To my grandmothers

AN ABSTRACT OF THE DISSERTATION OF

Chantalak Tiyayon for the degree of Doctor of Philosophy in Plant Physiology presented on October 15, 2008.

Title: A Microscopic and Phenological Study of Pollen Development and Bloom in Selected Cultivars of Hazelnut (*Corylus avellana*)

Abstract approved:

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Anita N. Azarenko

Pollen development is an important process in male flower development, the timing of which may be correlated with time of pollen shed in hazelnut (*Corylus avellana* L.). Early to very late blooming cultivars were identified and the relationship of microsporogenesis and microgametogenesis, and time of pollen shed were studied in nine hazelnut cultivars. Most advanced catkins from a single tree of each cultivar were collected each week from 4 Aug. to 6 Dec. 2002, and on 17 Jan. 2003, stained and analyzed by light microscopy. The phenology part of this dissertation studied the role of the chilling requirement as chill units (CU) and heat requirement as growing

degree hours (GDH) in pollen shed. Hazelnut twigs of three cultivars; ‘TGDL’, ‘Barcelona’, and ‘Hall’s Giant’ were collected at weekly intervals starting from early Fall 2006 through the time of anthesis in the field in winter 2007. Twigs were then held at a different constant temperature 0, 5, 10, 15, or 20 ^{o}C. Observing these twigs weekly, the time of anthesis (50% pollen shed) was recorded. A parallel study was conducted in more controlled conditions by collecting hazelnut twigs of the same three cultivars on 1 Nov. 2006 and holding them at 5 ^{o}C in a cold room. Five twigs of each genotype were brought out to room temperature at 5-day intervals in order to force them to bloom. Numbers of catkins that shed pollen were recorded every 5 days. From the results, we propose a model of hazelnut staminate flower development that begins with catkin differentiation concurrent with early stages of pollen development. Catkin length increased steadily and reached a lag phase at the end of microsporogenesis. While there is no external change, microspores continue microgametogenesis, the catkins are endodormant, unable to be induced to shed pollen, and accumulate chilling during this period of time. We propose that the chilling requirement is met when pollen reaches the mature stage and when catkins are in the latter stages of the lag phase of growth. At this point, GDH accumulate and catkins become ecodormant. As chilling continues to accumulate, the amount of GDH required for pollen to shed is reduced. A similar quantity of GDH was required for all genotypes from pollen grain maturity to catkin elongation.

A Microscopic and Phenological Study of Pollen Development and Bloom in Selected Cultivars of Hazelnut (*Corylus avellana*)

by

Chantalak Tiyayon

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Chantalak Tiyayon, Author

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CHAPTER 1

GENERAL INTRODUCTION

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1.1. Biology of Hazelnut

1.1.1. Taxonomy

Hazelnut is categorized in the Genus *Corylus*, Family Betulaceae (the birch family) (Lagerstedt, 1975; Thompson et al., 1996), or previously Family Corylaceae (the hazel family) (Menninger, 1977; Rosengarten, 1984). The name *Corylus* has originated from *korys* in Greek meaning a helmet, hood (Rosengarten, 1984), or bonnet (Lagerstedt, 1975), to which the husk resembles.

There are about 15 major species in this genus, all of which have edible nuts, spread across the temperate zones of North America, Europe, Northern Africa, and Asia (Menninger, 1977). In the United States, native species are *C. americana* Marsh. (American filbert), and *C. cornuta* Marsh. or *C. rostrata* (beaked filbert) (Rosengarten, 1984). Indigenous to Turkey and the Balkans is *C. colurna* L. (the Turkish tree hazel). The species grown in commercial orchards is European hazelnut, *C. avellana* (Thompson et al., 1996).

Hazelnuts have many names around the world. For example, “Avellana” in Spanish, “Aveleira” in Portuguese, “Bunduq” in Arabic, “Findik” in Turkish, “Haselnuss” in German, “Hasselnöt” in Swedish, “Hazelaar” in Dutch, “Lesnoi Orekh” in Russian, “Nocciola” in Italian, “Noisette” in French,

“Hashibami” in Japanese, and “Chên Tzu” in Chinese (Rosengarten, 1984). However, the term “hazelnut” is used worldwide for many *Corylus* species. There are other common names such as hazel, cob, cobnut, lambert nut, Lombardy nut, Spanish nut (Lagerstedt, 1975), and other scientific names that were given based on appearance, shape, husk, and origin (Bunyard, 1920).

In Oregon, hazelnuts have commonly been called filberts. The name “filbert” may have come from “full beard”, or *Vollbart* in German (Rosengarten, 1984), which refers to the long-leafy husk around the nut. It may also have evolved from St. Philibert’s Day that occurs on 22 Aug. which is coincident with the time the nuts start to ripen (Lyle, 2006). Also in the past, the “filbert” was often used for varieties where the husk is longer than the nut. The term “cob” referred to those nuts where the husk was about the same length as the nut. “Hazel” was the term for varieties where the husk was shorter than the nut (Menninger, 1977; Lyle, 2006).

1.1.2. Morphology

Reed (1976), as cited in Handbook of Nuts (Duke, 1989), gave a description of *C. avellana* as a deciduous shrub or small tree. Trees are generally up to 6 m tall and often thicket-forming. A very old, untrained, *C. avellana* tree in the northwestern U.S. was reported to be 8 m in height and 10 m in canopy diameter (Lagerstedt, 1975). Twigs are dark brown, smooth, with glandular-hair. Leaves are 5 to 12 cm long, orbicular, long-pointed, and hairy on both surfaces with double serrated-margins. Catkins, staminate

inflorescences, are 2-8 cm long, pendulous, and appear in clusters of 1 to 4 (Reed, 1976). Staminate catkins are borne at nodes on one year wood (Germain, 1994). Catkins are composed of 130-290 flowers; each has one bract and two bracteoles (Germain, 1995). There are four filaments bearing eight anthers in each catkin bract [Trotter, 1947 (in Italian) cited by Lagerstedt, 1975]. Pistillate flowers are bud-like, erect, and approximately 5 mm long. Fruits occur in clusters of one to 4. Nuts are 1.5-2 cm in diameter with brown color, and are enclosed by deeply lobed and irregularly toothed bracts as of variable length. Flowers bloom from January to March while nuts mature in the fall (Reed, 1976).

Hazelnuts are distinctive from other orchard crops because they are monoecious and anemophilous (wind pollinated), and bloom in midwinter (December to March in the northern hemisphere) (Germain, 1994). Each catkin produces 4 million (Kelley, 1980) to 40 million (Pisani et al., 1968) pollen grains. However, plants are generally self-incompatible. Most of the cultivars are also dichogamous. The staminate and pistillate flowering times may not overlap, and cultivars can be either protogynous or protandrous. Therefore cross-pollination with compatible pollinizers that shed pollen when female flowers are receptive is required for optimal nut production (Lagerstedt, 1975).

Hazelnut pollen is triangular or ellipsoidal in shape. It has three germinative pores, each located over a small bump in the pollen wall, or over a

small vacuole in some cases. The approximate size of pollen is 25-40 µm in diameter (Trotter, 1947 cited by Lagerstedt, 1975; Germain, 1995).

1.1.3. Staminate Flower Development

In France, the first sign of catkin differentiation occurs in mid-May, and a month later catkins are visible in the leaf axils. Free pollen grains are observed inside the anthers around mid-August (Germain, 1995). In Oregon, 'Barcelona' hazelnut catkins can be seen in the axils of basal leaves on the current season's stem in late June. Catkins grow rapidly in the first three months and then stay in a lag phase for approximately 10 weeks. After that, growth resumes until anthesis (Lagerstedt, 1975). Catkin abscission in the fall prior to anthesis has been observed at high temperatures (~25°C or greater) when catkins are presumed to be dormant (Woodroof, 1979; Mehlenbacher-personal communication).

Dimoulas (1979) did a comprehensive microscopic study on pistillate and staminate flower bud development of three hazelnut cultivars ('Ronde du Piemont' (TGDL), 'Fertile de Coutard' or 'Barcelona', and 'Merveille de Bollwiller' or 'Hall's Giant') in Bordeaux, France, from May to September. He found that male inflorescence differentiation starts early in the season and the process takes less than three months. In his study, catkin development was categorized into eight stages:

I. No sign of catkin differentiation in the apical meristem during the first half of May;

II. Initiation of the first catkin's bract inside the axial bud which becomes convex and the outline of the catkin evolves between 15 and 20 of May;

III. Catkin elongation inside the bud, open passage at the top between the scales of the bud, and the contour of the secondary catkin or future bud can be observed in mid-June;

IV. Emergence of catkins (which have reddish color at the tip) from the bud as a result of pedicel elongation that occurs during the second half of June;

V. Exiting of catkins out of the buds (approximately 10 mm long), initiation of the bracteoles inside the bracts, and gradual extension of the basal bracts to those at the tip of the catkin at the end of June and the beginning of July;

VI. The initiation of anthers, outline of anthers begins at the base of the catkin during the first and second week of July;

VII. Appearance of pollen mother cells (PMCs): anthers are completely formed and pollen sacs are seen with sporogenous cells in the middle happened on the third week of July; and

VIII. Formation of tetrads and pollen grain: meiosis occurs approximately two weeks after the appearance of the PMCs (the end of July to mid-

August). At this time, the catkins are 2-3 cm long. The formation of the pollen grains starts from the basal bracts to the top bracts of the same catkin.

There was roughly a week difference in the timing of these developmental processes among the three cultivars used in his study.

The process of microsporogenesis in hazelnut has not yet been observed in detail nor do we know if the timing of the various stages of pollen grain maturation is different between early, mid, and late pollen shedding cultivars.

1.2. Pollen Development

A review by Scott et al. (2004) stated that unlike most plants organs, which derive from meristem, the development of the anther is unusual in that the microsporangia (anther) arise from a single archesporial cell.

Pollen development consists of two processes, microsporogenesis and microgametogenesis. The former is the formation of microspores and the latter is the development of microspores into pollen grains.

1.2.1. Microsporogenesis

Microsporogenesis can be divided into two major phases, the formation of pollen mother cells (PMCs) which are also called microsporocytes or microspore mother cells, and the development of microspores from PMCs through meiosis.

Pollen mother cell formation: Davis (1966) described the general process of anther wall development and microsporogenesis in angiosperms. The initial periclinal division of archesporial cells forms the primary parietal cells on the outer portion and the sporogenous cells on the inner portion of the microsporangium. The primary parietal layer then divides periclinally, resulting in two secondary parietal layers, while the sporogenous cells enlarge into PMCs. The outer secondary parietal layer divides periclinally, forming an endothecial layer on the outside and a middle layer on the inside. The inner secondary parietal layer develops into the middle layer on the outside and the tapetal layer on the inside. However, anther wall layers in different plant species may develop differently, which would result in a different number of endothecial layers, middle layers, and tapetal layers.

There are two major types of tapetum, the glandular or secretory and the amoeboid tapetum. The glandular tapetum builds up endoplasmic reticulum and dictyosome-derived vesicles while the PMCs are in prophase. The tapetum begins to break down after meiosis, releasing its lysed cell walls and disintegrating cytoplasm into the locule. The amoeboid tapetum protoplasts stay intact when tapetal walls are lysed and intrude among the developing pollen grains (Esau, 1977). Of the 231 plants families listed by Davis (1966), 181 families have glandular tapetum, including the genus *Corylus*. Clément et al. (1998) reported that the tapetal cytoplasm is rich in ribosomes and rough endoplasmic reticulum (RER) saccules, but the locular

fluid of the PMC, which consists of neutral polysaccharides, pectins, and protein, could be detected only in small amounts.

Meiosis of PMCs to the release of microspores: Meiosis is one of the most complex events that occurs during gametogenesis because it involves the transition from a diploid to haploid state (Morohashi et al., 2003). Each of the PMC undergoes meiosis, resulting in a tetrad of haploid cells. These cells are held together by a callose (β-1,3-glucan) wall (Bewley et al., 2000). The synthesis of callose begins with initial deposition during the first meiotic prophase, and eventually spreading over the entire microsporocyte surface (Bhandari, 1984). The duration of meiosis can be as short as less than a day to as long as three months (Bennett et al., 1971). Sporopollenin is observed on the proUbisch bodies and on primexine matrix in the tetrad. Dictyosomes in the tapetum reach the maximal development at this stage (Clément et al., 1998). The tetrads are released by the action of callase (β-1,3-glucanase), an enzyme produced by the tapetal cells (Bewley et al., 2000).

The middle layers cannot expand themselves as the sporogenous cells are developing into PMCs, because their cells are unable to divide anticlinally so they are crushed into “inexorable endothecium” (Davis, 1966).

1.2.2. Microgametogenesis

After uninucleate microspores are released, they develop into pollen grains. The microspores undergo two mitotic divisions after release from the

callose wall. The first division is asymmetric and yields the vegetative cell, a larger cell, and the smaller generative cell. A second mitotic division of the generative cell yields two sperm cells (Bewley et al., 2000). Nuclear pore complex (NPC) density of the vegetative nucleus is twice as high as that of the sperm nuclei (Straatman et al., 2000). During pollen mitosis of the bicellular pollen grain stage, pollenkitt, composed of lipids and proteins, is located at the interface of the tapetal plasma membrane and loculus (Clément et al., 1998).

