flower potential and floral development by the time they reached the dormant period (leaf abscission), with no further induction or development during the quiescent period. The results suggested that some cultivars have minimal or no photoperiodic/temperature requirements to induce flowering. These studies indicated that genotypic variation in terminal and lateral floral induction, differences in floral development, and low or minimal inductive conditions (e.g. temperature, photoperiod) required for some cultivars may explain the ability of some cultivars to have a greater abundance and duration of flower production. The information provided for this study could aid the industry and gardeners in developing cultural practices (chemical treatments or pruning practices) to promote lateral bud-break throughout the growing season, thereby enhancing the production of flowers and extending the blooming season of some cultivars.

INDEX WORDS: Big-leaf Hydrangea, *Hydrangea macrophylla*, Flower induction, Flower development, Flower initiation, Hortensia, Cultivars, Re-flowering Hydrangea, Reblooming, Re-blooming and Pruning.

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DEDICATION

To Jo Anne, who have been a source of great blessings and whose support was crucial to my accomplishing this goal. To my brother Henry, my mother Marielos, to Doña Loli, my aunts Sergia, Marjorie, Gloria and, Lydia who always believed and support me on all my academic endeavors. Furthermore, I will dedicate this project in loving memory of my beloved father Lusvin and my grandmother María Evangelina, all of whom have given me a lifetime of love, support and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The family Hydrangeaceae includes mainly woody plants and comprises 17 genera and about 170 species (Roels, et al., 1997). Most of the genera of this family is plants of the Northern hemisphere, scattered throughout eastern Asia, especially China and Japan, but found in other countries such as Taiwan. In addition, they also inhabit the Americas and can be found from the U.S. Northeast, to Mexico, Costa Rica and the area of the Andes in Ecuador and Peru (van Gelderen and van Gelderen, 2004). *Hydrangea macrophylla* is one of the most well-known species in the genus and is known by the name of *Hortensia* and the synonyms *hortensis* and *opuloides* (Wallerstein and Rünger, 1985; McClintock, 1957). By examination of the native habitat of hydrangeas, Bailey (1992) reported that florists' Hydrangeas have evolved in a maritime climate with moderate temperatures, moderate to high humidity and extensive rainfall. *H. macrophylla* is characterized as a lush perennial, which is easy to grow, and has few pests and diseases. In addition, the plants tolerate moist soils, shade, acid or alkaline soils and coastal winds (Church, 1999).

Under outdoor conditions, *H. macrophylla* buds break early in the spring and shoots expand and develop until they show their inflorescences by early-mid June (depending on the cultivar). Flower-bud differentiation occurs at the apical buds (Zhou and Hara, 1989) and only later in well-developed axillary buds (Bowman-Price, 1999; Church, 1999; Lawson-Hall and Rothera, 1995; Wallerstein and Rünger, 1985) on the previous year's growth (Armitage and Laushman, 2003; Bowman-Price, 1999; Shanks et al., 1986). Both temperature and photoperiod are involved in stimulating inflorescence formation and 6-9 weeks of cool, short-day conditions of autumn lead to complete inflorescence formation (Bailey, 1992). Flower-bud differentiation

is followed by the quiescent period (Wallerstein and Rünger, 1985), and flower-buds enter a resting state and resume growth after winter-chilling and leaf-shedding.

Haworth-Booth (1984) speculated that *H. macrophylla*'s genetic relationship with the southern Asian species might account for the greater tenderness of some cultivars, as well as, the response to late-frost damage and pruning practices. Cold-hardiness is becoming very relevant since the susceptibility of *H. macrophylla* to cold-injury limits flowering potential in regions that are exposed to early fall frost and/or late-spring cold-snaps (Adkins, 2001; Adkins et al., 2002; Dirr, 2004; Reed, 2002). Flowering can also be limited due to mechanical damage, i.e., deer and/or careless pruning (Adkins et al., 2002; Church, 1999; Reed, 2002). In dealing with *H. macrophylla*, there is a lot of contradiction about pruning. For instance, van Gelderen and van Gelderen (2004) stated that *H. macrophylla* is one of the species that does not respond well to pruning due to the fact that this particular crop produces blooms on second-year stems and, pruning on a yearly basis would result in a shrub that rarely bore flowers. They also documented that the best show of flowers they had appreciated were in gardens where the secateurs remained in the workshed. In opposition to the previous stance, it is felt that correct pruning of *H. macrophylla* can not only benefit the plant in general, but result in increased flower production. For example, in spring or early-summer, Lawson-Hall and Rothera (1995) recommended cutting each stem back to just above the uppermost pair of new buds. To rejuvenate overgrown plants, Medic (1995) recommended pruning in the summer immediately after the plant has bloomed, removing one-third of the oldest growth and cutting the remaining stems to a few inches above the ground.

My study consisted of 3 experiments to determine where and when floral induction occurred in commercial cultivars and in the newer re-flowering hydrangeas. It also, determined

the patterns of floral development of the induced buds. Finally, it followed the effect of different pruning times on growth and development of re-blooming cultivars. In the first experiment, dormant 1-yr-old stems were collected after receiving natural outdoor floral inductive conditions. All terminal and lateral buds longer than 2 mm were measured, dissected, and floral induction was categorized microscopically. In the second experiment, cultivars with the capacity to produce a second flush of blooms or continuing flowering were evaluated to determine why those cultivars had such attributes. Four cultivars were evaluated: 'Penny Mac', 'Endless Summer', 'Madame Emilie Mouillèré' and 'Nikko Blue'. All of the plants were managed under the same outdoor nursery conditions and all were harvested at each of four key developmental intervals: 1) Pre-induction: late-summer, after completion of shoot expansion; 2) Post-induction: late-fall, following short days and cold temperature exposure; 3) Dormancy: winter, post-leaf abscission; 4) Post-dormancy: early-spring, just prior to bud break. At each sampling time, bud location (terminal and/or lateral) and stem origin (basal, lateral, terminal or secondary) was ascertained and recorded. As in the previous study, all buds longer than 2 mm were dissected under a stereomicroscope and the degree of floral induction was determined. The third study evaluated the response of re-flowering cultivars to different pruning times (late-summer, fall and late-fall). The same cultivars evaluated in the previous study were used for this assessment. Plants were pruned 3 to 4 inches above the soil line and maintained under nursery management conditions until they reached dormancy. Plants were forced in a heated greenhouse and growth and development was determined in mid-February and at the beginning of May.

CHAPTER 2

LITERATURE REVIEW

FLORAL INITIATION AND DEVELOPMENT

The physiology of flowering in ornamental plants is a rather poorly understood subject because it is a highly complex process involving many developmental stages. One of the reasons for such complexity is due to the interaction of the plants with the environmental conditions throughout the year; e.g., a plants' flowering development is impacted by seasonal climatic changes. The first stage in the flowering process is floral induction or evocation when the vegetative meristem becomes programmed to change into a reproductive one. It can be detected by determining increases in the synthesis of nucleic acids and proteins required for cell division and differentiation (Sedgley and Griffin, 1989). Floral initiation can also be detected by determining the morphological development that takes place in the in the bud. These changes are part of a multi-step process that is followed by the differentiation of floral structures. In addition, there are changes in the relative rates of mitoses in different meristematic domains, as well as, increases in the numbers of plasmodesmata across the entire floral meristem (Zik and Irish, 2003). In some species, flower initiation is manifested by changes in the size and shape of the shoot apical meristem; which takes on the form of a broad, low dome (Lamp et al., 2001). In *Rubus* for example, buds in vegetative phase have leaf primordia encircling the flat apical meristem. When the bud changes to reproductive development, the apices enlarge and sepal and petal primordia are initiated (Takeda and Wisniewski, 1989). During the early stages of inflorescence development, *H. macrophylla* produces pairs of partial inflorescence primordia successively as axillary buds on the primary inflorescence apex (Uemachi and Nishio, 2000).

In general the period between initiation and flowering is related to growth habit of the

plant, which in turn, is governed by the climatic range of the species. For example, temperate species may be evergreen in the case of gymnosperms or deciduous in the case of the angiosperms (Sedgley and Griffin, 1989). The deciduous species lose their leaves prior to winter during which little growth and development occurs. Cold-temperate species initiate their flowers in summer or autumn prior to the winter dormancy.

DORMANCY

Dormancy is a period during the life cycle of a plant when there is a little or no visible growth. Dormancy in shoots refers to a period of ceased growth and the presence of a resting bud that is typically enclosed in scales. The term is most commonly applied to temperate tree species which undergo a long dormant period during the cold winter months. This phenomenon is particularly noticeable in the case of deciduous species, that lose their leaves prior to the dormant period and are thus able to withstand subzero temperatures (Sedgley and Griffin, 1989). Wintering tissues of temperate woody plant species are known to display diverse freezing behaviors under subfreezing temperatures, such as extra-cellular freezing, deep super-cooling and extra-organ freezing (Price et al., 1997). The flower primordium is not damaged by freezing conditions because it appears to have an intrinsic resistance to ice nucleation in comparison to the bud scales and the pith, which freezes in preference to the delicate primordium. This is probably due to the cryoprotective compounds present inside the protoplast at elevated concentrations in order to avoid membrane damage; e.g., the higher sucrose levels in the flower primordia as compared with the bud scales and the vascular tissue below the bud (Sedgley and Griffin, 1989). Other cryoprotective compounds are cold-regulated proteins that help to stabilize membranes during a freeze-thaw cycle. For instance, there is a lipid-transfer protein homologue

(cryoprotectin) isolated from cold-acclimated cabbage leaves that protects isolated chloroplast thylakoid membranes from freeze-thaw damage (Hincha, 2002).

According to Seiler (2004), the development of shoot dormancy typically occurs in phases. The first phase is termed pre-dormancy. Pre-dormancy is reversible and the plant can resume growth if the plant is returned to favorable growing conditions. Following pre-dormancy, the plant enters true-dormancy where growth will not resume even if it is returned to optimal growing conditions. The plant is often defoliated at this point and a period of prolonged chilling is required before growth resumes. Temperate zone species experience a cessation of growth in response to shortening day length and/or lower temperatures. This is often marked by the setting of buds for the next year's crop. The plant enters in a phase known as endo-dormancy. Endo-dormancy is associated with changes in hormones and metabolic processes. In some cases, ABA and plant growth inhibitors increase, while levels of GA and promoters decrease. In addition, enzyme activity levels decrease. Chilling temperatures are needed for the plant to alter the ratio ABA: GA and levels of inhibitors and promoters of growth (Crassweller, 2000). The final stage of dormancy is post-dormancy. This stage is typical of late-winter and early-spring. In post-dormancy the bud is capable of growing, but it is still suppressed by adverse environmental conditions such as low temperatures (Sedgley and Griffin, 1989).

Floral development in dormant buds is not a well-understood process and can vary from crop to crop, cultivar to cultivar and even from one type stem to another. For example, in raspberries (*Rubus idaeus* L.), flower initiation and development in axillary buds change little during the late-December and early-January (Williams, 1959). In Blackberries (*Rubus* sp), the initiation of flower-buds can occur prior to the endo-dormant phase. Bud development may continue throughout the dormancy period and an increase in the complexity of floral organs

might occur. Axillary buds from the blackberry cultivar 'Black Satin' remained vegetative during the winter months (Takeda and Wisniewski, 1989).

JUVENILITY AND MATURATION IN WOODY PLANTS

Despite the conditions under which plants are grown, most species grow vegetative for sometime after being planted (Bernier et al., 1981a). All trees propagated from seed undergo a period of juvenility during which they will not flower (Sedgley and Griffin, 1989). This is known as the juvenile or maturation phase and in it, the plant is less sensitive to different conditions that eventually promote the floral transition. Juvenility is often characterized by a period of rapid vegetative growth, which slows considerably after maturity is reached. According to Meilan (1997), it is advantageous for a plant to delay reproductive growth, not only to compete for light and other resources but to produce sufficient photosynthetic capacity to support seed and biomass production. There have also been efforts made to determine the minimal leaf number that will provide assimilates so flowers can be induced. However, it is difficult to establish exact values for some plants. The number of nodes to first flower may be used as a measure of the length of the juvenile phase (Bernier et al., 1981a; Hackett and Sachs, 1967). It seems that the number of nodes and extension of the inter-nodes establishes a physical barrier (distance) separating the apex from the influences of the roots and allows the apex to become determined. For example, in blackcurrant, the apex is prevented from becoming reproductive due to gibberellins produced by the roots (Lyndon, 1990). In woody plants, once the juvenile phase is over, the plants reach the condition known as "ripeness-to-flower" (Bernier et al., 1981a) and are then able to respond to exogenous and endogenous floral inductive cues.

ENVIRONMENT AND OTHER FACTORS AFFECTING FLORAL INDUCTION

The time of flowering is a response to environment (Salisbury, 1963). In contrast to many annual plant species, flowering in most woody perennials does not appear to be under photoperiodic control. However, some herbaceous perennials show a marked need for specific photoperiods in order to induce flowers. For example, in *Dendranthema grandiflorum* cultivars, once the plant is prepared to be induced, floral morphogenesis development is determined by photoperiod. In this crop, plants grown under a 10 h photoperiod reached anthesis up to 7 days earlier than those grown in 8-12 h photoperiods. In 14-16 h photoperiods, plants initiated flower-buds but failed to reach anthesis (Lee et al., 2004).