The process when the generative nucleus undergoes mitosis to produce two sperm cells is called spermatogenesis. It can occur either in the pollen grain or in the pollen tube, depending on generative cell mitosis timing (Southworth and Russell, 2001). When division happens in the pollen grain, it becomes a tricellular pollen. If in the pollen tube, the pollen sheds as bicellular pollen (Rudall, 2007). Of 192 plant families, 137 families shed their pollen as bicellular and 55 families as tricellular (Davis, 1966). The pollen type of genus *Corylus* has not yet been reported.

After the first mitotic division in the pollen, the generative cell wall is reduced or completely disappears in some species (Southworth and Russell, 2001). In higher plants, the generative cell migrates to the interior of the vegetative cell and remains wrapped by the vegetative cell (Russell et al., 1996).

1.2.3. Pollen Grains

The mature pollen wall is composed of two layers, an inner, intine, and an outer, exine. The intine wall is largely pectocellulosic (Bedinger, 1992). The protein components of the intine are thought to be derived from gametophytic gene expression, whereas those of the exine are thought to be produced by the sporophytic tapetal layer (McCormick, 1991). The exine wall is composed of sporopollenin, a complex substance that is very resistant to degradation (Bedinger, 1992), which is currently thought to be a mixed polymer containing both phenolics and long-chain fatty acid derivatives (Bedinger et al., 1994). The exine is also reported to be synthesized of highly specialized extracellular matrix (ECM) which protects the pollen grain when it is released into the environment (Steiglitz, 1977).

In maize pollen, there is a high accumulation of proteins, such as actin and tubulin, after microspore mitosis. These proteins will be used in the growth of the pollen tube (Mascarenhas, 1990). Tryphine on the pollen surface is important for pollen hydration in some species. It may be required for direct or indirect signalling to the stigma (Bedinger et al., 1994). The protein content of pollen from wind-pollinated plants tends to be lower than from insect-pollinated plants, and can range from 10 to 30% among plant species (Burgett et al., 1989). Although hazelnut is considered a wind-pollinated crop (Germain, 1994), it is one of the pollen sources for honey bees in the Pacific Northwest (Burgett et al., 1989).

Pollen viability of some hazelnut cultivars varies from 50% to 70% (Barbeau, 1972; Romisondo, 1977). Pollen grains of *Corylus avellana* require 90-95% RH for 2-3 hours for optimum germination. Immediately after dehiscence from anthers, pollen grains can have a high germination rate (87%). However, the germinability is significantly reduced when pollen grains are stored under desiccating conditions for 12 hours at 18-20 ^{o}C and 27 ^{o}C, and is eliminated at 40 ^{o}C (Heslop-Harrison and Heslop-Harrison, 1985).

1.3. Phenology

1.3.1. Temperature Factor

One major factor that affects plant development and more specifically flowering is temperature (Ingram and McCloud, 1984). Faust (1989) reviewed research about time of bloom of temperate tree fruit and stated that most fruit trees enter a dormant period in late fall and resume growth in early spring. During this dormant or “rest” period plants cannot resume growth until the chilling requirement is met. Trees also have a heat requirement before bloom can occur.

Besides temperature factors, Faust (1989) concluded that natural climate and cultural practices such as irrigation, sprays, and bud scale removal in the previous and current growing season can all have an impact on ‘time of bloom’ of fruit trees. Hampson (1995) found that catkin density was reduced by 64-74% in ‘Ennis’ and ‘Barcelona’ hazelnut trees which had been

under heavy shade. However, flowering was not as sensitive to shading as yield.

1.3.2. Chilling Requirement and Heat Accumulation

Chilling Requirement

The chilling requirement has been studied in various temperate fruit and nut species. In pistachio, Küden et al. (1995) found that in their studies of two male and five female cultivars, male cultivars had lower chilling requirements than female cultivars. In 'Ahmadaghaei', 'Fandoghi-Ghafuri', and 'Chorouk' pistachio, the optimum chilling hours were 1000, 1200, and 1400 hrs at 4-5 ^{o}C, respectively (Esmaeilizadeh et al., 2006). In 12 almond cultivars, Rattigan and Hill (1986) found that 220-320 CU was required for breaking dormancy in flower buds and subsequent floral development.

Heat Accumulation

Spring temperatures are very important with respect to the time of bloom in temperate fruit trees. The amount of heat required for each plant varies upon physiology of each species (Faust, 1989). The heat requirement is normally calculated as growth (growing) degree days (GDDs) or growth (growing) degree hours (GDHs).

Richardson et al. (1975) defined one growing degree hour (GDH) as one hour at a temperature 1 ^{o}C above the base temperature of 4.5 ^{o}C. GDH is calculated by subtracting 4.5 ^{o}C from each hourly temperature between 4.5

and 25 ^{o}C. All temperatures above 25 ^{o}C are assumed equal to 25 ^{o}C, so the greatest accumulation for any one hour is 20.5 GDH.

Ashcroft et al. (1977) used statistical methods to calculate the CU and GDH requirements of the deciduous fruit trees 'Tilton' apricot, 'Italian' prune, 'Elberta' peach, 'Bing' cherry, 'Bartlet' pear, and 'Delicious apple'. Using temperature data and dates of full bloom from a period of six years, they were able to determine the CU required for rest and GDH required for full bloom in the above crops. They suggested that this method could be used to determine other phenological stages such as bud swell, but many years of data specific to that stage would have to be available.

Spiegel-Roy and Alston (1979) suggested that heat requirement alone would be an adequate criterion for pear cultivar selection in Israel. They made this suggestion based upon a weak correlation between chilling requirement and bloom date, and a strong correlation between heat requirement after chilling and bloom date. Similar results from experiments on peach and western sand cherry by Werner et al. (1988) suggested that the basis for the difference in time of bloom was due to a difference in the base temperature of heat accumulation and not related to chilling requirements. In addition, Gianfagna and Mehlenbacher (1985) suggested that late flowering in apple is not a result of high chilling requirement, but of high heat and high minimum temperature requirements for bud growth.

1.3.3. Modeling as Tool to Predict Bloom

Being able to estimate the time at which rest completion occurs would help growers determine whether specific cultivars of crops of interest can be grown in their area, when the growing degree hours accumulate enough to induce bud development, the time at which cultural practices such as irrigation should be applied, and the time at which the trees lose their cold hardiness and begin to grow with warm temperatures (Richardson et al., 1974).

Cumulative chilling and heat unit models have been created for various tree fruit species. Two important examples are the Chill-Units model (also known as Utah model) (Richardson et al., 1974) and the dynamic model (Erez et al., 1990).

Chill-Units Model

Richardson et al. (1974) developed the Chill-Units model for 'Redhaven' and 'Elberta' peach, where an hour of exposure to 6 ^{o}C equals one chill-unit (CU), and the CU value is lower as the temperature increases or decreases. The value of the chill-unit is presented in following paragraph. This model was tested and works well in Washington, Georgia and Utah.

< 1.4 ^{o}C	=	0	CU
1.5-2.4 ^{o}C	=	0.5	CU
2.5-9.1 ^{o}C	=	1	CU
9.2-12.4 ^{o}C	=	0.5	CU

12.5-15.9 ^{o}C = 0 CU

16-18 ^{o}C = -0.5 CU

> 18 ^{o}C = -1 CU

Aron (1975) could not duplicate the calculation successfully in California. Richardson et al. (1975) reported that this discrepancy is a result of the land-sea breeze effect along the California coast, where there is an air mass change twice each day. Such changes in air mass compromise the chill-unit method of synthesizing hourly temperatures.

The Dynamic Model

The dynamic model was developed in South Africa in a dormancy-breaking study of peach buds under controlled conditions (Erez et al., 1990). This model started as a two-step model (Fishman et al., 1987a) and evolved into the dynamic model over at least two decades.

The Two-Step Model was developed by Fishman et al. (1987a). This model describes thermal dependence of the dormancy breaking phenomenon, assuming that the level of dormancy completion is proportional to the amount of a certain dormancy breaking factor which accumulates in plants by a two-step process. “The first step represents a reversible process of formation of a precursor for the dormancy breaking factor at low temperatures and its destruction at high temperatures. The rate constants of this process are assumed to be dependent upon the temperature according to the Arrhenius

law. The second step is an irreversible cooperative transition from the unstable precursor to a stable dormancy-breaking factor. The transition is assumed to occur when a critical level of the precursor is accumulated."

Later, the same group of researchers, Fishman et al. (1987b) brought parameters in their model to use as computer simulation. They indicated that models used to predict completion of dormancy should describe four factors; (I) the bell-shaped dependence of the bud rest break on the temperature; (II) the dependence of the degree of chilling negation by high temperatures on cycle length; (III) the promotion of the chilling effect by short periods of high temperature or longer periods of moderate temperature in a daily cycle; and (IV) the dependence of the level of chilling negation by high temperature on the duration of the high temperature application in a daily cycle.

Erez et al. (1979a) found a negation effect of high temperature to chill hours in a daily cycle. In their experiment with 'Redhaven' and 'Redskin' peach, shoot cuttings were applied with daily temperatures of 16 hrs low: 8 hrs high of 6-15, 6-18, 6-21, and 6-24 ^{o}C with a constant temperature at 4 ^{o}C and non- chilled condition as controls. There was no budbreak in 6-21 and 6-24 ^{o}C cycles, so they concluded that temperatures 21 ^{o}C and above negate chilling and that negation to chilling occurred between 18 to 21 ^{o}C. Therefore, they suggested that the coefficient in the Utah model should have been -2 instead of -1. Erez et al. (1979b) found chilling negation when observing peach budbreak in different length cyclic treatments of chilling (4-6 ^{o}C) and high

temperature (24 ^{o}C). Cycling 2/3 day chilling: 1/3 day high temperature results in poorer budbreak than does a two-day chill: one-day high temperature or longer cycles. Further study by Couvillon and Erez (1985) revealed that the longer the exposure to high temperatures during dormancy, the greater the negative effect on budbreak.

The same group of researchers, Erez et al. (1979b), used the same type of plant materials to study cycle length on chill negation by high temperature. In their experiment, 2:1 low: high temperature cycles were applied to peach cuttings in 1, 3, 6, and 9 day cycles, with 4-6 ^{o}C as the low temperature and 24 ^{o}C as the high temperature. They found good budbreak in 6 and 9 day cycles and suggested that continuous 20-40 chill hours before the onset of high temperature was the time when cuttings are sensitive to heat. Similar research by Couvillon and Erez (1985) found that 20 and 21 ^{o}C caused almost complete negation of chilling. In 19 ^{o}C high diurnal cycle, budbreak was better than 20 and 21 ^{o}C, but worse than in the constant 4 ^{o}C control. They created a modulation factor using the equation $M = [(c-x)/c] [y/(y-24)]$ to define the degree of promotion or inhibition induced by each temperature treatment where:

M = Modulation factor per hour of high temperature

c = level of bud break at continuous low temperature

x = level of bud break induced by the temperature cycle

y = number of hours of chilling per day.

They also found that exposing rooted peach cuttings to short (2-4 hrs) at 20 ^{o}C enhanced the chilling effect.

Erez et al. (1990) then revealed the dynamic model, assuming that dormancy breaking occurs by a two-step process. First is the *formation and destruction* of the intermediate from the precursor, which is reversible. After the critical amount of precursor is reached, it needs *a portion of stable factor* in the second step.

Erez and Fishman (1998) state that poor budbreak results from the negation of chilling by high temperatures during the day, which changes the diurnal pattern, rather than from a lack of chilling at night.

1.3.4. Comparison between Models

Erez et al. (1990) compared the dynamic model with the chill-unit model at five locations in South Africa. They found that both models had good correlation in the coldest region, but had differences in the warm regions. From this comparison, the researchers suggested that the dynamic model is likely to be a better prediction in warm areas.

In apple, del Real-Laborde et al. (1990) used the chill-unit model and their model of different CU calculation to evaluate apple dormancy in Mexico. They assigned -1.5 to 1.0 chill unit accumulation fractions (CUAF) to temperature -2 to 24 ^{o}C. They concluded that in subtropical environments,

adjustments need to be made to the chill-unit model for accurate prediction of dormancy development.

1.4. Research Objectives

The aims of this research were two-fold. In order to better understand factors that influence staminate bloom in hazelnut, a comprehensive study was undertaken to characterize microsporogenesis and microgametogenesis at the microscopic level in nine cultivars ranging in their bloom time from early to late. The significance and interaction of chilling and growing degree hour accumulation on staminate bloom was studied to further our knowledge and ability to predict bloom date in hazelnut.

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CHAPTER 2

MICROSCOPIC STUDY OF POLLEN DEVELOPMENT IN NINE HAZELNUT GENOTYPES

Chantalak Tiyayon and Anita N. Azarenko

CHAPTER 2 MICROSCOPIC STUDY OF POLLEN DEVELOPMENT IN NINE HAZELNUT GENOTYPES

Abstract

Pollen development is an important process in male flower development, the timing of which may be correlated with time of pollen shed in hazelnut (*Corylus avellana* L.). I identified early to very late blooming cultivars and studied the relationship of microsporogenesis and microgametogenesis, to the time of pollen shed. The cultivars included in the study, listed in order of bloom date, were: 'Tonda Gentile delle Langhe' (TGDL), 'Tonda di Giffoni' (TDG), 'Barcelona', 'Hall's Giant', 'Creswell', 'Brixnut', 'Gem', 'Gasaway', and 'Contorta'. The largest catkins from a single tree of each cultivar were collected once per week from 4 Aug. to 6 Dec. 2002, and on 17 Jan. 2003. The catkins were fixed in formalin: alcohol: acetic acid (FAA) solution, embedded, sectioned, stained with Toluidine blue-O, and examined by light microscopy. Early blooming genotypes were more advanced than late genotypes in pollen development from catkin differentiation through pollen shed. Mature pollen grains were present at the time that the chilling requirement of each genotype was met. A similar number of growing degree hours was required for catkin elongation and anthesis, from the time pollen grains reached maturity, across all studied genotypes.

Introduction

The development of an adequate number of flowers, transfer of viable pollen, and maturation and fertilization of ovules are the primary determinants of fruit and nut set and yield of orchard crops. Germain (1994) explained that hazelnuts are distinctive from other orchard crops because they are monoecious, anemophilous (wind pollinated) and bloom in midwinter (December to March in the northern hemisphere). Hazelnuts typically are self-incompatible and dichogamous therefore cross-pollination with compatible pollinizers that shed pollen when female flowers are receptive is required for optimal nut production (Lagerstedt, 1975). Staminate flowers are borne in catkins at nodes on one year old wood (Germain, 1994). Each catkin produces 4 million (Kelley, 1980) to 40 million (Pisani et al., 1968) pollen grains. Pollen viability varies from very low to over 95%. The diameter of each pollen grain is approximately 25 to 40 µm and pollen can travel 14 to 21 m from the mother tree (Germain, 1995).

In Oregon, catkins can be seen in the axils of basal leaves in late June/early July. Catkins grow rapidly during the first three months and then enter a lag phase for approximately 10 weeks. Growth then resumes until anthesis in mid-December to February (Lagerstedt, 1975). A similar timing of catkin development of ‘Barcelona’ was reported by Germain (1994) in the Bordeaux region of France. Pollen mother cells (PMCs) were observed during the second half of July and meiosis occurred in the first half of August. During

mid-August, free pollen grains were observed, and finally pollen shed occurred 4 to 6 months after catkins were first visible.

The temporal differences in pollen development among hazelnut cultivars have not been well-studied. Mehlenbacher (1991) determined chilling requirements of several hazelnut cultivars considering 0-7 ^{o}C as chill hours in the field. Hazelnut twigs were cut starting on Oct. 16. His chilling requirements were reported as a range of two numbers. The second number was the accumulation of chill hours when hazelnut twigs shed pollen more than 50% within three weeks in the greenhouse after cutting. The first number was the number of chill hours of the previous cutting date. His study revealed chilling requirements that range from less than 100 hours for early blooming cultivars to over 1000 hours for late blooming cultivars.

Richardson et al. (1975) defined one growing degree hour (GDH) as one hour at a temperature 1 ^{o}C above the base temperature of 4.5 ^{o}C. GDH is calculated by subtracting 4.5 ^{o}C from each hourly temperature between 4.5 and 25 ^{o}C. All temperatures above 25 ^{o}C are assumed equal to 25 ^{o}C, so the greatest accumulation for any one hour possible is 20.5 GDH.

Ashcroft et al. (1977) used statistical methods to calculate the CU and GDH requirements of the deciduous fruit trees ‘Tilton’ apricot, ‘Italian’ prune, ‘Elberta’ peach, ‘Bing’ cherry, ‘Bartlet’ pear, and ‘Delicious’ apple. Using temperature data and dates of full bloom for a period of six years, they were able to determine the CUs required for rest and GDHs required for full bloom

in the above crops. They suggested that this method could be used to determine other phenological stages such as bud swell, but many years of data specific to that stage would have to be available.

Cytological study of pistachio reproductive buds by Behboodi (2002) indicated that staminate flower bud primordia occur in August, but there is no development until May of the following year. Dimoulas (1979) did a comprehensive microscopic study of the timing of catkin and female flower development of three hazelnut cultivars ‘Ronde du Piemont’ (also known as ‘TGDL’), ‘Fertile de Coutard’ (‘Barcelona’), and ‘Merveille de Bollwiller’ (‘Hall’s Giant’) from May to September. The catkins initiate inside the buds and develop continuously. He categorized catkin development into eight stages: first, no sign of catkin differentiation in the first half of May; second, initiation of the first catkin’s bracts inside the axial bud which becomes convex between May 15 and 20; third, catkin elongation inside the bud in mid-June; fourth, emergence of catkins, with reddish color at the tip, from the bud during the second half of June; fifth, catkin completely out of the bud and about 10mm in length at the end of June and beginning of July; sixth, the initiation of anthers during the first and second week of July; seventh, anthers completely formed and pollen sacs with sporogenous cells visible around the third week of July; and eighth, at the end of July to mid-Aug, PMCs were formed and developed into tetrads, then pollen grains when the catkins were 2 to 3 cm long.

The formation of the pollen grains starts on the bract's base and progresses to the top of the same catkin. Dimoulas (1979) found that there was roughly a one-week difference in the timing of each process between the three cultivars used in his study.

Pollen development is divided into two processes, microsporogenesis and microgametogenesis (Goldberg et al., 1993). Microsporogenesis is the formation of microspores and anther walls from archesporial cells, and microgametogenesis is the development of microspores into pollen grains (Blackmore and Knox, 1990; Goldberg et al., 1993).

Angiosperm microsporogenesis and anther wall development were described by Davis (1966), Stanley and Linskens (1974), Blackmore and Knox (1990). This process is initiated during the periclinal division of archesporial cells and the formation of primary parietal cells and sporogenous cells on the outer and inner portion of the microsporangium. The primary parietal layer then divides periclinally resulting in two secondary parietal layers, while sporogenous cells enlarge into pollen mother cells. After this, the outer secondary parietal layer divides periclinally, forming an endothecial layer and a middle layer. The inner secondary parietal layer develops into a middle layer and tapetal layer. The resulting structure has an outer endothecial layer, two middle layers, and an inner tapetal layer. Wall layers may occur differently in each plant species, causing different numbers of endothecial, middle, and tapetal layers.

Each of the pollen mother cells undergoes meiosis, resulting in a tetrad of haploid microspores. These cells are held together by a callose wall, composed of β-1, 3-glucan (Bewley et al., 2000). The tetrads of hazelnut are tetrahedral inside the callose wall (Davis, 1966). Callase enzyme produced by the tapetum causes the callose wall to disintegrate and release the microspores (McCormick, 2004).

Microspores undergo microgametogenesis to become pollen grains. The mitosis of a microspore is asymmetric, resulting in a vegetative nucleus and generative nucleus (Horvitz and Herskowitz, 1992). Hazelnut pollen has three apertures, and under each aperture, there is a structure called “oncus”, meaning a lens-shaped structure. The oncus is not resistant to acetolysis and occurs beneath the apertures of many kinds of pollen grains (Punt et al., 1994). The generative nucleus then undergoes another mitotic event, giving rise to two sperm cells (Southworth and Russell, 2001).

The pollen makes contact with the female tissue at pollination. The generative nucleus then undergoes mitosis as the pollen tube grows down the style. The generative nucleus of some plants undergoes mitosis before anthesis and is released as a 3-nuclei pollen grain (Shivanna and Johri, 1985). Hazelnut pollen shed as bi-nucleate (Bhattacharyya and Johri, 1998).

The purpose of this investigation was to determine the relationship of pollen development to the timing of staminate bloom in nine hazelnut genotypes with peak bloom dates from December until March. The

association of the time of bloom with various stages of microsporogenesis and microgametogenesis was studied. Additionally, we investigated the relationship of various stages of pollen development to chill unit and growing degree hour accumulation.

Materials and Methods

Plant Materials

The trees used in this study were located at the USDA-ARS National Clonal Germplasm Repository (NCGR), Corvallis, Oregon, USA, at lat. 45°32′56′N long. 123°13′02′W. Nine cultivars were selected to represent early [‘Tonda Gentile delle Langhe’ (TGDL)], mid-season [‘Tonda di Giffoni’ (TDG), ‘Barcelona’, ‘Creswell’], late (‘Hall’s Giant’, ‘Brixnut’, ‘Gem’), and very late (‘Gasaway’ and ‘Contorta’) catkin bloom periods and a range of very low to high chilling requirements (Appendix A). This classification was based on pollen shed and chilling requirement data reported previously by Mehlenbacher (1991). The three largest catkins were collected weekly from a single tree (to ensure genetic integrity) of each genotype from 4 Aug. through 6 Dec. 2002. A final catkin collection was done on 17 Jan. 2003. Catkins from selected dates (4 and 22 Aug.; 5, 19, and 26 Sept.; 10 and 24 Oct.; 22 Nov.; 6 Dec.; and 17 Jan.) were processed for microscopy. These catkins were kept in vials on ice and transported to the laboratory. The length, diameter, and fresh weight of catkins were recorded. Catkin development was observed in the field during the 2002-2003 growing season.

Microscopy

Catkins were cut into 1-2 mm thickness and preserved in formalin: alcohol: acetic acid (FAA) solution (formalin: 95% ethanol: glacial acetic acid: distilled water, 2:10:1:7 by volume). A section from the middle of each catkin was dehydrated through an ethanol series (50, 70, and 95%) for at least 8 hrs at each concentration under vacuum. Samples were embedded using a Technovit 7100, a Glycol Methacrylate Embedding Kit (Heraeus Kulzer GmbH & Co., Germany) that included a 1:1 infiltration solution: 95% ethanol, followed by two overnight infiltrations in 95% then 100% infiltration solution, respectively, and embedded at 65 ^{o}C. The plastic blocks were sectioned into 4-7 µm slices using a microtome (No. 820 Spencer Lens Co., USA). The sections were stained with 0.5% Toluidine blue-O (TBO) in citrate buffer for 5 min and cleared by dipping tissue slides in 95% ethanol four to five times. The tissue sections were then air-dried and sealed with an acrylic resin (Poly-Mount®, Polysciences, Inc., Pennsylvania) and a cover slip. Slides were viewed with an Axioskop2 plus microscope (Zeiss, Jena, Germany) and photographs were taken with a Pixera model PVC 100C at 400x and 630x (Pixera Corporation, California).

Pollen Development

Pollen development was categorized into eight stages (Fig. 2.1). The stages were characterized by the presence of 1) archesporial cells, 2) sporogenous cells with parietal layers, 3) pollen mother cells (PMCs) with a

distinct tapetal layer, 4) meiotic cells/tetrads, 5) uninucleate microspores, 6) young pollen grains with two nuclei, 7) pollen grains with a vegetative and a generative nucleus, and 8) catkin elongation and anthesis. Pollen development was observed from two catkins per cultivar on each date.

Chilling Unit and Growing Degree Hour Calculations

Cumulative chilling was calculated as the number of hours that the temperature was between 0 to 7 ^{o}C (Mehlenbacher, 1991), beginning on 1 Oct. 2002. Growing degree hours (GDH) were calculated using the following formula by Anderson et al. (1986) with a 4 ^{o}C baseline:

$$GDH = [(25\ ^{o}C - 4\ ^{o}C)/2]\ \{1+COS\ [\pi + \pi\ (\text{hourly temperature} - 4\ ^{o}C)/\ (25\ ^{o}C - 4\ ^{o}C)]\}.$$

GDH accumulation for each cultivar began on the date when the catkins were 2 to 3 mm emerged from the buds. GDH accumulation began on estimated catkin differentiation date (15 June 2002 for ‘TGDL’, ‘TDG’, and ‘Barcelona’; 22 June for ‘Creswell’, ‘Hall’s Giant’, ‘Brixnut’, and ‘Gem’; 30 June for ‘Gasaway’; and 7 July for ‘Contorta’).

Results and Discussion

Hazelnut Pollen Development

In general, pollen development in hazelnut cultivars used in this study followed the described process. The anther wall of hazelnut has a thick epidermis, which appears green with TBO stain, a layer of endothecium, two to three middle layers, and one or two tapetum layers [Fig. 2.1(3)]. At the

tetrad stage, the middle layers start to get crushed by the expansion of increased number of cells from the meiosis event and the tapetum cells start to secrete their contents [Fig. 2.1(4)].

The tapetal inner fluid is visible around microspores when they are released from the callose wall [Fig. 2.1(4)]. Tapetal layer remains as a peripheral of the pollen sac even when the tissue section tears off. As microgametogenesis progresses, the middle layers are completely crushed and are only visible as a dark purple layer lining the endothecium. At this time, the tapetum becomes vacuolated.

The tapetum stained well and can be seen as the inner wall layers surrounding PMCs [Fig. 2.1(3)]. Shivanna and Johri (1985) found that the tapetal cells have high levels of DNA, as much as 16 times that present in sporogenous cells. Multinucleate tapetal cells were observed in this study.

Pollen development of the early blooming cultivars was consistently ahead of the late blooming cultivars (Fig. 2.2 and 2.3). 'TGDL', the earliest blooming genotype, was at stage 3 on the first sampling date (4 Aug.). At this stage, pollen mother cells (PMC), distinct tapetal layers, middle layers, and endothecial layers were present. Only epidermal and archesporial cells (Stage 1) were present in the latest blooming cultivars, 'Gasaway' and 'Contorta'.