Bernier et al., (1981b) pointed out that the flowering stimulus may be a complex mixture that may include the known hormones and other translocated materials. In *Fuchsia hybrida*, photosynthetic irradiance increases shoot apex sucrose content and this raise has a positive correlation with the induction of flowering (King and Ben-Tal, 2001). In *Pyrus pyriflolia*, Japanese researchers postulated that higher activities of sugar catabolizing enzymes should enhance the capacity of buds to attract assimilates, thereby accelerating bud growth (Ito, et al., 2004). In *Pelargonium x hortorum*, flowering time depends on irradiance and temperature (Armitage and Wetzstein, 1984). In the case of *Bougainvillea* 'San Diego Red', flowering is influenced not only by temperature and daylength but also by light intensity. For this plant, most rapid flowering occurred when plants were grown under short days, high light intensity and moderate temperatures (Hackett and Sachs, 1967).

The effect of temperature on floral initiation also varies with the species. Sedgley and Griffin (1989) mentioned that in cool-temperate species, the relatively high temperatures of summer and early-autumn appear to promote initiation, whereas in warm temperate, subtropical

and tropical species a relative reduction in temperature is beneficial. In *Hydrangea macrophylla*, Litlere and Strømme (1975) demonstrated that short days (SD) hastened flowering at a high temperature, while a temperature range of 15-18^{o} C was optimal to induce floral bud formation. Quite the opposite, higher temperatures increased the number of leaf-pairs formed prior to bud initiation, and low-light intensities delayed bud formation and gave a high percentage of blind shoots.

ORIGIN AND GEOGRAPHICAL DISTRIBUTION OF HYDRANGEAS

Hydrangea origin of distribution includes temperate regions of Eastern-Asia and Eastern-North America and extends southward into the tropics of both hemispheres (McClintock, 1957). The tropical group has an evergreen habit and had its greatest development take place in Central and South-America (McClintock, 1957). They can be found Mexico, Costa Rica and the area of the Andes in Ecuador and Peru (van Gelderen and van Gelderen, 2004). The temperate group, on the other hand, has a deciduous habit and its greatest development took place in eastern Asia (McClintock, 1957) and can be can be found in China and Japan, but also can be found in other countries in that region (van Gelderen and van Gelderen, 2004).

The family Hydrangeaceae includes mainly woody plants and comprises 17 genera and about 170 species (Roels, et al., 1997). The florist's Hydrangea belongs to a group that includes approximately 23 species. Within that group, *Hydrangea macrophylla* is one of the most well-known species in the genus and from it, hundreds of named cultivars have been selected or developed (van Gelderen and van Gelderen, 2004). In recent years, the popularity of cultivating other species as ornamentals has increased (Reed, 2004). The common name for most cultivars is Hortensia and this name was used by several botanists until the end of the 19th century (van

Gelderen and van Gelderen, 2004). The plant is also known by the name Hortensia and the synonyms: *hortensis* and *opuloides* (Wallerstein and Rünger, 1985; McClintock, 1957).

According to Haworth-Booth (1984) *Hydrangea macrophylla* covers a wide range of garden hybrids that combines genetic material from wild maritime species as well as woodland - inland species. However, true *Hydrangea macrophylla* has a limited distribution to Honshu Island, Izu peninsula, Bōso peninsula and islands of Izu archipelago in Japan (Dirr, 2004). By examination of the native habitat of hydrangeas, Bailey (1992) reported that Florists' Hydrangeas have evolved in a maritime climate with moderate temperatures, moderate to high humidity and extensive rainfall. Haworth-Booth (1984), suggested, that the genetic input from inland species might account for the lack of cold-hardiness of some hydrangeas. Dirr (2004) mentioned that in his book about Hydrangea production, Bailey (1989) provided data about the climatic conditions from the native habitat of *H. macrophylla* that explains its sensitivity to certain extremes. Dirr wrote "Honshu enjoys more than 5 months of frost-free temperatures. The mean low and high temperatures are 31 and 47 ^{o}F in January and 72 and 85 ^{o}F in August, respectively. Annual rainfall is between 70 and 90 inches, while mean relative humidity during January and August are 65 percent and 85 percent respectably".

This plant became a popular garden and greenhouse plant in Europe after the importation of *Hydrangea macrophylla* into England from China by Sir Joseph Banks in 1789. However, prior to that time, the plant had been grown in Asian gardens for centuries. The plant is a prized crop for gardeners and greenhouse producers because it is a long-flowering summer shrub. Hydrangeas also have a wide range of different floral colors, lush perennial growth, ease of cultivation and few pests and diseases. In addition, some cultivars will tolerate coastal winds,

moist soils, shade, and thrive in either acid or alkaline soils (Church, 1999; Bowman- Price, 1999).

FLORAL INDUCTION ON HYDRANGEAS

Due to its importance as a greenhouse crop plant, a body of work has evaluated the phenology of flowering under controlled environmental conditions (Adkins and Dirr, 2003; Bailey and Weiler, 1984; Litlere and Strømme, 1975; Shanks et al., 1986). Before floral induction can occur, Wallerstein and Rünger (1985) mentioned that flower-bud differentiation requires a minimal number of leaves and an adequate amount of assimilates. The plant must have a minimum of 6 to 8 leaf pairs developed and an adequate amount of assimilates have to be accumulated (Yeh and Chiang, 2001). Flower induction is preceded by a series of morphological changes in the plant, i.e., shortening of internodes, lignification of the stem, thickening of leaves, retardation and cessation of shoot elongation (Wallerstein and Rünger, 1985). The transition from vegetative to a floral apex depends on the day-length and temperature. It occurs approximately 2 weeks before primordia can be observed (Wallerstein and Rünger, 1985).

It has also been reported, that the length of the photoperiod determines stem elongation and cyme expansion. Bailey and Weiler (1984) reported that a period of 8 to10 weeks under 8-hour photoperiods seemed sufficient stimulus for floral initiation and inflorescence development. However, additional exposure of plants (12-13 weeks) to SD induced leaf abscission and plant dormancy. In terms of the morphological progress of flower-bud differentiation in *Hydrangea* has been compared with that of azaleas (Bailey and Weiler, 1984). According to Criley (1985), azaleas maintain vegetative growth under long photoperiods and the transition to a floral apex under SD requires 4 to 6 weeks, depending on the day length and temperature.

In contrast to greenhouse directed studies, few reports have described environmental effects on flowering of outdoor plants. Growing under these conditions, nature provides/dictates the environment for hydrangea flower initiation and formation (Armitage and Laushman, 2003). When growing in temperate-zones and influenced by inductive conditions, the buds of this plant progress into flower-bud differentiation and show a defined seasonal pattern of dormancy. Shanks et al., (1986) reported that during the fall months, the cymose inflorescence undergoes initiation and partial development within the resting terminal bud, following defoliation and winter chilling. Wallerstein and Rünger (1985) mention that although the quiescent period is needed for floral induction, the floral bud continues developing during the quiescent period. After 1,000 to 1,200 chill hours (to satisfy bud dormancy), the warmer temperatures of late-winter/early-spring promote the buds to swell and break (Dirr, 2004). Leaves emerge and the cymes and each subtending leafy stem expand into full bloom in spring during May to June (Shanks et al., 1986).

Both temperature and photoperiod are involved in stimulating inflorescence formation in *H. macrophylla* (Bailey, 1992). However, several studies have shown variation in the optimal temperature for floral induction in hydrangea cultivars (Adkins, 2001; Shanks et al., 1986; Litlere and Strømme, 1975; Guo et al., 1995). According to Wallerstein and Rünger (1985) in hydrangeas, temperature is the main factor controlling flower-bud differentiation and the quiescent period. Light has a secondary effect interacting with temperature, or through its influence on overall shoot development. Other researchers have reported that in *H. macrophylla*, floral development continues in the dormant buds (Struckmeyer, 1950). According to Wallerstein and Rünger (1985), 'dormancy' is an essential stage for flower-bud development because during the quiescent period flower-buds continue to develop. Some cultivars might have

lower temperature and photoperiodic requirements (Adkins and Dirr, 2003; Shanks et al., 1986) and the developed buds from these cultivars are reported as being more sensitive to inductive conditions. For instance, Adkins (2001) pointed out that if hydrangea plants are left un-pinched, sensitivity to induction increases and flowers might get induced as early as August. Bailey (1992) noted that the fertile flowers develop during the autumn months and by early November dissected inflorescence buds contain fertile flowers.

Hydrangeas have two different types of inflorescences: lacecap and mophead (Fig. 1.1). A lacecap is characterized by a flat inflorescence with numerous fertile flowers. A mophead is characterized by a round inflorescence with numerous flowers with very large sepals. Flower development in later developmental stages is different for lacecaps and mopheads. In lacecaps, the axillary primordia initiated on each inflorescence apex develop into inflorescences. In mopheads, the axillary primordium develops into decorative florets. In both types of inflorescences, terminal florets which were non-decorative are initiated from the apical meristem on main inflorescence apices (Uemachi and Nishio, 2000).

FLOWER UNRELIABILITY

Although, hydrangeas have many attributes as an outdoor ornamental, the plant has a restricted geographic marketing range due to temperature constraints (Adkins, 2001; Adkins et al., 2002. Flower unreliability has been attributed to the susceptibility of new growth to freezing damage. For instance, -4° C or lower temperatures induced browning of flower-buds, leaf, and stem tissue, and may result in a flowerless season (Church, 1999; Dirr, 2004). Miller (1998) reported that varieties designated as suitable for outdoor use may be bud-hardy in Zone 6 (greenhouse forcing varieties are usually bud-hardy only in the lower part of Zone 7). There have

been several studies that evaluated flowering performance of various cultivars in different areas (Bir and Conner, 2000a; Bir and Conner 2000b; Reed, 2002). Unfortunately, the evaluations provided incongruous information on cold-hardiness and flowering potential (Dirr, 2004). In addition to cold injury, location and timing are also factors considered as part of the flowering unreliability in hydrangeas. Most of the literature reports that floral induction occurred in the terminal buds (Bir and Conner, 2002; Shanks et al., 1986, Zhou and Hara, 1989) on the previous year's growth (Bir and Conner, 2002; Bowman-Price, 1999; Shanks et al., 1986; van Gelderen and van Gelderen, 2004). However, there has been some speculation about induction occurring in the axillary buds as well (Adkins, 2001; Armitage and Laushman, 2003; Wilkinson and Hanger, 1992).

HYDRANGEA CULTIVARS WITH RE-FLOWERING POTENTIAL

Recently, there have been several reports of *H. macrophylla* cultivars that have the potential to produce a second flush of blooms or have the ability to continue producing flowers all season long. These cultivars have been called: free-flowering (Haworth-Booth, 1984), re-blooming (Bir and Conner, 2002) or remontant (Adkins, 2001; Bir and Conner, 2002; Dirr, 2004; Lindstrom et al., 2003; Reed, 2002). The reasons for this continuous and/or re-blooming capacity are not well-understood. However, a number of researchers have expressed several hypotheses to explain such behavior. For instance, re-flowering potential might be due to the propensity of some cultivars to release apical dominance allowing induced lateral buds to elongated and flower during the current season (i.e., late-summer/fall) that would ordinarily bloom next season but break and bloom due to favorable late-season conditions (Bir and Conner, 2002).

On the other hand, some cultivars, might have minimal or no photoperiodic and temperature requirements for flowering and have induced flower-buds forming on current season's growth throughout the season. The hypothesis based on low inductive requirements (photoperiod and temperature) is supported by several studies that have documented a genotypic variability in the threshold in temperature and day-length required to initiate flowering (Bailey and Weiler, 1984; Guo et al., 1995; Litlere and Strømme, 1975) allowing some cultivars to produce flower-buds under different conditions. Other researchers have reported that some cultivars can bloom on newly formed stems (Bir and Conner, 2000; Adkins, 2001; Lindstrom et al., 2003). For example, Armitage and Laushman (2003) claimed that some *H. macrophylla* cultivars seem to be able to form buds on new stems without the required cold temperature to induce flowering. Bailey and Weiler (1984) evaluated different cultivars and determined that cultivar Sister Therese, bloomed freely regardless of photoperiod.

PRUNING HYDRANGEAS

Lawson-Hall and Rothera (1995) mentioned; "The urge to prune *Hydrangea macrophylla* shrubs is so strong that many misguided actions are taken by eager, ill-informed gardeners with itchy secateurs, resulting in uneven growth and impeded flowering." Other authors have suggested that generally speaking, the hard pruning of established hydrangeas does more harm than good and may easily be fatal (Haworth-Booth, 1984). Prudent and timely pruning can bring out the best in most ornamental plants by improving their health, shape and ultimately result in plants that require less maintenance. Nevertheless, in the case of pruning *Hydrangea macrophylla* shrubs, the topic is surrounded by controversy. In the industry there are a lot of discrepancies about proper pruning requirements among the different cultivars

(Conwell et al., 2002). The genotypic variation on hydrangeas has induced a lot of discrepancies in the industry as to proper pruning requirements among different cultivars (Conwell et al., 2002). The problem becomes more complex since, the current literature on pruning hydrangeas is generally limited to specific species other than *H. macrophylla* e.g., *H. paniculata*, *H. arborescens*, *H. quercifolia* (Bowman-Price, 1999; Dunwell et al., 2001). In the case of *H. macrophylla*, literature only accounts for pruning practices in popular varieties and old cultivars.