Two weeks later on 22 Aug., ‘TGDL’ and ‘TDG’ were at Stage 4. The PMC had undergone meiosis. ‘Barcelona’ and ‘Hall’s Giant’ had PMCs with a clear tapetal layer (Stage 3). ‘Creswell’, ‘Brixnut’, ‘Gem’, and ‘Gasaway’ were between Stages 2 and 3 (PMC visible but partial tapetal layer). ‘Contorta’ had sporogenous cells present with parietal layers (Stage 2).

On 5 Sept., TGDL was at the microspore stage (Stage 5). TDG also had microspores present, but they were less advanced than those seen in TGDL as evidenced by remaining tetrads. ‘Barcelona’ was at the tetrad stage (Stage 4). PMCs and tetrads were present in the developing ‘Hall’s Giant’ anthers. The rest of the cultivars were at the PMC stage with the exception of ‘Contorta’, which was slightly less developed into the PMC stage.

Two weeks later on 19 Sept., TGDL was at the microspore stage (Stage 5). Some of the microspores were in the microgametogenesis process, having undergone mitosis. They contained two nuclei and some of the pollen grains were vacuolated (Stage 6). Microspores were present in all of the mid-season blooming cultivars as well as in ‘Hall’s Giant’ (Stage 5). The rest of the cultivars were at the tetrad stage (Stage 4) with an exception of ‘Gasaway’ whose tetrads had just been released from the callose wall.

A week later on 26 Sept., TGDL, TDG, and ‘Barcelona’ had young pollen grains containing two nuclei (Stage 6). Other later blooming cultivars were at the microspore stage (Stage 5) with ‘Hall’s Giant’ slightly advanced,

with some of the microspores already becoming young pollen grains with two nuclei (Stage 6).

On 10 Oct., TGDL reached the mature pollen stage (Stage 7), in which the generative nucleus is visible and the vegetative nucleus is diffused. Also, the pollen grain cytoplasm had filled the vacuole, causing the grains to be devacuolated. All other cultivars had vacuolated young pollen grains with two nuclei (Stage 6), with an exception of the latest blooming cultivar, 'Contorta'. In 'Contorta', the majority of anther contents were microspores (Stage 5), with some Stage 6 present.

Two weeks later, on 24 Oct., all cultivars were at the young pollen grain stage except for TGDL that had already reached mature pollen grain stage. Some pollen grains of TDG and 'Barcelona' were mature.

One month later on 22 Nov., all cultivars were at the mature pollen stage (Stage 7), except for 'Gasaway' and 'Contorta', in which some of the generative nuclei were not yet diffuse (Stage 6).

On 6 Dec. and 17 Jan., all cultivars had achieved the mature pollen stage. 'TGDL' had completely shed its pollen (Stage 8). It was difficult to find nuclei, especially vegetative, in the pollen grains, because they were obscured by the dense cytoplasm inside the pollen (Dunwell and Sunderland, 1974).

Relationship of Catkin Growth and Pollen Development with Cumulative Chilling Units and Growing Degree Hours

Catkin length, stages of pollen development and purported chill unit requirements are shown in Fig. 2.3. Catkin length increased continuously from the first obvious signs of undeveloped staminate flowers until it reached a plateau which was coincident with the tetrad and microspore stages [Fig. 2.3 (Stages 4 to 5)]. During the autumn and simultaneous with the plateau in catkin growth, the pollen continued its maturation through Stages 6 and 7 (microgametogenesis). Microspores underwent asymmetric mitosis resulting in larger size vegetative nucleus and smaller size generative nucleus. Vegetative nuclei further lost their globular shape and became diffused or elongated. Finally there was a rapid increase in length as the rachis of catkins elongated and pollen was shed (Stage 8).

The development of the pollen grains to the mature pollen stage (Stage 7) was generally coincident with the attainment of the purported chilling requirements (Mehlenbacher, 1991) (Fig. 2.3). The earliest cultivar to reach the mature pollen stage was TGDL. It reached Stage 7 prior to 16 Oct. when chilling hour accumulation began. However, genotypes did not shed pollen *in situ* until weeks or months later. These data indicate that sufficient growing degree hours (GDH) may also accumulate in order for hazelnut catkins to complete the final stages of maturation and elongation.

Chill hours and GDH of important stages are shown in Appendix B. Approximately 30,000 GDH were required from the beginning of catkin differentiation to the lag phase of catkin growth and there is little difference in GDH among cultivars to reach this lag phase (Fig. 2.3). The same trend was observed for pollen to reach maturity, which took approximately 33,000 to 37,500 GDH. Early blooming cultivars generally required more GDH than late cultivars. Male flowers of early blooming/low chilling requirement hazelnut cultivars need very little cold temperature to develop from archesporial cells into mature pollen. However, a longer period of heat accumulation was required to release pollen. Faust (1989) stated that as the plants accumulate more chilling, they will respond to GDH faster. The heat requirement of catkins may involve specific genes that activate after certain GDH have been fulfilled to trigger catkin elongation.

Egea et al. (2003) showed that chilling requirements had more effect on the timing of flowering than did heat for a variety of almond cultivars. Luza and Polito (1988) suggested that budbreak in winter-deciduous trees is a function of time and temperature. They discussed that budbreak is thought to depend on two temperature-dependent processes, chilling requirement for the breaking of dormancy, and after chilling is met, growth will resume after exposure to warm temperature. Our results are consistent with this hypothesis. Luza and Polito (1988) also comment that different types of tissue may respond differently to temperature.

Werner et al. (1988), in their study of the relationship between chilling requirements and post-rest heat accumulation and time of bloom in peach and western sand cherry, suggested that an important determinant of bloom time between these species may be the base temperature for heat accumulation. Degradi-Hoffman et al. (1996) validates this finding in almond with earlier blooming cultivars showing lower base temperatures for bloom progression.

Conclusions

In contrast to most of the cultivars which started microsporogenesis prior to August, the very late blooming cultivars (‘Gasaway’ and ‘Contorta’) started the process at least two weeks later. The time at which a genotype reached stages 4 and 5 was well-correlated with the time of bloom and the chilling requirement.

Based on this study, we would like to propose the following model for pollen maturation, chilling requirement, and GDH in hazelnut (Fig. 2.4). The length of the catkin increases with each stage of pollen development until the tetrad and microspore stages are reached (Stage 4-5) at which time the growth curve plateaus. During this time chilling hours accumulate and microgametogenesis proceeds (Stage 5-7). Once pollen reaches maturity (Stage 7), the chilling requirement has been met. This has lead us to propose that the anther itself may not have any need for GDH, but the vegetative tissues such as the rachis will require a certain amount of GDH to elongate and dehisce pollen from the anthers. Therefore, catkins will remain at this

stage until adequate GDH have accumulated for the rachis to elongate rapidly and shed pollen (Stage 8).

Acknowledgements

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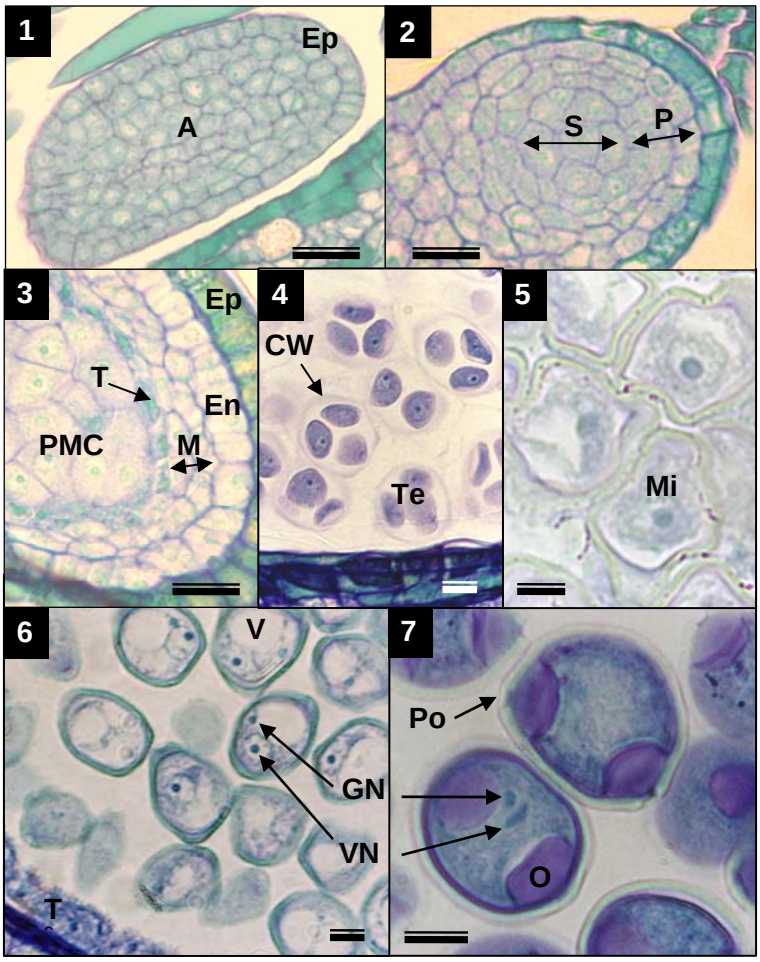

Fig. 2.1. Stages of hazelnut pollen development; (1) young anther with archesporial cells and epidermis, (2) sporogenous cells with parietal layers, (3) pollen mother cells with clear tapetal layer, middle layers, and endothecium, (4) meiosis/tetrad with tapetum that starts secretion, middle layers that begin to get crushed, and endothecium, (5) free microspores with secreting tapetum and crushed middle layers, (6) vacuolated young pollen grains with two nuclei, vacuolated tapetum, and most likely completely crushed middle layers, and (7) pollen grain which devacuolated, generative nucleus visible, but vegetative nucleus often diffused and difficult to observe, onci are larger that those in young pollen grains. Bar=10µ. A=archesporial cells, CW=callose wall, En=endothecium, Ep=epidermis, GN=generative nucleus, M=middle layers, Mi=microspores, O=oncus, P=parietal layers, PMC=pollen mother cells, Po=pore, S=sporogenous cells, T=tapetum, Te=tetrads, V=vacuole, VN=vegetative nucleus.

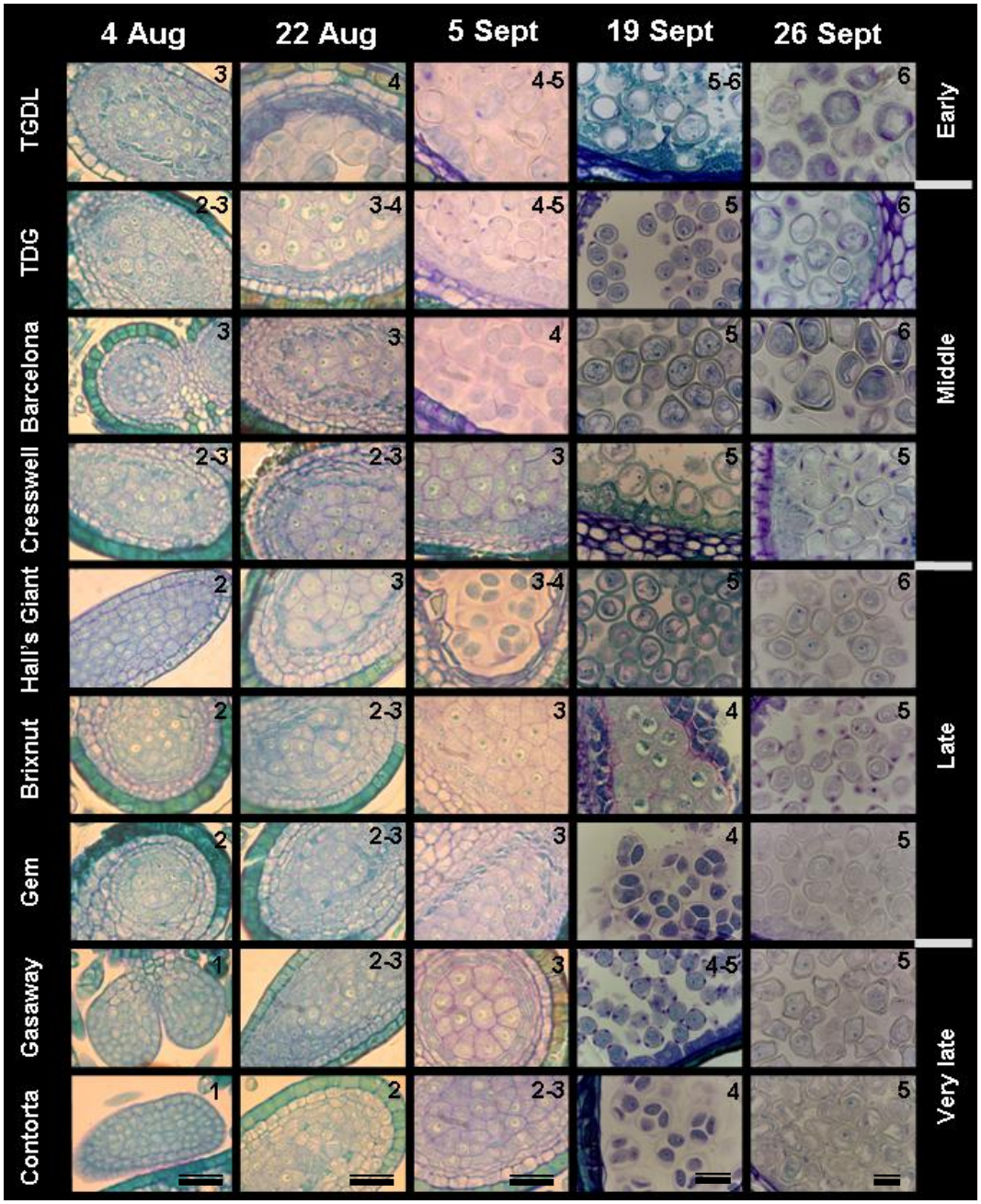

Fig. 2.2. Stages of microsporogenesis and microgametogenesis during pollen development of nine hazelnut cultivars. Microsporogenesis; (1) archesporial cells and epidermis, (2) sporogenous cells with parietal layers, (3) pollen mother cells (PMC) and layer of tapetum, (4) meiosis/tetrads, (5) microspores: and microgametogenesis; (6) young pollen grains with two nuclei. Bar=10μ (bar sizes are the same for photographs in the same column).

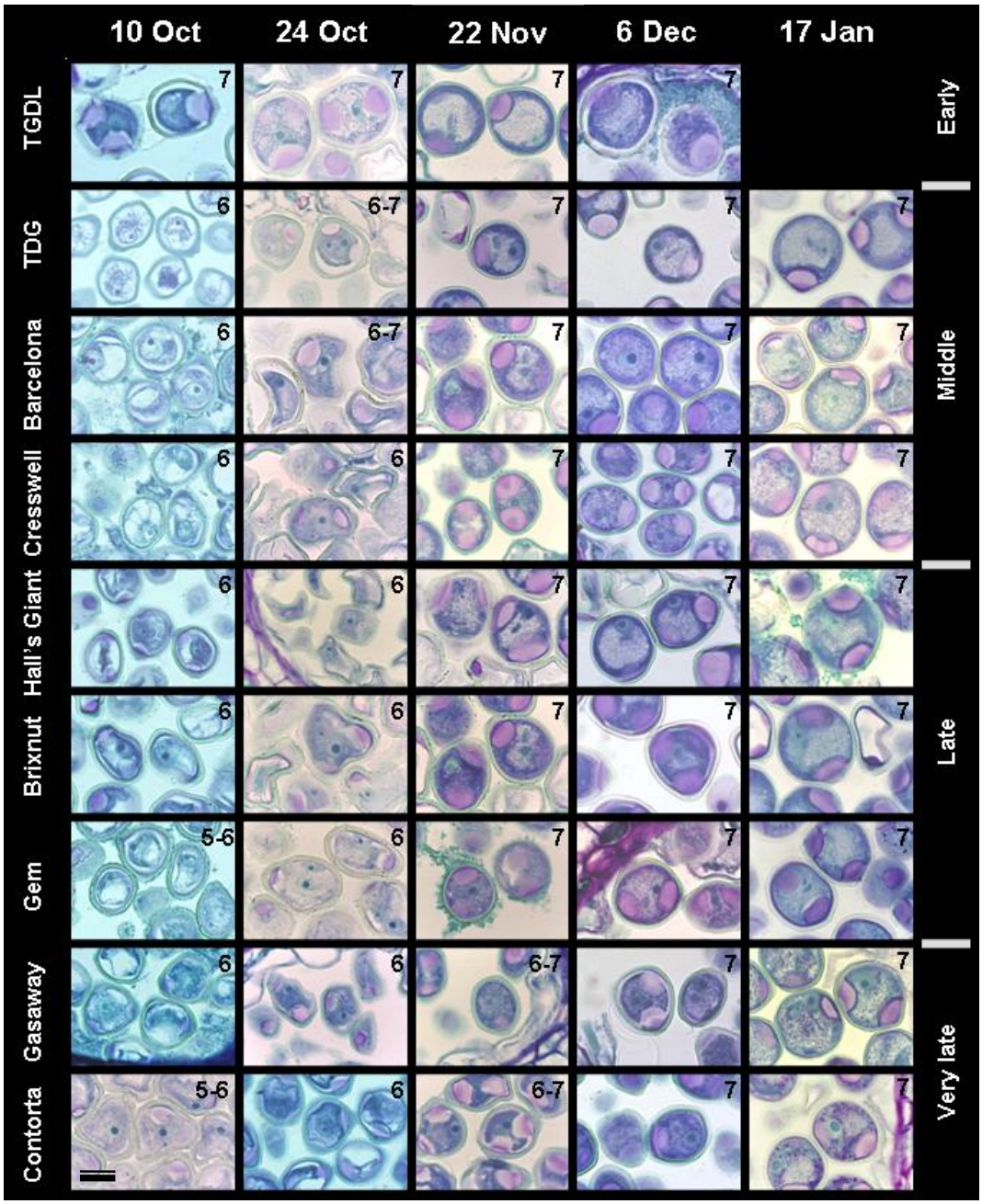

Fig. 2.2 (continued). Stages of microgametogenesis during pollen development of nine hazelnut cultivars. (6) young pollen grain with two nuclei, and (7) pollen grain with vegetative nucleus and generative nucleus. Bar = 10μ for all photographs

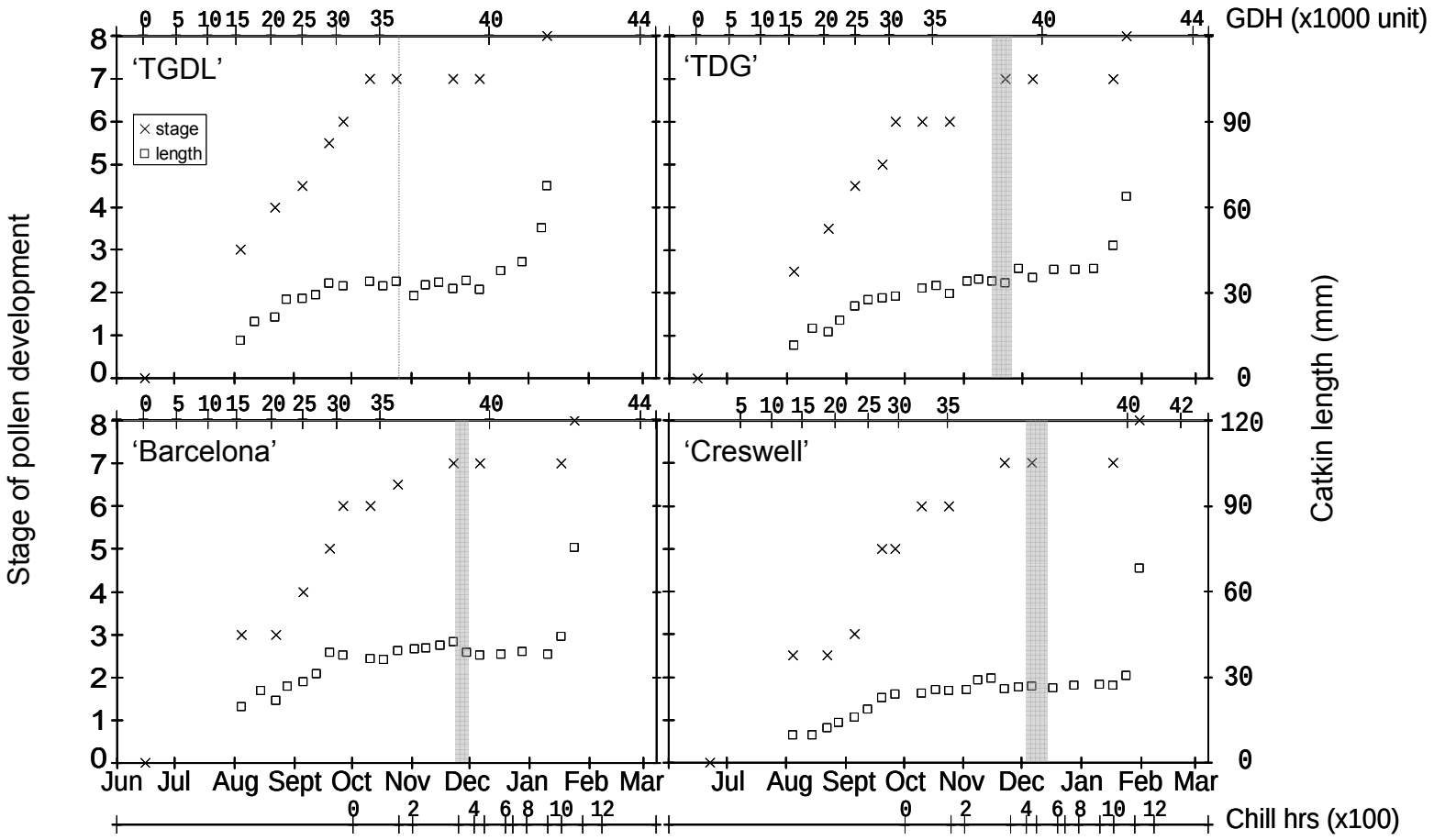

Fig. 2.3. Stage of hazelnut pollen development and catkin length in relation to calendar date, cumulative chill hours, and growing degree hours (GDH). The first cross (x) on an x-axis indicates the first visible presence of staminate flowers. The vertical grey lines are chilling requirements of each cultivar reported by Mehlenbacher (1991). Data are not shown for 'Brixnut'

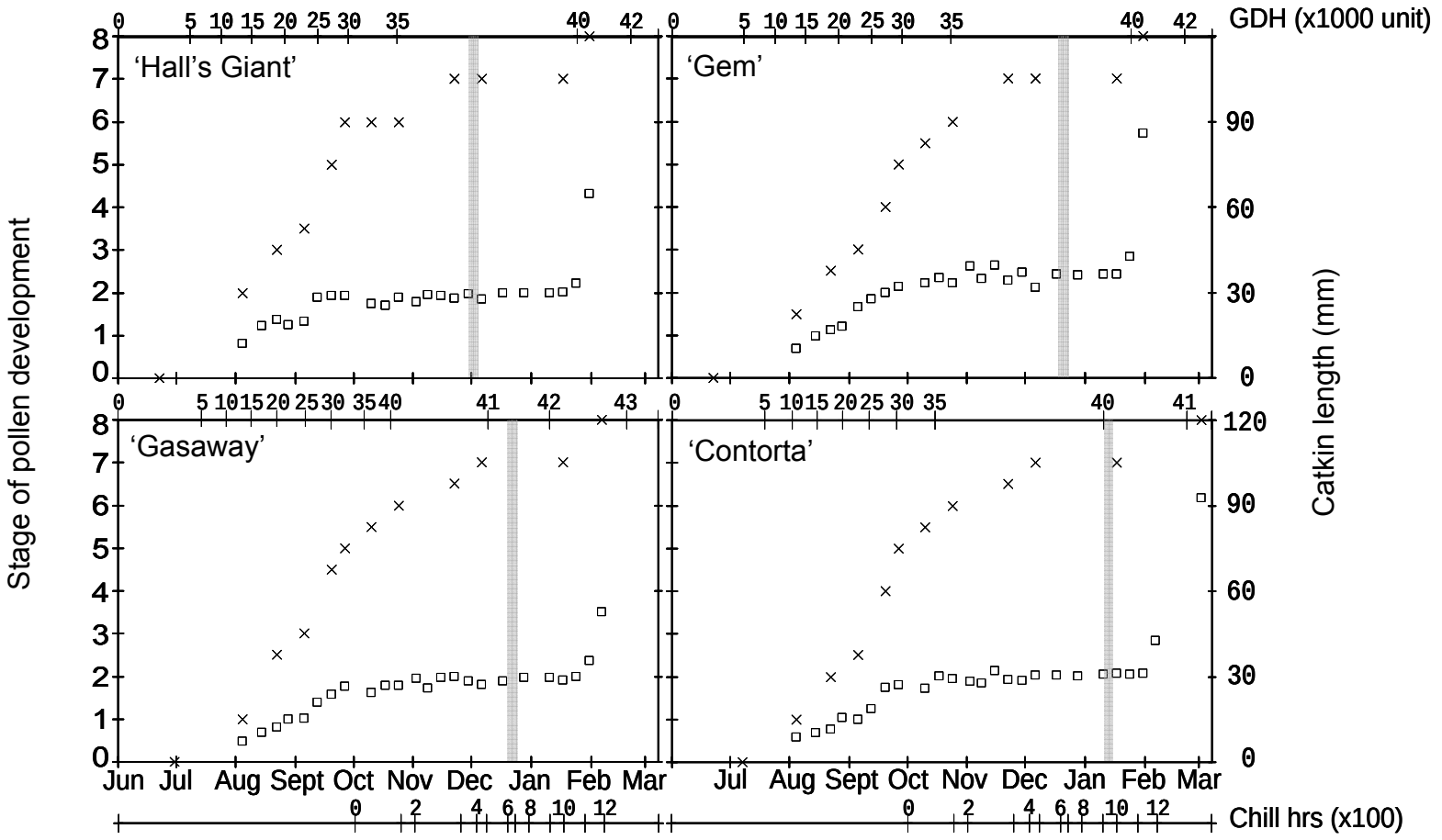

Fig. 2.3 (continued). Stage of hazelnut pollen development and catkin length in relation to calendar date, cumulative chill hours, and GDH. The first cross (x) on an x-axis indicates the first visible presence of staminate flowers. The vertical grey lines are chilling requirements of each cultivar reported by Mehlenbacher (1991). Data are not shown for ‘Brixnut’