Even though winter-kill is a major problem with hydrangea flower production, there are several other factors that can adversely affect the plant. Armitage and Laushman (2003) mentioned that induced buds are also sensitive to mechanical damage. In some areas, gardeners are plagued by herbivores (deer) that during the early-spring are very attracted to the tender new growth of the terminal buds of hydrangea. This causes a major problem, since most cultivars develop their flower-buds on the tips of the branches from the previous year's growth (Bir and Conner, 2002; Bowman-Price, 1999; Shanks et al., 1986; van Gelderen and van Gelderen, 2004; Zhou and Hara, 1989). Another major factor contributing to flower failure in hydrangeas is pruning. Van Gelderen and van Gelderen (2004) stated that *H. macrophylla* is a plant that does not respond well to pruning and pruning on a yearly basis would result in a shrub that hardly ever produced flowers.

In opposition to the previous stance, it is felt that correct pruning of *H. macrophylla*, should actually increase flower production and stimulate the production of longer stems (Conwell, 2002). However, pruning time and severity depends on what is the objective of applying this cultural practice. One of the common pruning practices consists of the removal of old stems and dead-flowers (deadheading) in late-winter/early-spring, before growth begins

(Howard-Booth, 1984). To rejuvenate overgrown big-leaf Hydrangeas, Medic (1995) recommended cutting out one-third of the oldest growth immediately after the plant had bloomed in the summer. At the same time, he further suggested cutting the remaining stems to a few inches above the ground. However, it should be noted that pruning this crop too late in the fall (after September) can be harmful. New growth, both vegetative and reproductive will not have proper time to develop and mature.

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A


B

Fig. 1.1. *Hydrangea macrophylla* inflorescence type. **A**. Lacecap. **B**. Mophead.

CHAPTER 3

GENOTYPIC VARIATION IN FLOWER-BUD DEVELOPMENT IN *HYDRANGEA MACROPHYLLA*[1]

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Subject Area: Developmental Physiology

Genotypic variation in flower-bud development in *Hydrangea macrophylla*

ADDITIONAL INDEX WORDS: flower induction, flower development, flower initiation, Big-leaf Hydrangea, Hortensia, cultivars.

ABSTRACT.

The general doctrine of flowering in *Hydrangea macrophylla* (Thunb.) Ser. is that floral induction occurs during the previous season on last year's growth and usually within the stem's terminal bud. However, it has been noted that hydrangea cultivars widely differ in their relative abundance and duration of flower production. The objective of this study was to determine how developmental flowering patterns compared among different hydrangea genotypes. Flowering was characterized in 18 cultivars by assessing flower initiation in dormant buds of 1-yr-old stems that were collected after receiving natural outdoor floral inductive conditions. All terminal and lateral buds longer than 2 mm were measured; dissected and floral developmental stage was categorized microscopically. Flower development was very consistent in terminal buds and occurred in 100% of the terminal buds for all of the cultivars with the exception of 'Ayesha' (33%). In contrast, lateral buds showed a wide variation in flower development among different genotypes. For example: 'Ayesha', 'Blushing Pink', 'Freudenstein', and 'Nigra' had 10% or less lateral buds with floral initials. 'All Summer Beauty', 'David Ramsey', 'Kardinal', 'Masja', and 'Nightingale' showed high levels of floral induction (> 92% of lateral buds induced). Within a cultivar, flower development was more advanced in terminal than lateral buds. We found a

correlation between bud size (length) and floral stage development for some cultivars. However, low r-square values suggest that there are other factors that influence floral induction and should be considered. This study shows that floral induction and development in lateral buds varies markedly among cultivars, and may provide insight into causes for differences in the amount and duration of flowers produced within a growing season.

INTRODUCTION

Hydrangea macrophylla (Thunb.) Ser. is by far the most well-known species in the genus, and from it, hundreds of named cultivars have been selected or developed over the years throughout the world (van Gelderen and van Gelderen., 2004). Its popularity lies in part to its versatility as both a florist and landscape plant. In the garden, the plant forms a rounded/mounded shrub composed of erect, usually unbranched stems that can reach 2 meters. *H. macrophylla* is a desirable ornamental plant because it has immense variation in flower sizes, shapes and colors. In addition, it performs well in areas that require low maintenance because it has characteristics such as ease of cultivation, few pests and diseases, shade tolerance and adaptability to alkaline and acid soils.

The center of distribution of this genus covers the eastern Himalayas, southern China, and Japan (McClintock, 1957). According to Haworth-Booth (1984), the parentage of Hydrangeas is related to wild maritime species, as well as, some woodland Asian species. He speculated that the genetic relationship with the southern species might account for the greater tenderness of some varieties. Low cold-hardiness in many cultivars is very relevant since the susceptibility of *H. macrophylla* to cold injury limits flowering potential in regions that are exposed to early fall frost and/or late spring cold-snaps (Adkins, 2001; Adkins et al., 2002; Dirr 2004; Reed, 2001). If the terminal flower-bud is killed by low temperatures, flowering can be delayed until new flower-buds can be produced (Church, 1999; Reed, 2002).

The general doctrine of flowering in *H. macrophylla* is that floral induction occurs during the previous season on last year's growth (Zhou and Hara, 1988). Plants initiate inflorescences under the cool, short-day conditions of fall, and then bloom in spring with the resumption of growth, making its flowering pattern similar to that of azaleas (Bailey and Weiler, 1984).

However, hydrangea cultivars exhibit great variability in the abundance and duration of flower production. Recent reports of cultivars with the attribute of displaying flowers throughout the growing season (Adkins and Dirr, 2003; Dirr, 2004; Haworth-Booth, 1984; Lindstrom et al., 2003; Reed, 2002) have caught the attention of the industry, breeders, researchers, and hydrangea enthusiasts.

Detailed developmental studies evaluating flowering patterns in *H. macrophylla* are lacking, as are evaluations of how flower induction and development compare in different cultivars. The objective of this study was to determine how developmental flowering patterns compare among 18 *H. macrophylla* genotypes after exposure to natural inductive conditions. As part of the study we try to determine the location and frequency of floral develop, i.e., the extent of flower bud induction in terminal and lateral buds, and the degree of variability in different genotypes. Such fundamental information about flower induction patterns can be used to develop cultural practices to promote enhanced flower production.

MATERIALS AND METHODS

PLANT MATERIALS. Flowering was characterized in 18 cultivars of *H. macrophylla* (Table 3.1). Stems were harvested from plants growing in the hydrangea collection at the University of Georgia Shade Garden on the UGA campus, Athens, GA, or at the Center for Applied Nursery Research in Dearing, GA. For each cultivar, four to ten 1-yr-old dormant shoots (Fig. 3.1) with terminal buds were collected during the second week of February 2003. All shoots were exposed to natural outdoor floral inductive conditions (short days and low temperatures during fall and winter). Shoots were brought back to the lab to microscopically assess the extent of flower initiation and development within the terminal and lateral buds.

ELECTRON MICROSCOPY. Representative apices were fixed and prepared for scanning electron microscopy to document observations characterized for floral developmental stages. Tissues were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, dehydrated through an ethanol series, and critical point dried through CO_2 using a Sandri-780 critical point drying apparatus (Tousimis Research Corporation, Rockville, MD, USA). Samples were mounted on aluminum stubs, further dissected if necessary, sputter-coated with gold, and observed with a scanning electron microscope (JSM-5800, JEOL, Tokyo, Japan).

STEM, BUD, AND REPRODUCTIVE CHARACTERIZATION. The length and node number were recorded for each stem. Stem caliper was determined by measuring the diameter at the base of the stems. Location, size (length and diameter) was recorded for all terminal and lateral buds longer than 2 mm (Fig. 3.1). Axillary buds longer than 2 mm were dissected by removing bud scales and leaf primordia to expose the shoot apex. Buds were examined under a stereomicroscope to determine if meristems were vegetative or reproductive, and to rate the stage of floral development. Apices were classified according to stage of development (Table 3.2) ranging from those having a vegetative apex (Stage 1) to those with differentiated florets with expanded sepals enclosing the floral apex (Stage 5) (Fig.3.2).

QUANTITATIVE AND STATISTICAL ANALYSIS. All morphological and developmental data was compared using mean separation by Student-Newman-Keuls ($P < 0.05$) with the PROC GLM function of SAS® (SAS Institute Inc., Cary, N.C.). Bud length and floral stage development were analyzed using Pearson Correlation Coefficients. Descriptive statistics of the data were generated with Excel Graph Wizard (Microsoft, Redmond, Wash.) and Sigma Plot® 8.0 (SPSS Inc. Chicago, IL).

RESULTS

TERMINAL BUD DEVELOPMENT. Terminal buds exhibited advanced stages of floral development. Flower induction was consistent with all cultivars achieving 100% induction of the terminal buds with the exception of 'Ayesha' with 33% induction (Table 3.3). Mean floral bud development stages ranged from 2.8 to 5.0. However, with the exception of 'Nigra' and 'Ayesha', there were few significant differences among cultivars. 'Nigra' (with a mean stage =2.8) had significantly less advanced floral development than all cultivars except for 'Ayesha' and 'Compacta'.

LATERAL FLORAL INDUCTION AND DEVELOPMENT. Lateral buds exhibited a wide range in the percentage of flower induction among cultivars (Table 3.3). For example, 'Kardinal', 'David Ramsey', 'Nightingale', 'All Summer Beauty', 'Masja', and 'Penny Mac' showed over 90% of lateral buds with floral primordia. In contrast, 'Ayesha', 'Blushing Pink', 'Freudenstein', and 'Nigra' showed 10% or fewer lateral buds with floral initials. Other cultivars were intermediate in the percentage of induction. In general, the stage of floral development in induced buds was not significantly different among cultivars. Most of the induced buds showed a maximum stage mean of 2.1 (apical meristem has an open, dome-shaped form) to 3.5 (inflorescence is well-formed and expanding).

BUD SIZE AND FLOWER DEVELOPMENT. Correlation analysis was conducted to determine if there was a relationship between bud size (length) and floral development stage. In several cultivars, bud length and floral stage were positively correlated with longer buds exhibiting more advanced floral development (Table 3.4). Although correlation coefficients were highly significant in some cases, r-square values were characteristically low (0.04 to 0.46). Low r-square values in the correlation between bud length versus floral development indicated

that within a cultivar, flower development is explained largely due to factors other than bud size (length). Other factores such as genotypic variation, bud position, stem, cultivar sensitivity and exposure to low temperatures can influence bud size. Our data show that cultivars varied in mean bud length (Table 3.4) but this was necessarily not associated with floral developmental stages (Table 3.4) neither. 'Blushing Pink' had large buds with a mean length of 18.9 mm (Table 3.4) yet meristems on lateral buds were not florally induced. In the case of 'Ayesha', bud length had a mean of 11.3 mm with a floral development value of 1.2 and only 10% of the buds were induced. 'David Ramsey' on the other hand, showed smaller lateral bud sizes (mean 6.5 mm) but had a great degree of floral development (3.1) and showed 95% of buds induced.

DISCUSSION

In this study, we documented that in *H. macrophylla*, flower induction occurs in both terminal and lateral buds. With the exception of one cultivar, flower-bud induction was observed in 100% of the terminal buds. The breaking of terminal buds when shoot growth resumes in the spring would correspond to the production of high flower numbers. Flower-bud induction among the 18 cultivars proceeded similarly to the descriptions provided by several authors, i.e., under inductive conditions, *H. macrophylla* initiated flower-bud differentiation at the apical buds (Shanks et al., 1986; Wallerstein and Rünger, 1985; Yeh and Chiang, 2001). For instance, Zhou and Hara (1988) found that the leaves and inflorescences of flower-bearing hydrangea shoots were normally preformed in the terminal bud during the previous year. In our study, we found that terminal bud induction occurred in most of the evaluated buds (100%). However, we also found that a cultivar like 'Ayesha' showed a low percentage of induction (33%). The occurrence of floral primordia in lateral buds has previously been speculated (Adkins, 2001; Armitage and

Laushman, 2003; Dirr, 2004), but not verified. Zhou and Hara (1988), in their evaluations of the structure of winter buds indicated a lack of flower initiation in lateral buds. (It should be noted that Zhou and Hara did not reveal the cultivars used in their evaluation).

Our observations were that flower induction in lateral buds varied markedly according to genotype from those exhibiting over 90% induced buds to those with less than 10% or 0% induction. The variability in floral induction in lateral buds among genotypes may help to explain why some cultivars differ in their blooming seasons and why some cultivars are more reliable bloomers than others. Cultivars with flower buds confined primarily to terminal buds may be restricted in their blooming to the period of early season shoot expansion. Furthermore, if terminal flower-buds are lost due to mechanical damage, cold-injury or careless pruning, little or no bloom would occur that season. In our study, 'Ayesha' would be predicted to have limited bloom as only 33% of terminal buds had develop inflorescences and only 10% of lateral buds were reproductive. Similarly, it could be projected that 'Freudenstein', 'Blushing Pink', and 'Nigra' would exhibit unreliable blooming. Although these cultivars had all terminal buds induced, the percentage of floral induction in lateral buds was low. In contrast, cultivars like 'David Ramsey', 'All Summer Beauty', and 'Penny Mac' exhibited high percentages of floral induction in lateral buds and would have the capacity to reliably flower even if terminal buds were lost from cold or injury.