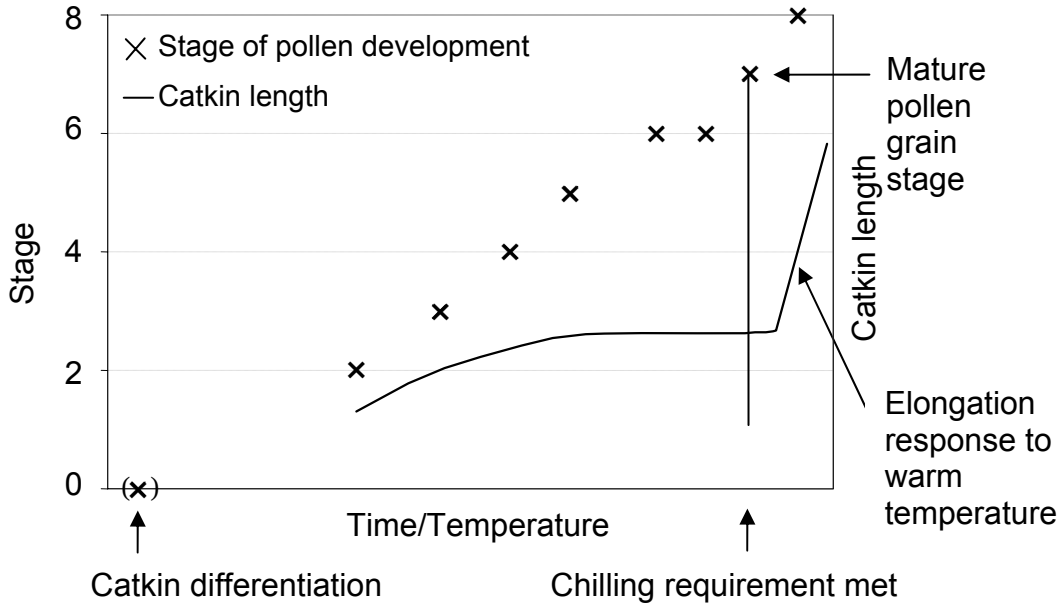

Fig 2.4. The model describing the relationship of hazelnut catkin length and stages of pollen development to time and temperature

Table 2.1. Bloom time and chilling requirement of hazelnut cultivars categorized from Mehlenbacher, 1991

Chilling requirement	Bloom time			
	Early (before 1/8)	Middle (1/8 to 1/21)	Late (1/22 to 2/18)	Very late (2/19 and later)
Very low (less than 100 hrs)	'TGDL'			
Low (100-240 hrs)		'Tonda di Giffoni'		
Medium (240-365 hrs)		'Barcelona'	'Hall's Giant'	
Medium/high (365-480 hrs)		'Creswell'	'Brixnut'	
High (480-680 hrs)			'Gem'	'Gasaway'
Very high (more than 680 hrs)				'Contorta'

CHAPTER 3

STAMINATE BLOOM PHENOLOGY IN THREE CULTIVARS OF HAZELNUT

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CHAPTER 3 STAMINATE BLOOM PHENOLOGY IN THREE CULTIVARS OF HAZELNUT

Abstract

Knowledge of floral biology and understanding factors that influence pollen shed are important for optimizing cultural practices and yields, and for crop breeding. This study aims to determine the role of temperature, the chilling requirement and growing degree hours (GDH) in the release of dormancy and anthesis of the staminate flowers of hazelnut. Hazelnut twigs of three cultivars; 'Tonda Gentile delle Langhe' ('TGDL'), 'Barcelona', and 'Hall's Giant' in Corvallis, Oregon, were collected at weekly intervals starting from early fall 2006 through the time of anthesis in the field in winter 2007. 'TGDL', 'Barcelona' and 'Hall's Giant' represent early, mid-season and late blooming cultivars, respectively. Twigs were held at five different constant temperatures: 0, 5, 10, 15, and 20 ^{o}C. These twigs were observed weekly and the time of anthesis (50% pollen shed) was recorded. Hazelnut twigs of the same three cultivars were also collected on 1 Nov. 2006 and held at 5 ^{o}C. Five twigs of each genotype were placed at room temperature (20-22 ^{o}C) at 5 day intervals to force bloom. Four different chilling models; cumulative chill hours at 0 – 7^{o}C, chill portions, cumulative chill unit based on the Utah model, and cumulative chill unit based on a modified Utah model, were used to estimate the amount of chilling needed to enable pollen shed. Growing degree hours were calculated from when catkins were 2-3 mm in length and began on 15

June 2006. We hypothesized that the number of chill units (CU) and GDH required for anthesis of staminate flowers was genotype-dependent. Time of staminate bloom was largely dependent on the chilling requirement of each cultivar. 'TGDL', 'Barcelona', and 'Hall's Giant' achieved 50% pollen shed at 168, 217, and 819 CH, respectively. When twigs were placed in high temperatures (15, and 20 ^{o}C) after this minimum amount of chilling was accumulated to break dormancy and induce catkins to shed pollen, up to 53,000 GDH were required to induce bloom. As chilling accumulated, whether in the field or in the cold room, anthesis of staminate flowers occurred with a lesser accumulation of GDH. Similar results were obtained across the cultivars and indicated that once the minimum chilling requirement was satisfied, the heat requirements for bloom were fairly constant across genotypes.

Introduction

The development of an adequate number of flowers, transfer of viable pollen, maturation, and fertilization of ovules are the primary determinants of fruit and nut set and yield of orchard crops. Staminate flowers of hazelnut are borne in catkins at nodes on one year old wood. Each catkin produces 4 million (Kelley, 1980) to 40 million (Pisani et al., 1968) pollen grains. Pollen viability varies from very low to over 95%. Germain (1995) recorded that the diameter of each pollen grain is approximately 25 to 40 µm and pollen can travel at least 14 to 21 m from the mother tree but the density declines.

Germain (1994) explained that hazelnuts are distinctive from other orchard crops because they are monoecious, anemophilous (wind pollinated) and bloom in midwinter (December to March in the northern hemisphere). Plants are self-incompatible and typically dichogamous, therefore cross-pollination with compatible pollinizers that shed pollen when female flowers are receptive is required for optimized nut production. In hazelnut production, choosing crop cultivars and pollinizers greatly depends on time of catkin elongation (TCE) and time of stigma exsertion (TSE) (Yao and Mehlenbacher, 2000). In that study, Yao and Mehlenbacher (2000) evaluated 17 morphological and phenological traits in 41 parental genotypes for three years and 35 progenies for two years. TCE and TSE, which are important phenological traits, are greatly influenced by temperature. They also found that TCE and TSE vary from year to year, but there are small differences in the range of bloom time among trees in the same area.

One major factor that affects plant development and more specifically flowering is temperature (Ingram and McCloud, 1984). Faust (1989) concluded that events in the natural environment and cultural practices such as irrigation, chemical sprays, and bud scale removal which occur in the previous and current growing season, all have an impact on the 'time of bloom' of fruit trees.

Bloom prediction is important in fruit and nut tree management (Hamer, 1986). Richardson *et al*. (1974) proposed two factors that are involved in the

resumption of growth; a chilling requirement which is measured in 'chill units' (CU); and the heat sum requirement for floral development measured as growing-degree-hour (GDH). CU expresses the relative effectiveness of various temperatures in releasing dormancy and models have been proposed for predicting dormancy release. Also, bloom date was postulated to be influenced by "post-rest" chilling (Couvillon and Hendershott, 1974).

GDH is the linear accumulation of hourly temperatures above a threshold growth temperature. Data from research in apple, cherry, peach, and pear by Couvillon and Erez (1985) showed that bloom at low temperatures by certain species could be due to GDH accumulation at temperatures below 4.5 ^{o} C. Rattigan and Hill (1986) also found from over seven years of a study of 12 almond cultivars that 220-320 CU and heat sum requirements from 5300 to 8900 GDH above 4.5^{o}C were required for breaking dormancy in flower buds and subsequent floral development.

Spiegel-Roy and Alston (1979) suggested that the heat requirement alone would be adequate when selecting pear cultivars (in Israel). They made this suggestion based upon a weak correlation between chilling requirement and bloom date, and a strong correlation between heat requirement after chilling and bloom date. Similar results from the experiments in peach and western sand cherry by Werner et al. (1988) suggested that the basis for the difference in time of bloom is due to a difference in the base temperature of heat accumulation and is not related to chilling requirements. In addition,

Gianfagna and Mehlenbacher (1985) suggested that late flowering in apple is not a result of a high chilling requirement, but from high heat and high minimum temperature requirements for bud growth.

Research by Alcalà and Barranco (1992) in olive suggested that the model which considered heat units accumulated before flowering was the most accurate way to predict flowering time. Less accurate methods compared the number of days with a higher mean temperature than the threshold temperature from beginning of the heat accumulation date until full bloom; and the method of correlation between the mean maximum temperature of selected months and the date of full bloom. The most appropriate threshold temperature for heat accumulation was 12.5 ^{o}C. This threshold is higher than 4.5 ^{o}C in peach (Richardson et al., 1975) and apple (Hamer, 1986), and 4 ^{o}C in tart cherry (Anderson et al., 1986).)

Mehlenbacher (1991) compared 58 genotypes of hazelnut and determined that the chilling requirement of catkins ranged from less than 100 to between 860 and 990 hours. In his experiment, chilling hours were considered from 0 to 7^{o} C and chilling requirements were reported as a range between two numbers. The former number was of chilling hours accumulated one week prior to completion of dormancy or rest. The latter was the number of hours accumulated on the cutting date on which dormancy was considered to be complete. Rest was considered to be complete when 50% of catkins or

more shed pollen after cutting them from the field and forcing them in the greenhouse for 3 to 4 weeks.

Degree-day models are useful in predicting biological events in the development of plants and poikilothermic ("cold-blooded") animals (Higley et al., 1986). By keeping a running total of the accumulated degree-days, and comparing this total to the number of degree-days required for an event (such as an egg hatching), Higley et al. (1986) stated that the date of the event can be predicted.

Wickman (1981) related degree-days to the growth of the tussock moth and the phenology of the host tree, white fir. Heat units were accumulated by subtracting the threshold temperature from the daily mean temperature, where the daily mean temperature is the sum of the maximum and minimum temperature divided by two. Each degree above the threshold is a degree-day. In this case, the threshold temperature was 5.6 ^{o}C, and the negative values were counted as zero.

Cumulative chilling and growing degree hour models have been created for various tree fruit species. The Chill-Units model (also known as Utah model) was developed by Richardson *et al.* (1974) for 'Redhaven' and 'Elberta' peach, where an hour of exposure to 6 ^{o}C equals 1 chill-unit (CU), and the CU value is lower as the temperature increases and decreases. This model, as stated in Faust (1989), established CU values for the following temperatures:

< 1.4 °C	=	0 CU
1.5-2.4 °C	=	0.5 CU
2.5-9.1 °C	=	1 CU
9.2-12.4 °C	=	0.5 CU
12.5-15.9 °C	=	0 CU
16.0-18.0 °C	=	-0.5 CU
18.1-21.0 °C	=	-1 CU
21.1-23.0 °C	=	-2 CU

This model was tested and found to adequately predict the chill unit requirements of peaches in Washington, Georgia, and Utah.

The Dynamic model was first developed as the Two-Step model by Fishman et al. (1987a). This model describes thermal dependence of the dormancy-breaking phenomenon, assuming that the level of dormancy completion is proportional to the amount of a certain dormancy-breaking factor which accumulates in plants by a two-step process. “The first step represents a reversible process of formation of a precursor for the dormancy breaking factor at low temperatures and its destruction at high temperatures. The rate constants of this process are assumed to be dependent upon the temperature according to the Arrhenius law. The second step is an irreversible cooperative transition from the unstable precursor to a stable dormancy breaking factor. The transition is assumed to occur when a critical level of the precursor is accumulated.

After more than a decade, the same group of researchers, Erez et al. (1990), created the Dynamic model to predict peach bloom time in South Africa. The model assumes that dormancy breaking occurs by a two-step process. The first step is the formation and destruction of the intermediate from the precursor, which is reversible. After the critical amount is reached, it needs a portion of stable factor in the second step. This “Dynamic model” was compared with the Chill-Unit model in five locations in South Africa (Erez et al., 1990). They found that both models had a good correlation in the coldest region, but had differences in warm regions. From this comparison, the researchers suggested that the “Dynamic model” is likely to be more effective in warm areas.

Richardson et al. (1975) created a GDH model for estimating rest completion in peach trees, where they defined one GDH as an hour at a temperature 1 ^{o}C above the base temperature of 4.5 ^{o}C. GDHs were calculated by subtracting 4.5 ^{o}C from each hourly temperature between 4.5 and 25 ^{o}C. All temperatures above 25 ^{o}C were assumed equal to 25 ^{o}C. From this calculation method, the greatest accumulation for any one hour is 20.5 GDH.

Anderson et al. (1986) proposed a growing degree model using an asymmetric curvilinear model with GDH accumulation between 4°C and 25°C (base and optimum). To determine GDH accumulation, they applied the following formula:

$$GDH = [(25\ °C - 4\ °C) / 2] \{1 + \cos [\pi + \pi\ (\text{hourly temperature} - 4\ °C) / (25\ °C - 4\ °C)]\}.$$

Additionally, they concluded that in 'Montmorency' tart cherry, an adjusted chill unit model in combination with this curvilinear model for GDH calculation adequately predicted spring bud phenology in the field.

The optimum chilling temperature and the baseline temperature for the resumption of growth after the chilling requirement are not known for hazelnut. Accurate chilling temperatures and heat unit accumulation models would be helpful for bloom prediction in this crop. This study was undertaken to establish chilling unit requirements and the role of GDH accumulation on staminate flower development in hazelnut.

Materials and Methods

In Situ Chilling and Growing Degree Hour Accumulation until Anthesis

Three hazelnut cultivars; 'Tonda Gentile delle Langhe' (TGDL), 'Barcelona', and 'Hall's Giant' (early, mid-season and late blooming cultivars, respectively), were used in this study. Trees were located at the OSU Vegetable Research Farm (VRF), OR (lat. 44°34´27´N long. 123°14´19´W) and the USDA National Clonal Germplasm Repository (NCGR) in Corvallis, OR (lat. 45°32´56´N long. 123°13´02´W). Twenty twigs that were at least 50 cm in length, having at least 15 catkins and 10 vegetative buds were randomly cut from trees, 10 twigs of each cultivar at each location. They were collected weekly from 2 Oct. until 50% of pollen shed was observed on the tree in the

field. Twigs were divided into five groups and placed in 0 and 5 ^{o}C cold rooms (OSU), and in 10, 15, and 20 ^{o}C growth chambers. Four twigs of each cultivar were placed in the dark at constant temperatures in 0 and 5 (±1) ^{o}C cold rooms, 10 (±1), 15 (±1) and 20 (±1) ^{o}C growth chambers. The twigs were observed weekly and the numbers of catkins that elongated and shed pollen were recorded until 50% pollen shed was reached. The twigs were re-cut and water changed weekly throughout the experimental period.

Controlled Chilling and Growing Degree Hour Accumulation until Anthesis

Hazelnut twigs were cut from three cultivars on 1 Nov. 2006 from W and J Orchard, Inc., Albany, OR (lat. 44^{o}42´15´N long. 123^{o}07´57´W) (80 twigs of TGDL, 100 of 'Barcelona', and 120 of 'Hall's Giant'). The twigs were kept in a 5 ^{o}C (±2 ^{o}C) cold room in the dark and in water. Five twigs were randomly brought out every 5 days to the laboratory and held at room temperature (20-22 ^{o}C). Every 5 days the number of catkins that elongated and shed pollen was recorded, the water was changed, and stems were re-cut under water.

Chill Unit and Growing Degree Hour Calculations

Four chilling calculation methods were used in this study. The first method was that used by Mehlenbacher (1991) where each hour accumulated between 0 to 7^{o}C was summed (chill-hour or CH). Second, the Chill-Units model (Richardson et al., 1974), which weighted the contribution of temperatures above and below the optimum temperatures, was used. Chill

portions were also calculated using the model developed by Erez *et al.* and Southwick (2006 unpublished and proprietary data). Lastly, the data suggested that hazelnut is likely to accumulate chilling at lower ranges than some temperate fruit and nut species. Therefore, we developed a fourth method of chilling calculation as the sum of weighted chill units at temperature between -1.9 ^{o}C and 16.1 ^{o}C, assuming that any temperature outside of this range accumulates 0 CU.

< -1.9 ^{o}C = 0

-1.9-1.0 ^{o}C = 0.5

1.1-4.0 ^{o}C = 0.75

4.1-7.0 ^{o}C = 1

7.1-10.0 ^{o}C = 0.75

10.1-13.0 ^{o}C = 0.5

13.1-16.0 ^{o}C = 0.25

>16.1 ^{o}C = 0

Growing degree hours (GDH) were calculated using the following formula by Anderson *et al.,* (1986) with a 4 ^{o}C baseline:

$$GDH = [(25\ ^{o}C - 4\ ^{o}C) / 2] \{1+COS [\pi + \pi (\text{hourly temperature} - 4\ ^{o}C) / (25\ ^{o}C - 4\ ^{o}C)]\}.$$

GDH accumulation for each cultivar began on the date when the catkins had emerged from the buds and were 2 to 3 mm in length. Although the date of 'Hall's Giant' catkin differentiation (time at which GDH begins to accumulate)

was approximately one week later than ‘TGDL’ and ‘Barcelona’ as reported in the previous study, cumulative GDH calculation in this study began on 15 June 2006 for all cultivars.

Results and Discussion

In Situ Chilling and Growing Degree Hour Accumulation until Anthesis

‘TGDL’ twigs that were cut on the first two sampling dates (2 and 10 Oct.) did not shed pollen within 8 weeks of observation. Therefore, the data from these sampling dates are not presented graphically in Fig 3.1A. The potential for catkin elongation and pollen shed began on 16 Oct. [51 chill hours (CH) calculated in the field]. However, at all temperatures, the pollen shed percentage was lower than 50%, which was not considered complete anthesis. The first cutting date when catkins on the twigs attained 50% pollen shed was 23 Oct. (168 CH) but only at 20 °C and it took 5 weeks. On 6 Nov. (217 CH) twigs held at 15 °C and 20 °C shed pollen at 5 and 4 weeks, respectively. All catkins elongated and reached 50% or more pollen shed on twigs harvested on 13 Nov. (253 CH). At 5, 10, 15, and 20 °C, it required 7, 4, 2, and 3 weeks, respectively. From this date forward, anthesis was reached more quickly at each subsequent sampling date. ‘TGDL’ catkins cut between 11 and 18 Dec. (680 and 721 CH), attained nearly 100% pollen shed at all temperatures except 5 ^{o}C within the first week of observation. In the field,

‘TGDL’ catkins started to elongate on 11 Dec. and were shedding pollen between 18-26 Dec. 2006.

A very similar pattern was observed in ‘Barcelona’ but shifted later by one to two weeks (Fig 3.1.B). Twigs cut on 30 Oct. (168 CH) and held at 20 °C reached anthesis within 6 weeks. The first cutting date when all twigs were able to reach 50% shed was 27 Nov. (430 CH), and the date catkins began to elongate in the orchard was 8 Jan. (968 CH)

Catkins on the twigs of ‘Hall’s Giant’ often fell prematurely or dried up when sampled before 4 Dec. (567 chill hours). On 4 Dec. some anthesis was observed at 20 °C, however, on less than 10% of the twigs. Pollen shed from ‘Hall’s Giant’ catkins occurred consistently on twigs sampled after 26 Dec. (819 CH) at all temperatures (Fig 3.1C). Anthesis on this date occurred in 2-3 weeks at 15 and 20 °C, 4-5 weeks at 10 °C; and 7-8 weeks at 5 °C. The shortest amount of time until pollen shed at all temperatures was observed from twigs cut on 29 Jan. (1,807 CH) and occurred within 1-3 weeks from sampling. In the field, ‘Hall’s Giant’ catkins began to elongate in early February (1,364 CH).

The number of days required for twigs of each cultivar to achieve 50% anthesis at different temperatures declined as the winter progressed (Fig 3.2). Generally, twigs held at higher temperature reached anthesis before those held at lower temperature. Interestingly, ‘TGDL’ and ‘Barcelona’ twigs held at

15 and 20 ^{o}C responded similarly across the cutting dates indicating that the optimum threshold for growth of catkins may be around 15 ^{o}C. As chilling increased in 'Hall's Giant', the number of days to anthesis became more similar at later sampling times when twigs were held at the 15 and 20 ^{o}C.

Staminate flowers of 'TGDL', 'Barcelona' and 'Hall's Giant' typically bloom in late December, mid-January and mid- to late February, respectively (McCluskey, unpublished data). If we assume that optimum chilling is when the least amount of heat is required for pollen shed, then we can predict the time of bloom by setting y=0 (y is the number of days to anthesis from the cutting date) and determine the point at which bloom occurs. These dates, 21 Dec. 2006 for 'TGDL', 15 Jan. 2007 for 'Barcelona', and 12 Feb. 2007 in 'Hall's Giant' are roughly correlated with field observations (Table 3.1). In Table 3.1, the regression equation of each cultivar came from the pool of data from all temperatures. The upper and lower bounds of 95% confidence interval were used to compare slopes of the above cultivars. The slopes of all three cultivars were not statistically different. This result may imply that once the chilling requirement is met, a very similar amount of GDH is required for all cultivars. This statement is contradictory to the comment in Chapter 2. However, the approach in this Chapter is more sensible.

As chill units accumulated (in the field and while in the growth chambers/cold rooms), the amount of heat required for anthesis declined for all cultivars and models (Fig. 3.3a, b, c, and d). In both 'TGDL' and 'Barce-

lona', when chill hour accumulation was relatively low and twigs were placed at 15 and 20 °C, more heat was required to achieve anthesis (Fig. 3.3a). For twigs held at the 5 and 10 °C, the number of GDH required for anthesis decreased slightly as CU increased, except 'Barcelona' at 10 °C. 'Hall's Giant' catkins with low CU accumulation abscised when placed in 15-20 °C. If optimum chilling is defined as the point at which the least amount of heat is required to induce anthesis, then approximately 38,000 to 40,000 GDH were required for anthesis for all three cultivars.

Hazelnut catkins appear to accumulate chilling over a wider range of temperatures than 0-7 °C. This is apparent because at 10 °C, lower GDH was required to induce catkin bloom. Therefore, three additional methods that consider the level of chilling accumulation at different temperature ranges were compared for their efficiency in estimating bloom time.

The relationship of chill portions, as calculated by the Dynamic model (according to Southwick 2004, unpublished and proprietary), to GDH at which 50% of the catkins shed pollen, is illustrated in Fig. 3.3b. Again, as chill portions accumulated, less GDH were required to achieve pollen shed. The use of chill portions resulted in a better correlation between GDH and anthesis than that calculated with CH. The fit of the exponential equation was slightly improved when using the Utah model (Fig. 3.3c). The relationship of chilling and GDH to pollen shed from our weighted chill unit calculation method (Fig. 3.3d) had a slightly higher r^2 value than the Dynamic and Utah models, except

in ‘Hall’s Giant’ where the r^2 values were nearly the same. Appendix C illustrates a comparison of the different chilling calculations and cumulative growing degree hours (GDH).

Controlled Chilling and Growing Degree Hour Accumulation until Anthesis

Twigs that were collected on 1 Nov. 2006 had been exposed to approximately 196 CH or 403 CU in the field. Ten percent of the ‘TGDL’ catkins were able to shed some pollen when forced at 20 ^{o}C (Fig. 3.4). ‘Barcelona’ and ‘Hall’s Giant’ catkins required an additional 240 and 600 CH, respectively, to be able to shed pollen when forced (CH= CU in cold room study since 1 hr at 5 ^{o}C equal 1 CU). ‘TGDL’ twigs and catkins that received 436 CH (10 days at 5 ^{o}C + 196 CH prior to cutting) or 643 CU had 50% or more of the catkins shedding pollen within two weeks of 20 ^{o}C exposure or ~42,500 cumulative GDH. At 1156 CH (40 days at 5 ^{o}C + 196CH prior to cutting) or 1363 CU, ~38,000 GDH were required to achieve 50% anthesis.

When twigs were held in the cold room and allowed to accumulate additional chilling before being moved to 20 ^{o}C, the number of days and cumulative GDH until anthesis decreased (Fig. 3.5 and 3.6). While pollen shed could be induced at a minimum chill level, this was often accompanied by catkin desiccation and abscission as shown in Fig. 3.5. ‘TGDL’, the low chilling requirement cultivar, had greater catkin retention at 20 ^{o}C with only a short period (10 days) of 5 ^{o}C of chilling. On the other hand, ‘Hall’s Giant’,

which has a much higher chilling requirement and required an additional 50-60 days of chilling to have the least amount of catkin abscission.

In Table 3.2, the regression lines from Fig. 3.5 are compared. The upper and lower bounds of 95% confidence interval were used to evaluate the differences between slopes of the above cultivars. The slopes of all three cultivars are not statistically different. Since twigs accumulated chilling while they were kept at constant 5 ^{o}C and accumulate heat when they were brought out to force at room temperature, the CU and GDH amount for staminate bloom can be calculated by the sum of CU and GDH under control condition and CU and GDH they were exposed to prior to when they were cut on 1 Nov 2006 (Fig. 3.6).

The relationship of cumulative CU and GDH in controlled condition shown in Fig. 3.6 is remarkably similar to those in the semi-controlled study (Fig. 3.3d). Table 3.3 provides a comparison of the curves within Fig. 3.3d and Fig. 3.6. In both studies, 'TGDL' curve is not statistically different from 'Barcelona', and both 'TGDL' and 'Barcelona' are different from 'Hall's Giant'. In addition, the curves of the same cultivar from both studies are also not statistically different.