The presence or absence of flower primordia within dormant buds can provide an indication of a cultivar's potential for flowering, i.e., a shoot will not display an inflorescence if floral induction has not occurred in the apex. However, it should be noted that an induced floral initial may not necessarily develop into a flower. Prerequisite is that a bud breaks and shoot expansion is sufficient for floral expression. Other factors such as the degree of shoot apical

dominance, and the availability of physiological factors to support shoot expansion and growth are pivotal. In container-grown hydrangeas, Yeh and Chiang (2001) found that defoliation and root restriction affected shoot growth, bud size and flowering. Furthermore, it is possible that some genotypes may be capable of inducing flower primordia during the current season's growth in the absence of apparent short day or low temperature conditions (Orozco-Obando and Wetzstein, unpublished observations).

In cultivars having floral induction limited to terminal buds, late-season pruning should be avoided to circumvent removing flower-buds. Likewise, freeze injury to terminal buds would be catastrophic to bloom. Cultivars with floral induction occurring within numerous lateral buds could have very different cultural requirements. For example, late-season pruning to improve plant habit would be acceptable. The uppermost axillary buds of the flower-bearing shoot often begin expanding into new lateral shoots when the flowering phase has ended (Zhou and Hara, 1989). If lateral buds have floral primordia, strategies to enhance lateral bud break and expansion of late-season shoots could be used to promote recurrent blooming.

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Table 3.1. List of cultivars evaluated in the study.

All Summer Beauty	General Vic. de Vibraye
Ayesha	Lilacina
Blushing Pink	Kardinal
Charm Red	Masja
Compacta	Nightingale
David Ramsey	Nigra
Dooley	Nikko Blue
Freudenstein	Penny Mac
Lady Fugino	Veitchii

Table 3.2. Stages of *H. macrophylla* inflorescence development.

Stage	Characteristics
1	Vegetative apex. Meristem is covered by the upper pair of leaf primordia
2	Transition to a floral apex. Apex is broadened, swollen, dome-shaped and the upper pair of leaf primordia are separated
3	Individual floral primordia are defined within the inflorescence
4	Sepal primordial are initiated on flowers
5	Florets are well-defined; sepal primordia enclose the floral apex

Table 3.3. Percentage of floral induction and mean floral development in terminal and lateral buds.

Cultivar	Terminal buds		Lateral buds	
	%	Floral stage of induced buds	%	Floral stage of induced buds
All Summer Beauty	100 (7) [z]	4.3 ab [y]	92 (36) [z]	3.4 a [y]
Ayesha	33 (9)	3.0 cd	10 (38)	2.1 b
Blushing Pink	100 (4)	4.0 abc	0 (13)	--
Charm Red	100 (10)	4.3 ab	83 (102)	2.8 ab
Compacta	100 (9)	3.4 bcd	86 (6)	2.6 ab
David Ramsey	100 (7)	4.4 ab	95 (21)	3.4 a
Dooley	100 (5)	4.0 abc	82 (31)	2.5 ab
Freudenstein	100 (5)	3.9 abc	10 (20)	3.0 ab
Lady Fugino	100 (4)	4.3 ab	71 (49)	2.4 ab
General Vic. De Vibraye	100 (5)	4.9 a	88 (31)	3.2 a
Kardinal	100 (9)	3.9 abc	88 (29)	3.5 a
Lilacina	100 (4)	4.0 abc	73 (25)	2.8 ab
Masja	100 (6)	4.5 ab	100 (66)	2.9 ab
Nightingale	100 (6)	4.2 ab	100 (52)	3.2 a
Nigra	100 (5)	2.8 d	0 (19)	--
Nikko Blue	100 (4)	5.0 a	81 (16)	3.1 ab
Penny Mac	100 (6)	4.3 ab	93 (42)	2.9 ab
Veitchii	100 (6)	4.5 ab	59 (28)	2.4 ab

[z] The numbers in parenthesis indicate the number of buds evaluated.
[y] Means with the same letter are not significantly different (Student Newman-Keuls Test. $P \geq 0.05$).

Table 3.4. The relationship between bud length and floral stage for different *Hydrangea* cultivars.

Cultivar	Bud length (mm)	Mean floral stage	Bud quantity	Correlation coefficient [y]	Significant level	r^2
Ayesha	11.3 + 4.8	1.2 + 0.5	38	0.21	0.2009	0.04
All Summer Beauty	9.2 + 3.7	3.0 + 1.2	36	0.68	< 0.0001	0.46
Blushing Pink	18.9 + 5.2	1.0 + 0.0	13	--	--	--
Charm Red	9.6 + 4.4	2.5 + 0.8	102	0.21	0.0338	0.04
Compacta	3.6 + 2.4	2.3 + 0.8	6	0.61	0.1977	0.37
David Ramsey	6.5 + 3.7	3.1 + 1.1	21	0.48	0.0262	0.23
Dooley	5.7 + 3.0	2.1 + 0.8	31	0.56	0.0010	0.31
Freudrestein	8.9 + 4.2	1.2 + 0.6	20	0.26	0.2584	0.07
General Vic. de Vibraye	11.3 + 3.4	2.9 + 0.8	31	0.39	0.0278	0.15
Kardinal	10.2 + 5.7	3.4 + 0.6	29	0.61	0.0004	0.37
Lady Fugino	5.2 + 3.0	2.0 + 0.8	49	0.19	0.1920	0.04
Lilacina	8.5 + 4.3	3.2 + 1.3	25	0.56	0.0035	0.31
Masja	9.0 + 3.5	2.9 + 0.2	66	0.24	0.0532	0.06
Nightingale	8.5 + 4.5	3.1 + 0.6	52	0.40	0.0034	0.16
Nigra	8.2 + 2.5	1.0 + 0.0	19	--	--	--
Nikko Blue	10.7 + 5.9	2.7 + 1.0	16	0.65	0.0062	0.42
Penny Mac	10.3 + 4.2	2.6 + 0.7	42	0.62	< 0.0001	0.38
Veitchii	10.0 + 4.3	1.9 + 0.9	28	0.27	0.1633	0.07

[y] Coefficient is based on Pearson Correlation Coefficients analysis (Cody, 1991).

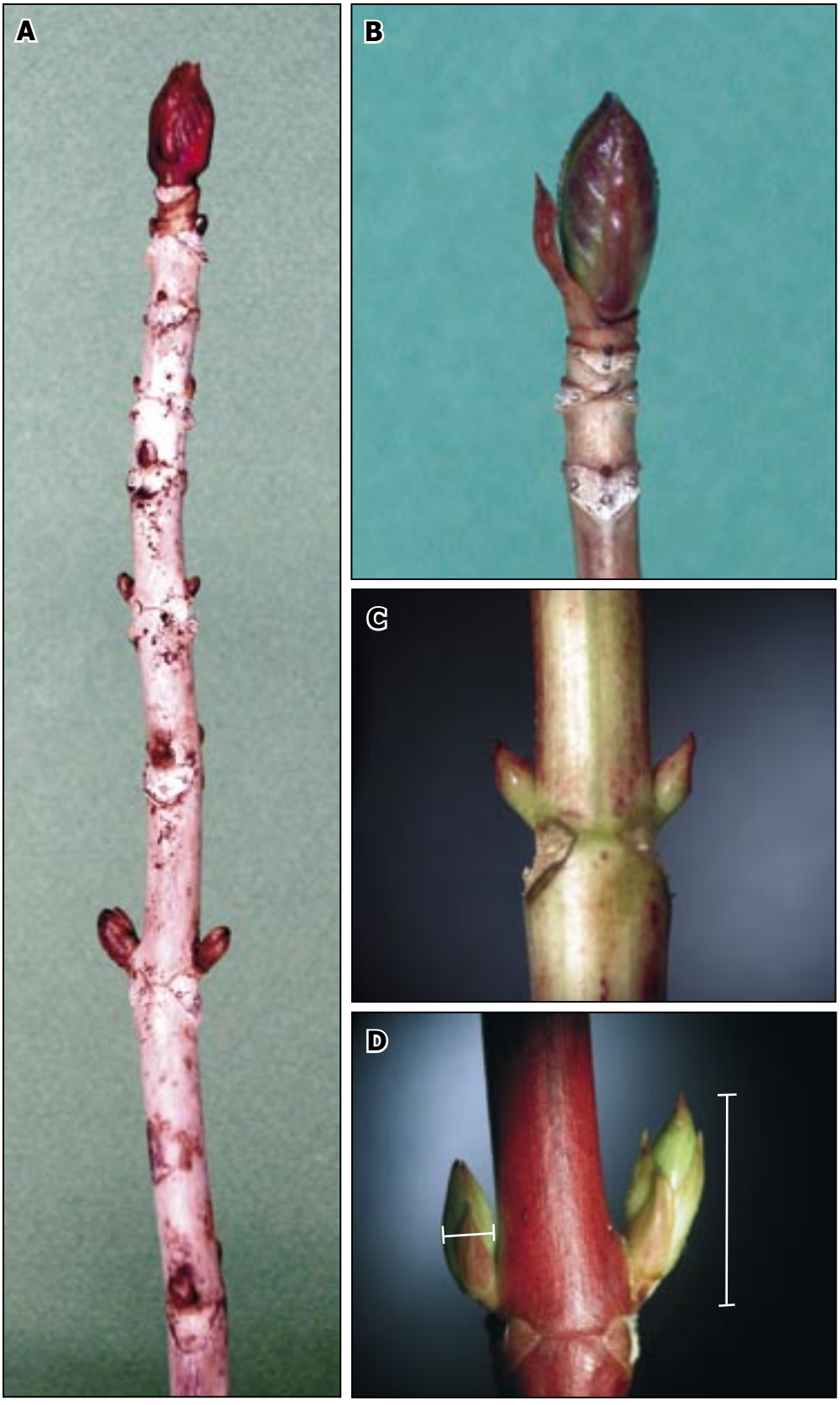
A
B
C
D

Fig. 3.1. Pictures of *Hydrangea macrophylla* dormant stem, terminal, and axillary buds. **A**. Dormant shoot. **B**. Terminal bud. **C**. Axillary small buds. **D**. Lateral buds showing how bud diameter was determined (bud to the left) and how the length of the bud was measured (bud to the right).

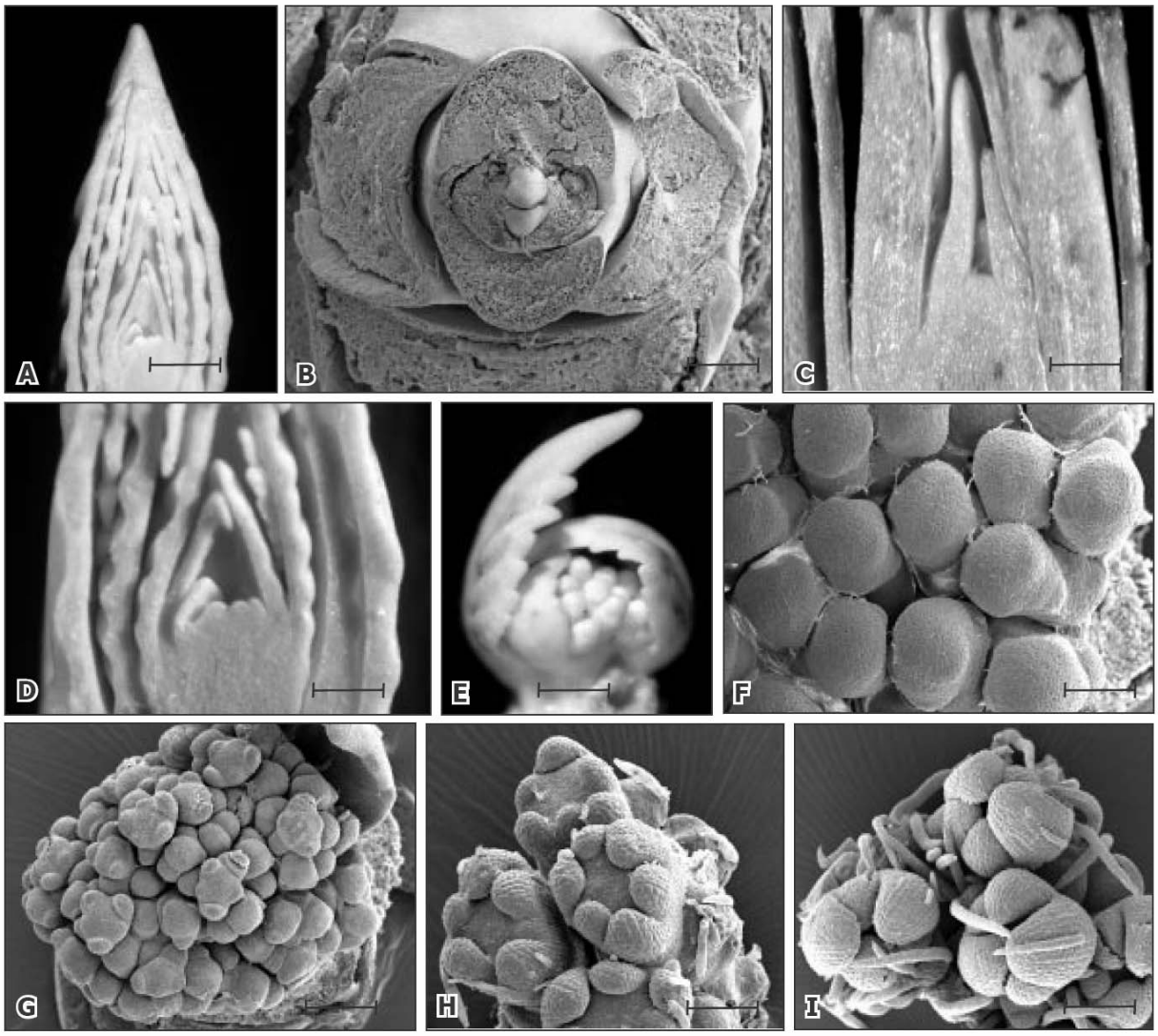
A
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Fig. 3.2. Light and scanning electron microscope pictures of meristems from *Hydrangea macrophylla* illustrating different developmental stages. **A**. Longitudinal section of a bud. Bar = 1000 µm. **B**. Vegetative meristem, stage 1. Bar = 200 µm. **C**. Meristem in early stages of floral induction, stage 2. Bar = 200 µm. **D**. Early stages of inflorescence primordia, stage 3. Bar = 260 µm. **E**. Floral apices, stage 3. Bar = 750 µm. **F**. Flower primordia close up, stage 3. Bar = 150 µm. **G**. Flowers showing early sepal formation, stage 4. Bar = 175 µm. **H**. Flowers showing more developed sepals, stage 4. Bar = 100 µm. **I**. Flowers with well-formed sepals enclosing the floral apex, stage 5. Bar = 200 µm.

CHAPTER 4

CHARACTERIZATION OF SEASONAL AND SPATIAL PATTERNS OF FLORAL INITIATION AND DEVELOPMENT IN *HYDRANGEA MACROPHYLLA* CULTIVARS WITH RE-BLOOMING POTENTIAL[1]

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Subject category: Developmental Physiology

Characterization of Seasonal and Spatial Patterns of Floral Initiation and Development in *Hydrangea macrophylla* Cultivars with in Re-blooming Capacity

Additional index words: flower induction, flower development, flower initiation, Big-leaf Hydrangea, Hortensia, Re-blooming Hydrangea, Re-flowering Hydrangea.

ABSTRACT

Recently, the release of hydrangea cultivars with the capacity to produce a second flush of blooms has created high expectations in the ornamental plant industry. However, the lack of fundamental information on flower development in big-leaf hydrangea does not allow a descriptive explanation of why re-blooming capacity occurs. The objectives of this study were to characterize the timing and location of flower initiation and development in several *H. macrophylla* cultivars throughout an annual cycle. Four cultivars with re-flowering capacity were evaluated: 'Penny Mac', 'Madame Emilie Mouillèré', 'Endless Summer' and 'Nikko Blue'. All of the plants were managed under the same outdoor nursery conditions and harvested at one of four key developmental periods: 1) late-summer, after completion of shoot expansion; 2) late-fall, following short days and cold temperature exposure; 3) early-winter, post-leaf abscission; 4) late-winter, just prior to bud break. At each sampling time, bud location (terminal versus lateral) and stem origin (basal, lateral, terminal or secondary) were ascertained and recorded. All buds longer than 2 mm were dissected under a stereomicroscope and the presence or absence of floral induction and development stage were determined. Floral primordia were found to be initiated

within axillary buds at the first sampling period, with percent of induction varying among cultivars from 4% in ‘Madame Emilie Mouillèré’ ‘Endless Summer’ to 47% in ‘Endless Summer’. The data suggested that some hydrangea cultivars may have minimal or no photoperiodic/temperature requirements for flowering. Most cultivars reached their maximum percentage of flowering at the fall harvest except for ‘Madame Emilie Mouillèré’. No significant floral development occurred during winter, and stem type had no effect on the percentage of induced buds.

INTRODUCTION

Hydrangeas are plants from the Northern Hemisphere, scattered throughout eastern Asia, especially China and Japan, but found in many other countries in that region including Vietnam (van Gelderen and van Gelderen, 2004). The plant is known by the name Hortensia and the synonyms: *hortensis* and *opuloides* (McClintock, 1957; Wallerstein and Rünger, 1985). The florist's hydrangea belongs to the family Hydrangeaceae and includes 17 genera and about 170 species (Miller, 1998; van Gelderen and van Gelderen, 2004). Also known as big-leaf hydrangea, *H. macrophylla* has been a popular garden and greenhouse plant since 1789 when Sir Joseph Banks imported it into England from China (McClintock, 1957). Today the florist hydrangea is widely cultivated throughout both the old and new worlds, and the plant is a prized crop for gardeners and greenhouse producers. Some of their attributes include: long-flowering summer shrub, a wide range of different floral colors, lush perennial growth, ease of cultivation, and few pests and diseases. In addition, some cultivars will tolerate coastal winds, moist soils, shade, and thrive in either acid or alkaline soils (Church, 1999; Bowman-Price, 1999).

Because of its importance as a greenhouse crop plant, a body of work has evaluated the phenology of flowering under controlled environmental conditions (Adkins, 2001; Adkins and Dirr, 2003; Bailey and Weiler, 1984; Litlere and Strømme, 1975; Shanks et al., 1986). Flower induction is preceded by a series of morphological changes in the plant, i.e., shortening of internodes, lignification of the stem, thickening of leaves, and retardation and cessation of shoot elongation (Wallerstein and Rünger, 1985). In addition, before floral induction can occur the plant must have a minimum of 6 to 8 leaf pairs developed and an adequate amount of accumulated assimilates (Yeh and Chiang, 2001). The transition from a vegetative to a floral apex depends on the day length and temperature. Litlere and Strømme (1975) demonstrated that

short days (SD) hastened flowering at a high temperature, while a temperature range of 15-18^{o} C was optimal to induce floral bud formation. In contrast, higher temperatures increased the number of leaf-pairs formed prior to bud initiation. Low light intensities delayed bud formation and gave a high percentage of blind shoots. It has also been reported, that the length of the photoperiod determines stem elongation and cyme expansion. Bailey and Weiler (1984) reported that a period of 8 to10 weeks under 8-hour photoperiods seemed sufficient stimulus for floral initiation and inflorescence development. However, longer exposure of plants (12 to 13 weeks) to SD induced leaf abscission and plant dormancy.

In contrast to greenhouse directed studies, few reports have described environmental effects on flowering of outdoor plants. Some researchers have suggested that flower bud differentiation in hydrangea is similar to that of azaleas (Bailey and Weiler, 1984). Azaleas maintain vegetative growth under long photoperiods and the transition to a floral apex under SD requires 4 to 6 weeks, depending on the day length and temperature (Criley, 1985). The time frame for initiation is approximately 4 weeks and it is manifested by changes in the size and shape of the shoot apical meristem. These changes are explained by cell activity in the central zone of the apical meristems, then the cells become active and the apex begins to increase in height, and then to broaden. In *Hydrangea macrophylla*, flower induction occurs approximately two weeks before primordia can be observed (Wallerstein and Rünger, 1985) and it has been suggested to occur in terminal buds (Zhou and Hara, 1988) and in lateral buds (Orozco and Wetzstein, 2004). In terminal buds during the fall months, the cymose inflorescence undergoes initiation and partial development, following defoliation and winter chilling (Shanks et al., 1986). After the dormant period, the warmer temperatures of late-winter/early-spring promote the buds

to swell and break (Dirr, 2004). Leaves emerge and the cymes and each subtending leafy stem expand into full bloom in the spring during May to June (Shanks et al., 1986).

Although, hydrangeas have many attributes as an outdoor ornamental, this plant has a restricted geographic marketing range due to low cold-hardiness (Adkins et al., 2002; Reed, 2002). In the majority of hydrangea cultivars, the new growth is very susceptible to late-spring frost. For instance, minus 4° C or lower temperatures cause browning of flower-bud, leaf, and stem tissue, and may result in a flowerless season (Church, 1999; Dirr, 2004). Miller (1998) reported that varieties designated as suitable for outdoor use may be bud-hardy in Zone 6 (greenhouse forcing varieties are usually bud-hardy only in the lower part of Zone 7). Several researchers have studied the flowering performance of various cultivars in different areas (Bir and Conner, 2000 ab; Bir and Conner, 2000 b; Reed, 2002). Unfortunately, the evaluations provided inconsistent information on cold-hardiness and flowering potential (Dirr, 2004).

It has been suggested that in most hydrangeas, the terminal bud on previous year's wood provides the new season's flowers (Bowman-Price, 1999; Church, 1999; Lawson-Hall and Rothera, 1995; Shanks et al., 1986). However, there have been several reports of *H. macrophylla* cultivars that have the potential to produce a second flush of blooms or have the ability to continue producing flowers all season long. These cultivars are being called: free-flowering (Haworth-Booth, 1984), re-blooming (Bir and Conner, 2002; Wetzstein and Orozco, 2003) or remontant (Adkins, 2001; Bir and Conner, 2002; Dirr, 2004; Lindstrom et al., 2003; Reed, 2002). The reasons for this continuous and/or re-blooming capacity are not well understood. However, a number of researchers have expressed several hypotheses to explain such behavior. For instance, re-blooming potential might be due to the elongation of flower-buds that were induced during the current season (i.e., late-summer/fall) and that would

ordinarily bloom next season, but break and bloom due to favorable late-season conditions (Bir and Conner, 2002). Some cultivars may have minimal or no photoperiodic and temperature requirements for flowering and have induced flower buds forming on current season's growth throughout the season. The inductive environment threshold required to initiate flowering appears to vary between cultivars. For instance, floral induction has been observed in terminal buds on new growth of greenhouse grown 'Penny Mac', which were not exposed to short days or cold temperatures (Armitage, Orozco-Obando, and Wetzstein, personal observation). In order to characterize flowering in hydrangea and define re-blooming capacity, it is necessary to determine the flowering potential of *H. macrophylla* cultivars. This can be accomplished by determining when flower induction occurs, defining floral development stages in induced buds, and identifying the spatial distribution of induced buds. The objective of this study was to assess the timing and location of flower initiation and development of lateral buds from four *H. macrophylla* cultivars with re-blooming capacity.

MATERIALS AND METHODS

PLANT MATERIALS. Based on previous studies, four cultivars were selected to determine genotypic differences in flowering initiation and development in plants exposed to normal outdoor inductive conditions. 'Penny Mac', 'Endless Summer', 'Nikko Blue' and 'Madame Emilie Mouillèré' were selected for their re-blooming potential (Adkins, 2001; Adkins and Dirr, 2003; Bir and Conner, 2002; Dirr, 2004; Lindstrom et al., 2003; Wetzstein and Orozco, 2003). Plants were obtained from a commercial grower (McCorkle Nurseries. Dearing, Georgia). The plants were grown in 18 liters plastic containers and maintained outdoors under shade cloth (30% transmittance). Plants were top dressed twice with 45 grams of a slow-release 19N-6P-12K fertilizer formula (Osmocote®, Scotts-Sierra Co., Marysville, Ohio). To control powdery mildew (*Leveillula taurica*), the plants were sprayed with Triforine (Funginex, Ortho. San Francisco, CA). All plants were overhead irrigated as needed.

MEASUREMENTS AND ANALYSIS. Following the resumption of new growth in the spring, individual shoots were marked with latex paint to indicate current season's growth. Plants were destructively harvested at one of four times during the year at periods representing key developmental stages (Table 4.1), with each harvest consisting of 4 plants per cultivar. At each harvest, shoot length of the current season's growth for each plant was measured. Stem diameter was also measured (at the base of each stem). Buds were categorized as terminal vs. axillary. Terminal buds were located at the upper part of the stem and axillary buds were located at each node (Fig. 4.1). Shoot type was ascertained based on the origin of the shoot, i.e., basal, terminal or lateral (Fig. 4.1). For example, basal shoots were those that had their origin at the base of the plant, close to the soil line. Terminal stems were those that broke from the apical bud of the main stem. Lateral shoots originated from the elongation of axillary buds.

To determine lateral floral bud induction, buds greater than 2 mm in length were dissected. Bud scales and leaf primordia were excised with a scalpel to expose the shoot apex. Apices were examined under a stereomicroscope to assess the presence or absence of floral induction, and to rate the developmental stage of each bud, based on a numerical rating system (Table 4.1 & Fig. 3.2) ranging from those that had no floral induction, i.e., a vegetative apex (Stage 1) to those with differentiated floral apices containing flowers with developed sepals (Stage 5).

EXPERIMENTAL DESIGN. For this study, 64 plants were set up in a randomized complete block design. This design consisted of 4 cultivars x 4 reps/cultivar x 4 harvest dates. All data were subjected to analysis of variance and Student Newman-Keuls mean separation (SAS® Institute, 2004. Cary, NC).

RESULTS AND DISCUSSION

FLORAL BUD INDUCTION. Terminal induction was not reported because those buds were exposed to last year's inductive conditions. On the other hand, seasonal changes in lateral flower-bud induction on four cultivars were observed (Table 4.3). The first harvest occurred prior to the onset of cool temperatures (Fig. 4.3). Nonetheless, low to moderate levels of flower induction ranging from 4 to 47% were observed in lateral buds. At Harvest II (October 15), after the plants had been exposed to typical conditions of the fall weather, the percentage of floral buds increased significantly in all cultivars except for 'Nikko Blue'. 'Madame Emile Moullièré' exhibited a 17-fold increase. In contrast, the percentage of floral buds in 'Endless Summer' increased 1.6 fold, but reflects the already high levels of induction observed for this cultivar at Harvest I. By Harvest III when plants abscised their leaves and entered their dormant period (December 15), 'Madame Emile Moullièré' exhibited a significant increase in the percentage of induced buds. No change was observed in the other three cultivars indicating they had reached their maximum level of induction by Harvest II. By Harvest IV (February 15) the percentage of induced buds in 'Madame Emile Moullièré' did not increase, indicating that this cultivar had reached its maximum percentage of induction by Harvest III.

Cultivars differed in percentage of floral induction as indicated by floral stage (Table 4.4). At Harvest I, 'Madame Emile Moullièré', 'Nikko Blue' and 'Penny Mac' showed no significant differences in percentages of induction. However, 'Endless Summer' had a significantly higher percentage of induction, approaching 50%. By Harvest II, with the exception of 'Nikko Blue', all the cultivars had reached a similar percentage of floral induction. 'Nikko Blue' continued to exhibit a lower percentage of induced buds compared to the other cultivars throughout the experiment. At Harvest IV, floral induction in 'Nikko Blue' was only 50-60% of that of the

other cultivars indicating that this cultivar has a lower potential to flower than the other 3 cultivars. These results agree with work that has shown variation in the percentage of axillary induced buds among different cultivars during the dormant period (Orozco-Obando and Wetzstein, 2004).

Several researchers that have evaluated other cultivars under controlled conditions have shown that flower differentiation is influenced by temperature and to a lesser degree by photoperiod. For instance, Wallerstein and Rünger (1985) mentioned that floral induction in hydrangeas occurs at moderate to low temperatures with photoperiodic as a secondary effect. Furthermore, Bailey and Weiler (1984) reported that temperatures above 21º C under continuous light delays floral initiation, and plants remain vegetative and actively growing. On the other hand, Shanks and his colleagues (1986) reported that long photoperiods have a marked effect on shoot extension (increased internodes lengths) and size of the inflorescences. Contrary to the previous findings, in this study the percentage of induced buds prior to lower temperatures and short days demonstrates that re-flowering cultivars meristems can become reproductive even at temperatures reported to delay floral initiation and maintain vegetative growth.

FLORAL BUD DEVELOPMENT. The rate of floral development in lateral buds varied over time (Table 4.3). At Harvest I, induced buds exhibited limited development. Although the apex was broadened, few apices had defined floral primordia. Floral development in ‘Madame Emile Moullièré’ showed an accumulative response as the season progressed. No significant changes were observed at Harvest IV demonstrating that this cultivar reached its maximum floral development at leaf-abscission. In the case of the other three cultivars, all showed significantly more advanced flower development at Harvest II at which time they had reached their maximum level of floral development. Comparing the cultivars to each other

(Table 4.4), at Harvest I, all the induced buds had similar development where meristems were exposed and raised showing a dome shape (floral development stage ranged from 2.0 to 2.3). At Harvest II, the mean floral development in 'Madame Emile Moullièré' was lower than the other three cultivars. 'Penny Mac' and 'Nikko Blue' were not different, and 'Endless Summer' showed a significantly more advanced level of development than other cultivars. By Harvest III, all the cultivars had reached their maximum floral development and had caught up with 'Endless Summer'. At Harvest IV, mean flower stage was the same in three of the cultivars (the exception was 'Nikko Blue').

Although, floral bud induction and development in hydrangea has been compared to that of azaleas (Bailey et al., 1986), hydrangea's patterns are different. According to Criley (1985), azaleas require a total of 10-12 weeks from initiation to development of flowers. At the end of such period (when dormancy occurs), flowers reach a stage of development where they have elongated style and ovaries containing ovules. In order to match those characteristics, by December 15 (when plants reach dormancy), induced hydrangea buds should have reached a high level of floral development (Stage 5). However, the dissected meristems showed flowers beginning to form sepals (Stage 4). None of the cultivars evaluated showed evidence of a significant increase in floral development during the dormant period. Similar patterns of floral development have been described for other crops. In raspberry (*Rubus idaeus* L.), flower initiation and development in axillary buds changed little during late-December and early-January (Williams, 1959). In blackberries (*Rubus sp*), reproductive bud development during the dormancy period varies from one cultivar to another (Takeda et al., 2002). Takeda and Wisniewski (1989) reported that axillary buds from the 'Black Satin' blackberry remained vegetative during the winter months.

STEM TYPE AND PERCENTAGE OF INDUCED BUDS. In hydrangea, current season's growth can be derived from expansion of terminal, lateral or basal buds. The strength of apical dominance in shoots within a genotype will impact the shoot growth patterns. We tried to determine if there was a relation between the stem type and the percentage of induced lateral buds at different sampling periods-harvest time (Table 4.5). Regardless of the type of shoot where the axillary bud was produced (i.e., basal, lateral or terminal stems), there were no significant differences in the percentage of induced buds from the different stems. In terms of harvest time, the only cultivar that behaved differently was 'Nikko Blue'. This cultivar showed significant variation among stem types at Harvest III. Terminal stems demonstrated a greater percentage of induced buds followed by lateral and basal shoots, respectively. The variation for this isolated case is hard to explain in terms of biological factors. However, a vigorous terminal stem's growth might out-compete the other types of shoots for spatial distribution (light) and carbohydrate allocation (stronger sink). Consequently, buds located on those stems might have had greater stimulus and resources for floral induction. Knowing the spatial differentiation can be of great importance in horticultural practices, particularly as it relates to pruning. For example, Haworth-Booth (1984) recommended the removal of basal shoots from free-flowering cultivars to prevent the plant's energies from being expended or sending up too thick a sheaf of young shoots from the base. By removing the basal shoots, the nourishment that would have gone to many buds would now be invested in fewer shoots that would now hold a better chance to develop, mature and survive the cold temperatures of the following winter. Since, the data suggest that stem-type does not have any effect on the percentage of induced buds (for most of the cultivars), the evaluation of changes in cultivar practices (e.g., pruning) should focus on the timing rather than the spatial distribution and/or type of stems pruned.

Re-flowering hydrangea cultivars have the ability to produce multiple axillary flower buds along the stem (Orozco-Obando and Wetzstein, 2003). In some cases, every branch can carry several corymbs (inflorescences) and new shoots can flower during the same season (Haworth-Booth, 1984). Our studies indicated that the re-blooming capacity can be due to minimal inductive conditions required for some cultivars (e.g., 'Endless Summer') and the induced buds can reach advanced levels of floral development. The breaking of buds with high levels of floral development would allow the plant to remain flowering for an extended period of time. The information provided by this study could aid the industry and gardeners in developing cultural practices (chemical treatments or pruning practices) to help promote lateral bud-break throughout the growing season; thereby, enhancing the production of flowers and extending the blooming season.

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Table 4.1. Harvest times determined by developmental characteristics in *H. macrophylla* cultivars.

Harvest	Date	Characteristics of shoots
I	August 15	Summer collection. Shoots expansion is complete. Axillary buds have develop with little or no inductive conditions received
II	October 15	Late-summer/fall collection. Shoots had been exposed to short days and cooler temperature
III	December 15	Late-fall collection. Leaves have abscised, buds are dormant
IV	February 15	Late-winter collection. Just before bud break. Some bud swell may have occurred

Table 4.2. Stages of *H. macrophylla* inflorescence development.

Stage	Characteristics
1	Vegetative apex. Meristem is covered by the upper pair of leaf primordia
2	Transition to a floral apex. Apex is broadened, swollen, dome-shaped and the upper pair of leaf primordia are separated
3	Individual floral primordia are defined within the inflorescence
4	Sepal primordial are initiated on flowers
5	Florets are well-defined; sepal primordia enclose the floral apex

Table 4.3. Effect of harvest date on percent floral induction and floral stage for induced lateral buds.

Harvest	% Floral buds				Floral stage of induced buds			
	Madame	Penny	Nikko	Endless	Madame	Penny	Nikko	Endless
I	4 c [y]	17 b	12 b	47 b	2.0 c	2.0 b	2.3 b	2.3 b
II	67 b	62 a	36 ab	76 a	2.9 b	3.2 a	3.2 a	3.5 a
III	82 a	80 a	56 a	94 a	3.6 a	3.7 a	3.2 a	3.7 a
IV	83 a	80 a	48 a	92 a	3.7 a	3.6 a	2.9 a	3.5 a

[y] Means (within columns) with the same letter are not significantly different (Student Newman - Keuls $\leq$ 0.05).

Table 4.4. Effect of cultivar on percent floral induction and floral bud stage.

Cultivar	% Floral buds				Floral stage of induced buds			
	I	II	III	IV	I	II	III	IV
Madame	4 b [y]	67 a	82 ab	83 a	2.0 a	2.9 c	3.6 a	3.7 a
Penny	17 b	62 a	80 ab	80 a	2.0 a	3.2 b	3.7 a	3.6 a
Nikko	12 b	36 b	56 b	48 b	2.3 a	3.2 b	3.2 a	2.9 b
Endless	47 a	76 a	94 a	92 a	2.3 a	3.5 a	3.7 a	3.5 a

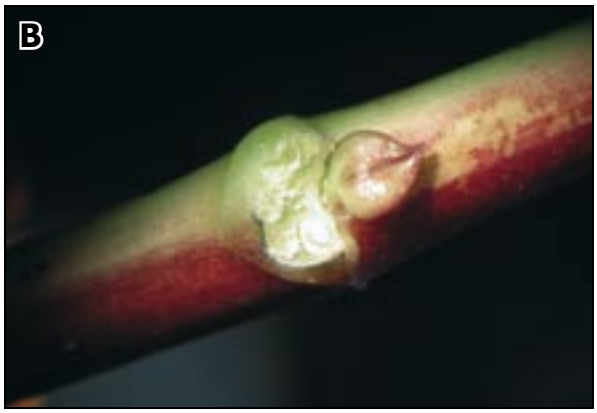
[y] Means (within the column) with the same letter are not significantly different (Student Newman - Keuls ≤ 0.05).

Table 4.5. Effect of stem type on percentage floral induction for four cultivars of *H. macrophylla* cultivars at different harvest dates.

Cultivar	Stem type	Harvest			
		I	II	III	IV
Madame E.	**Basal**	3 a [y] (6) [z]	61 a (3)	71 a (7)	77 a (10)
	Lateral	6 a (11)	72 a (10)	88 a (13)	94 a (15)
	Terminal	3 a (12)	75 a (19)	82 a (17)	72 a (17)
Endless Summer	**Basal**	71 a (4)	72 a (5)	-- [z]	94 a (13)
	Lateral	49 a (35)	69 a (36)	97 a (66)	93 a (73)
	Terminal	52 a (32)	86 a (31)	100 a (24)	83 a (14)
Nikko Blue	**Basal**	19 a (5)	42 a (8)	39 c (5)	45 a (11)
	Lateral	15 a (12)	42 a (42)	64 b (46)	53 a (44)
	Terminal	7 a (12)	48 a (19)	78 a (17)	68 a (11)
Penny Mac	**Basal**	-- [z]	74 a (4)	86 a (4)	69 a (4)
	Lateral	12 a (11)	42 a (19)	70 a (14)	70 a (25)
	Terminal	21 a (15)	65 a (17)	87 a (19)	93 a (8)

[y] Means with the same letter within a column are not significantly different (Student Newman Keuls $\leq$ 0.05).
[x] The number in parenthesis represents the number of stems.
[z] Data not included because quantity was very low.


A


B


C


D


E

Fig. 4.1. Pictures of *Hydrangea macrophylla* shoot and bud types. **A**. Terminal bud. **B**. Axillary bud. **C**. Basal shoot breaking. **D**. Terminal shoot. **E**. Lateral shoot.

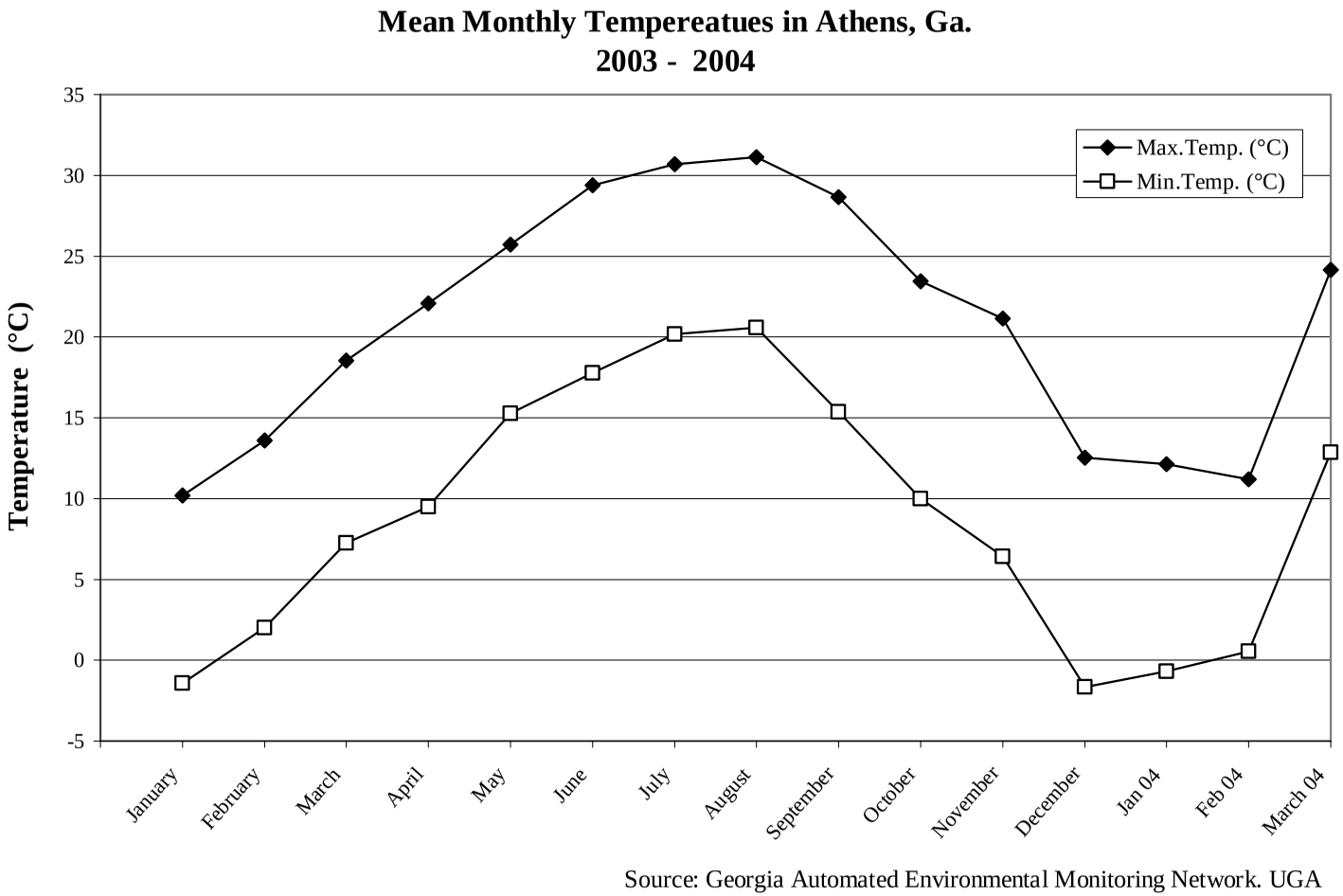


Fig. 2.3. Minimum and maximum mean monthly temperatures in Athens, Georgia (2003-2004).

Fig. 4.2. Minimum and maximum mean monthly temperatures in Athens, Georgia (2003- 2004).

CHAPTER 5

EFFECT OF PRUNING TIME ON GROWTH AND DEVELOPMENT OF RE-FLOWERING *HYDRANGEA MACROPHYLLA* CULTIVARS[1]

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Subject Area: Developmental Physiology

Effect of Pruning Time on Growth and Development of Re-flowering *Hydrangea macrophylla* Cultivars

ADDITIONAL INDEX WORDS: Re-blooming Hydrangea, Pruning, Cultural Practices, Big-leaf Hydrangea, Hortensia.

ABSTRACT

Prudent and timely pruning practices can bring out the best in most ornamental plants by improving their health and shape. Nevertheless, in the case of *Hydrangea macrophylla* shrubs, the topic of pruning is surrounded by controversy and in some cases; improper pruning may result in a flowerless season. An alternative to avoid this problem may be the use of cultivars that have the potential for re-flowering and/or cultivars that have the potential to produce a second flush of inflorescences. The objective of this study was to determine growth and development of re-flowering *Hydrangea macrophylla* cultivars after pruning at 3 different dates. Four re-flowering cultivars, 'Penny Mac', 'Endless Summer ™', 'Nikko Blue' and 'Madame Emile Mouillèré' were included in the research. Containerized plants were grown outdoors and pruned in either late-summer (August 15), autumn (October 15) or late-fall (December 15). On December 15, all plants were moved into a heated glasshouse to be forced. Plants were evaluated at the time of transfer into the greenhouse and again in February and May. Stems were characterized based on the length, caliper, number of nodes, origin, number and types of inflorescences (mature, young, visible-bud, faded, dead or none). At the different evaluations,

total growth and number of stems from ‘Endless Summer’ and ‘Madame Emilie Mouillèré’ did not show signs of being affected by the different pruning times. On the contrary, ‘Penny Mac’ exhibited greater growth after the late-summer pruning but no differences in terms of number of stems. At the May evaluation, the total growth and number of stems of ‘Nikko Blue’ seemed to have been stimulated by the late summer pruning. Total number of inflorescences varied among cultivars and evaluations periods. ‘Madame Emilie Mouillèré’ and ‘Nikko Blue’ did not show differences at the different evaluations/pruning times. At the February evaluation, ‘Endless Summer’ showed a number of inflorescences after having been pruned in late-summer. At the same evaluation date, ‘Penny Mac’ showed a higher production of inflorescences after the late-fall pruning than any of the other pruning dates/evaluation times. Cultivars with re-blooming capacity such us: ‘Penny Mac’ and ‘Endless Summer’ appear to have a greater potential to recover from improper pruning, late-spring frost and/or mechanical damage of induced terminal buds. Consequently, their use should be encouraged especially for areas where environmental conditions reduce the flowering reliability of the crop.

INTRODUCTION

Prudent and timely pruning can bring out the best in most ornamental plants by improving their health, shape and, ultimately resulting in plants that require less maintenance. Nevertheless, in the case of pruning *Hydrangea macrophylla* shrubs, the topic is surrounded by controversy. For instance, Haworth-Booth (1984) commented that in general, hard-pruning of established hydrangeas does more harm than good and may easily be fatal. In the industry discrepancies exist about proper pruning requirements among different cultivars (Conwell et al., 2002). In order to schedule proper pruning practices for this species, a basic knowledge of the origin of the plant, the physiology of its flowering and cultivar variation must be contemplated. Although there are some tropical evergreen species, most of the cultivated species of hydrangea are from temperate regions and they have a deciduous habit. The largest center of distribution of the genus can be found in eastern Asia (McClintock, 1957) and different species can be found scattered in countries such as: China, Japan and, Vietnam (van Gelderen and van Gelderen, 2004). However, true *H. macrophylla* is limited to some areas of Japan (Dirr, 2004). On the other hand, Haworth-Booth (1984) mentioned that cultivated hydrangea (*Hydrangea macrophylla*) includes a wide range of garden hybrids that combines genetic material from wild maritime species as well as other woodland species. In addition, he suggested, that the genetic input from inland species, might account for the lack of cold-hardiness of some hydrangeas.

Adkins (2001) reported that hydrangeas thrive in maritime regions and in regions where winter temperatures remain above 23° C, in which case the plant grows and flowers. However, in most of the hydrangea cultivars new growth is very susceptible to late-spring frost (Adkins et al., 2002; Reed, 2002). For example, -4° C or lower temperatures causes browning of flower buds, leaf, and stem tissue, and may result in a flowerless season (Church, 1999; Dirr, 2004).

Miller (1998) reported that varieties designated as suitable for outdoor use may be bud-hardy in Zone 6. As a consequence, this ornamental cannot be successfully marketed as a reliable flowering shrub in areas prone to late-spring frost. In addition to cold damage, Armitage and Laushman (2003) mentioned that induced buds are also sensitive to mechanical damage (i.e., deer problems and/or careless pruning).

To improve pruning practices genotypic variation should be taken into consideration as well as the patterns of floral initiation and development. For example, the common conception is that floral induction occurs in the terminal buds (Shanks et al., 1986, Zhou and Hara, 1989) on the previous year's growth (Bowman-Price, 1999; Shanks et al., 1986; van Gelderen and van Gelderen, 2004). However, recent studies have demonstrated that axillary buds can also be induced along the entire stem (Wetzstein and Orozco, 2003). In addition, several reports have documented genotypic variability in the threshold of temperature and day length required to initiate flowering (Bailey and Weiler, 1984; Guo et al., 1995; Litlere and Strømme, 1975). Recent research (Orozco-Obando et al., 2005a) on floral induction and development in hydrangeas has reported the initiation of reproductive meristems under environmental conditions previously cited as promoters of vegetative growth (i.e., high temperatures). In addition, the occurrence of inflorescence development on newly formed shoots has been observed (Armitage, Orozco-Obando and Wetzstein, 2003. Personal observations).

Pruning hydrangeas is not a well-documented topic. For instance, the current literature on pruning hydrangeas is limited to specific species other than *macrophylla*, e.g., *H. paniculata, H. arborescens, H. quercifolia* (Bowman-Price, 1999; Dunwell et al., 2001). van Gelderen and van Gelderen (2004) stated that *H. macrophylla* does not respond well to pruning and pruning on a yearly basis would result in a shrub that hardly ever produced flowers. In opposition to the

previous stance, it is believed that correct pruning of *H. macrophylla* should increase flower production and stimulate the production of longer stems (Conwell, 2002). Pruning time and severity depends on the objective of applying this cultural practice. For example, for regular maintenance it has been suggested to prune the shrub in summer after it blooms (Medic, 1995). To remove old stems and dead inflorescences, Haworth-Booth (1984) recommended pruning the plant in late-winter/early-spring, before growth begins. To rejuvenate overgrown big-leaf hydrangeas, Medic (1995) recommended removing one-third of the oldest growth and cutting the remaining stems to a few inches above the ground during the summer immediately after the plant has bloomed. Conwell et al. (2002) evaluated the effects of pruning cultivars in two different ways: cut back to old wood (half) and 2-3 inches from the soil line (renewal). Plants pruned back to old wood produced 15% more inflorescences than renewal pruning and 23% more than no pruning.

Most of the literature available only accounts for pruning practices in popular varieties and old cultivars. However, there is much discrepancy in the industry about proper pruning requirements among different cultivars (Conwell et al., 2002). Furthermore, only a few authors have referred to the response of cultivars with re-flowering potential as it relates to different pruning times. The objective of this study was to determine the response of four re-flowering *Hydrangea macrophylla* cultivars (‘Penny Mac’, ‘Nikko Blue’, ‘Endless Summer’ and, ‘Madame Emile Moullièré’) after having been pruned on 3 different dates (late-summer, fall or late-fall).

MATERIALS AND METHODS

Based on previous studies, four cultivars with re-flowering potential were considered: 'Penny Mac', 'Endless Summer', 'Nikko Blue' and 'Madame Emile Mouillèré'. Twenty plants of each cultivar were obtained from a commercial grower. The plants were grown in 18 liter plastic containers and maintained outdoors under shade-cloth (45% transmittance). Plants were top-dressed twice with 45 grams of a slow-release 19N-6P-12K fertilizer formula (Osmocote®, Scotts-Sierra Co. Marysville, Ohio) to control Powdery Mildew (*Leveillula taurica*); the plants were sprayed with Triforine (Funginex, Ortho. San Francisco, CA.) as needed. All plants were overhead irrigated as needed. For this study, 64 plants were set up in a randomized complete block. This design consisted of 4 cultivars x 4 reps (pots) per cultivar x 3 pruning dates. The pruning times were defined as late-summer (August 15), autumn (October 15) and late-fall (December 15). After each pruning, the plants were placed back in the shade-house where they were maintained until the last week of December, by which time the plants had reached a dormant stage (abscised leaves). After leaf abscission (third week of December), the plants were brought into a double-layer polyethylene-covered greenhouse to be forced. The pots were placed on greenhouse benches and irrigated on a regular basis. The plants were top-dressed with 45 grams of a slow-release 19N-6P-12K-fertilizer formula (Osmocote®, Scotts-Sierra. Marysville, Ohio) and sprayed with Triforine (Funginex, Ortho. San Francisco, CA) as needed. In late-March, a frame was constructed over the bench and covered with a 30% shade-cloth to provide protection to the plants from the late afternoon sun. To evaluate general performance of the plants to the pruning dates (growth, number of stems and number of flowers), plants were evaluated three times: before forcing (December 20), second week of February and first week of May. It should be noted that the last evaluation coincided with a commercial marketing period.

Bailey (1992) and Midcap (2003) mentioned that growers forced hydrangeas for early spring sales and Mother's Day market (May). At each evaluation, stems were characterized based on the length, caliper, number of nodes, and origin. Presence or absence of a terminal flower was noted according to the development of the inflorescence and placed into 5 categories: non-visible flower-bud, visible flower-bud, young inflorescence, mature inflorescence and faded/senescent inflorescence (Fig. 5.1).

All data was subjected to descriptive statistical analysis (Microsoft Office 2003 Excel version 11), analysis of variance and Student Newman-Keuls' mean separation test (Statistical Analysis Software. SAS Institute, N.C).

RESULTS AND DISCUSSION

Total growth of 'Endless Summer' and 'Madame Emile Mouillèré' did not show signs of being affected by the different pruning times at any harvest (Table 5.1). On the other hand, despite small but significant differences, 'Penny Mac' responded best to late-summer pruning. 'Nikko Blue' exhibited greater total growth in May after being pruned in late-summer. In terms of number of stems, 'Endless Summer', 'Madame Emile Moullièré' and 'Penny Mac' did not appear to have been affected by pruning dates. At the May evaluation, 'Nikko Blue' showed a greater number of stems from the late-summer (August 15) pruning. These results support the findings of Conwell et al. (2002) who reported that pruning 'Nikko Blue' in August produced the most visually appealing plants in comparison with plants pruned at different times (May through September). Visually appealing evaluation was based on flower number and quality from a retail customer's viewpoint (Conwell et al., 2002).

In our study, number of inflorescences varied among cultivars and evaluation periods. 'Madame Emile Mouillèré' and 'Nikko Blue' did not show differences at the different evaluation/pruning times. At the February evaluation, 'Endless Summer' showed a significant amount of inflorescences after have been pruned in late-summer. On the same evaluation date, 'Penny Mac' showed a greater production of inflorescences after the late-fall pruning in comparison with the other pruning dates.

Proper pruning not only helps to rejuvenate a plant (Haworth-Booth, 1984), but also increases the number of inflorescences and length of the stems (Conwell et al., 2002). Nonetheless, the appropriate pruning depends on time of the year and the cultivar's propensity to set multiple lateral floral buds and to re-bloom. The first and most common form of pruning *H. macrophylla* cultivars is dead-heading (Lawson-Hall and Rothera, 1995). Dead-heading is implemented at the beginning of the spring, before growth is initiated and when the possibilities for late-spring frosts have been reduced.

Cultivars with re-blooming capacity are less affected by improper pruning techniques and/or mechanical damage than cultivars without re-blooming potential. Haworth-Booth (1985) recommended pruning non-reflowering cultivars in the summer. He argues that those cultivars contain a stronger infusion of the woodland species and they do not have the capacity to flower from side shoots if the terminal bud is winter-killed, and.l even if the terminal bud is not damaged, it will not always flower. Summer pruning usually is implemented after the plant has flowered. Pruning at this time removes apical dominance allowing other buds (axillary and/or basal buds) to expand and the plant's energy will be invested on the new growth rather than the production of seeds. Summer pruning allows the new growth enough time to mature and for some cultivars, will allow axillary buds to be induced. Cultivars with re-blooming capacity have

a greater potential to recover from an improper pruning, late-spring frost and/or mechanical damage of induced terminal buds. According to Haworth-Booth (1984), these types of plants can bloom even if only 5–10 cm of old wood survives. Consequently, their use should be encouraged especially in areas where environmental conditions reduce the flowering reliability of the crop.

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Table 5.1. Effect of pruning date on total growth, number of stems and inflorescences on four *H. macrophylla* cultivars.

Evaluation	Pruning date	Cultivars					
		'Endless Summer'			'Madame Emile Moullièré'		
		Total Growth [w x] (cm)	Stem number [y]	Flower Number [z]	Total Growth (cm)	Stem number [z]	Flower number
December	Late-summer	133 a	13 a (49)	6 ab	198 a	11 ab (44)	1.0 a
	Fall	27 a	12 a (46)	1 b	63 a	6 b (23)	1.0 a
February	Late-summer	152 a	21 a (81)	8 a	139 a	26 a (105)	1.0 a
	Fall	40 a	14 a (55)	5 ab	113 a	11 ab (42)	2.0 a
	Late-fall	62 a	14 a (56)	6 ab	165 a	16 ab (62)	4.0 a
May	Late-summer	157 a	19 a (77)	6 ab	142 a	25 ab (100)	8.0 a
	Fall	52 a	13 a (53)	6 ab	135 a	12 ab (46)	4.0 a
	Late-fall	86 a	15 a (60)	5 ab	166 a	13 ab (50)	5.0 a

		'Penny Mac'			'Nikko Blue'		
		Total Growth [wx] (cm)	Stem number [y]	Flower number [z]	Total Growth (cm)	Stem number	Flower number
December	Late-summer	212 ab	10 a (39)	3 ab	123 b	9 b (34)	1.0 a
	Fall	74 c	6 a (24)	3 ab	43 b	7 b (27)	1.0 a
February	Late-summer	220 ab	19 a (76)	3 ab	184 b	25 ab (76)	0.3 a
	Fall	103 bc	12 a (48)	4 ab	84 b	14 ab (56)	3.0 a
	Late-fall	61 c	16 a (62)	8 a	88 b	12 ab (50)	3.0 a
May	Late-summer	247 a	19 a (77)	1 b	287 a	30 a (90)	1.0 a
	Fall	133 abc	12 a (48)	1 b	63 b	9 b (37)	0.3 a
	Late-fall	68 c	14 a (54)	4 ab	97 b	13 ab (40)	1.0 a

[w] Total growth values were obtained by adding all the lengths of the different stems (cm).
[x] Means with the same letter are not significantly different (Student Newman-Keuls ≤ 0.05).
[y] Number in parenthesis represents the total number of stems.
[z] Flower number represents the mean value of total mature inflorescences, young inflorescences, and visible buds.

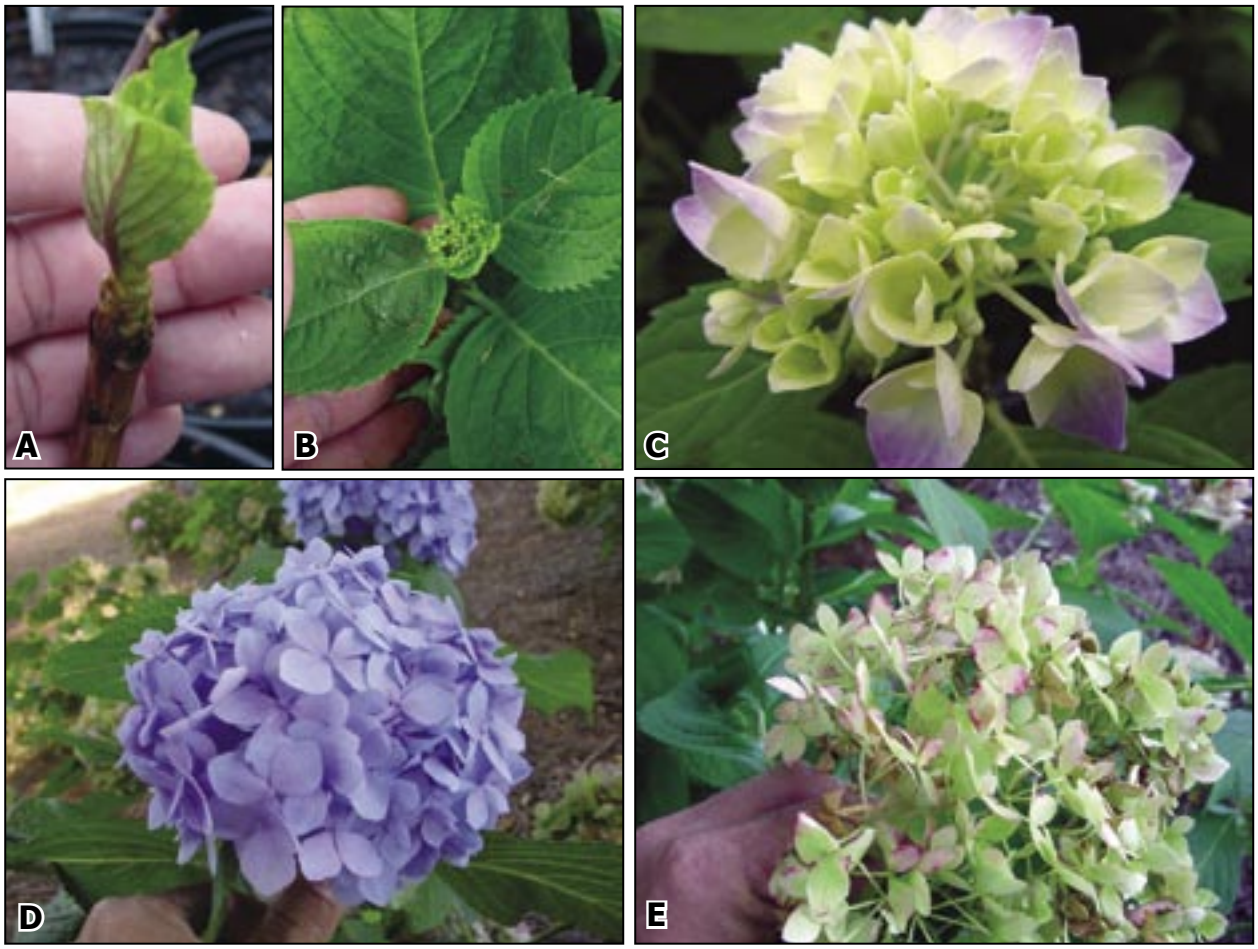
A
B
C
D
E

Fig. 5.1. Pictures of *Hydrangea macrophylla* inflorescence categories. **A**. Non-visible flower. **B**. Visible flower bud. **C**. Young inflorescence. **D**. Mature inflorescence. **E**. Faded/senescent inflorescence.

CHAPTER 6

CONCLUSIONS

The general doctrine of flowering in *Hydrangea macrophylla* (Thunb.) Ser. is that floral induction occurs during the previous season on last year's growth and usually in the stem's terminal bud. However, it has been noted that hydrangea cultivars widely differ in their relative abundance and duration of flower production. The objectives of this study were to determine how developmental flowering patterns compared among different genotypes, to characterize the seasonal and spatial patterns of floral initiation and development in cultivars with re-flowering capacity and, to determine the effects of different pruning time on growth and development of such cultivars.

The first study allowed me to determine that flower development was very consistent in terminal buds, and occurred in 100% of the terminal buds for all of the cultivars evaluated with the exception of 'Ayesha' (33%). In contrast, lateral buds showed a wide variation in flower development among different genotypes. For example, 'Ayesha', 'Blushing Pink', 'Freudenstein', and 'Nigra' had 10% or fewer lateral buds with floral initials. 'All Summer Beauty', 'David Ramsey', 'Kardinal', 'Masja', and 'Nightingale' showed high levels of floral induction (> 92% of lateral buds induced). Within a cultivar, flower development was more advanced in terminal than lateral buds. I also found a correlation between bud size (length) and floral stage development for some cultivars. However, lower r-square values suggest that there are other biological factors that influence or are correlated with floral induction that should be considered.

In the second study, floral primordia were found to be initiated within axillary buds at the first sampling period, with levels of induction varying among cultivars from 4% in 'Madame

Emilie Mouillèré' to 47% in 'Endless Summer'. This data suggests, that some cultivars may have minimal or no photoperiodic/temperature requirements for flowering. Most cultivars reached their maximum percentage flowering at Harvest II except for 'Madame Emilie Mouillèré'. No significant floral development occurred during winter. Stem type had no effect on the percentage of induced buds.

In the third study, total growth and number of stems from 'Endless Summer' and 'Madame Emilie Mouillèré' did not show signs of being affected by the different pruning times at any evaluation. However, 'Penny Mac' exhibited greater growth after late-summer pruning than the other pruning times, but no differences in number of stems. 'Nikko Blue' plants evaluated in May showed that late-summer pruning may stimulate its total growth and number of stems. Total number of flowers (mature, young and visible bud) varied in cultivars and evaluation periods. 'Madame Emilie Mouillèré' and 'Nikko Blue' did not show differences at the different evaluation/pruning times. At the February evaluation, 'Endless Summer' showed a significant increase in flowers after having been pruned in late-summer. At the same evaluation, 'Penny Mac' showed a higher production of flowers after the late-fall pruning. Despite the variation in the cultivars responses, the data suggests that the late-summer pruning provided the plants enough time to develop stems that would be better adapted for the winter dormancy period. Consequently, an increase in flower production and a better looking plant can be expected for the next flowering season.

The variation in floral induction and development in terminal and lateral buds may provide insight into causes for differences in the amount and duration of flowers produced within a growing season. In addition, the minimal requirements for floral inductive conditions shown by re-flowering cultivars, as well as, the high percentage of floral induced lateral buds, suggest

that these cultivars will be more amenable to recovery from winter injury, improper pruning and/or mechanical damage of terminal buds. These characteristics could provide opportunities for the development of cultural practices (i.e., pruning, chemical treatments) to remove apical dominance of terminal buds. As a result, the lateral induced buds would have the opportunity to elongate enhancing the potential of higher floral production as well as a more extended blooming season. Finally, re-flowering cultivars represent a great outdoor plant especially for areas where environmental conditions reduce the flowering reliability of the crop.