These two studies confirm that although a minimum chilling will enable pollen shed, additional chilling will reduce the GDH requirement. These results may imply two things. First, once the minimum requirement is met, a similar amount of GDH is needed for all three cultivars. Second, the optimal

chilling requirement of ‘TGDL’ and ‘Barcelona’ are similar, but the optimal chilling of ‘Hall’s Giant’ is higher than the previous two cultivars.

Mehlenbacher (1991) estimated the CH requirement for ‘TGDL’, ‘Barcelona’ and ‘Hall’s Giant’ to be <100, 240-290, and 290-365 CH; and characterized their bloom period as early, middle, and late, respectively. We estimated CU from the year of 1989-1990 from mean daily temperatures (the only temperature data set available) and multiplied by 24. The amount of CU for ‘TGDL’, ‘Barcelona’, and ‘Hall’s Giant’ were 570, 1020-1086, and 1056-1122, respectively. The chilling requirement from this study is greater than that reported by Mehlenbacher (1991). In 2006-2007 growing season, chilling at when anthesis occurred in the field were 1170-1313 CU for ‘TGDL’, 1535-1614 CU for ‘Barcelona’, and 1969-2079 for ‘Hall’s Giant’. CU’s are substantively different between these two studies. We propose that there is a minimum and optimum chilling requirement. The minimum chilling requirement was that observed by Mehlenbacher (1991). The optimum chilling requirement would consider catkin retention and the reduced number of GDH required for bloom.

In ‘Siberian C’ peach, Young and Houser (1980) reported that the end of the chilling requirement is when pollen meiosis is completed. However, Felker and Robitaille (1985) did not find relationship between meiosis and the chilling requirement in cherry. In this study, the chilling requirement appears to be related to a stage of pollen development, but it is not certain when

chilling accumulation begins and ends. Chilling may need to accumulate until the pollen is mature since twigs with catkins in the pollen meiosis stage did not elongate and abscised when placed at warm temperatures.

Lang et al. (1987) separated dormancy into three types; paradormancy, endodormancy, and ecodormancy. Paradormancy is regulated by buds or organs elsewhere on the plant such as in apical dominance. Endodormancy is the classic stage of dormancy where growth is controlled by plant growth substances within the bud itself. In order for growth to resume, the plants must be exposed to cool temperatures to satisfy what is called the chilling requirement. Ecodormancy is the cessation of growth induced by environmental factors such as low temperatures. Based on our results, we propose that the endodormant period of staminate hazelnut flowers occurs during the lag phase period of catkin length when pollen grains are completing microgametogenesis. Once pollen grains complete their develop-ment, the catkin enters into the ecodormant period where catkin elongation and pollen shed is limited by the amount of GDH that accumulate.

Higley et al. (1986) worked with DEGDAY which is a program for calculating degree-days and considers numerous factors. They suggested eight factors that affect degree-days for both insect and plants which were 1) substrate availability 2) enzyme availability 3) approximations and assumptions in laboratory estimates of development 4) approximations in calculating the developmental minimum 5) approximations in calculating or not

calculation the developmental maximum 6) approximations in using single values for developmental thresholds 7) thermoregulation, and 8) propriety and limitations of temperature data used in calculations. If the calculations accounted for all these factors, prediction efficiency of the model would be improved.

This study provides a simple way to estimate pollen shed in hazelnut. Even though this study considers only CU and GDH amount for estimation, the models of three hazelnut cultivars are able to predict anthesis period. This more accurate calculation of the chilling requirement and GDH calculation is a better tool to predict staminate bloom in hazelnut.

Conclusions

Hazelnut catkins appear to accumulate chilling at temperatures between 5 and 15 °C as evidenced by the lower amount of GDH required to induce catkin elongation (Fig. 3.3a). This led us to evaluate three other chilling models; the chill portion or Dynamic model, the chill unit or Utah model, and our weighted chill unit model. These models considered the ability for temperatures above 7 °C to contribute to the chilling accumulation. The role of increasing chilling and the consequent reduced amount of GDH required to induce bloom became clear (Fig. 3.3b, c, d). Our model was most predictive of this relationship.

GDH calculations in this study used 25 °C as the optimum growth temperature for catkins. 'TGDL' and 'Barcelona' catkins that were placed in 15 and 20 °C growth chambers reached anthesis in a similar amount of time (Fig. 3.2). One would have predicted a more rapid response, or fewer days to anthesis at the 20 °C if the optimum growth temperature was 25 °C. Our data indicates that the optimum temperature for catkin elongation may be close to 15 °C.

We propose that staminate flowers of hazelnut have a minimum and an optimum chilling requirement. Prior to receiving the minimum chilling requirement, catkins do not elongate and shed pollen (Fig. 3.5). They desiccate and abscise. When the minimum chilling requirement is met, catkins can be induced to elongate and shed pollen, however, a greater number of GDH are required to induce bloom (Fig. 3.4). We mark this as the end of the endodormant phase of the catkin. As the catkin is transitioning from the endodormant phase to its ecodormant phase, some catkins may not have acquired adequate chill and could still desiccate and abscise (Fig. 3.5). This is in contrast to the staminate buds that continue to receive chilling and accumulate GDH. When the optimum chilling requirement is met, the least amount of GDH is required to induce bloom (Fig. 3.3a, b, c, d and 3.6).

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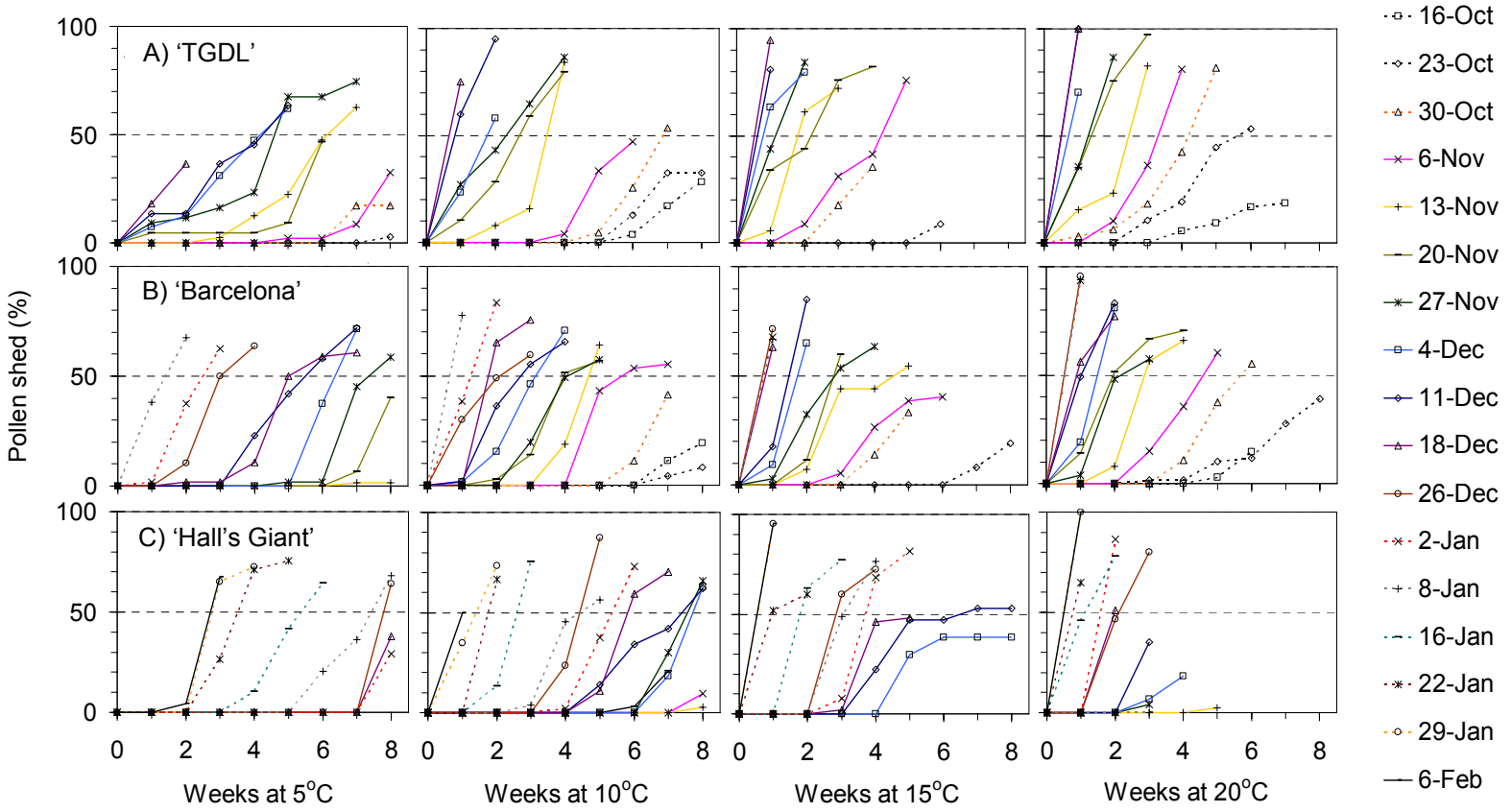

Fig. 3.1. Percent pollen shed of three hazelnut cultivars, A) 'TGDL', B) 'Barcelona', and C) 'Hall's Giant', cut weekly from 2 Oct. 2006 to 6 Feb. 2007, placed at five different temperatures and observed for up to 9 weeks or until the twigs reached 50% anthesis (n=4)

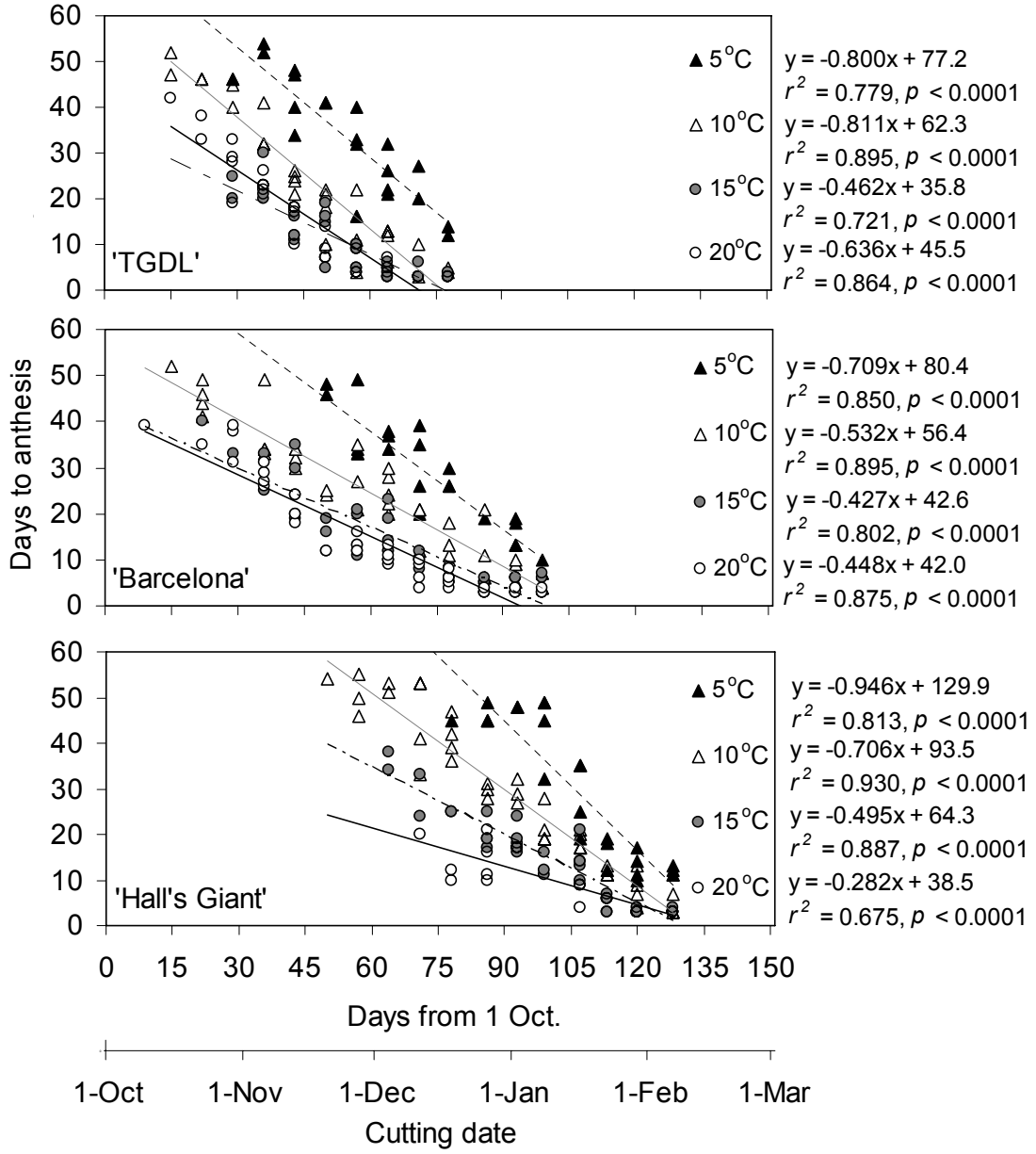

Fig. 3.2 Number of days required for three hazelnut cultivars to reach anthesis after cutting at weekly intervals and holding twigs at 5, 10, 15, and 20 °C relative to sample day interval from 1 Oct. 2006.

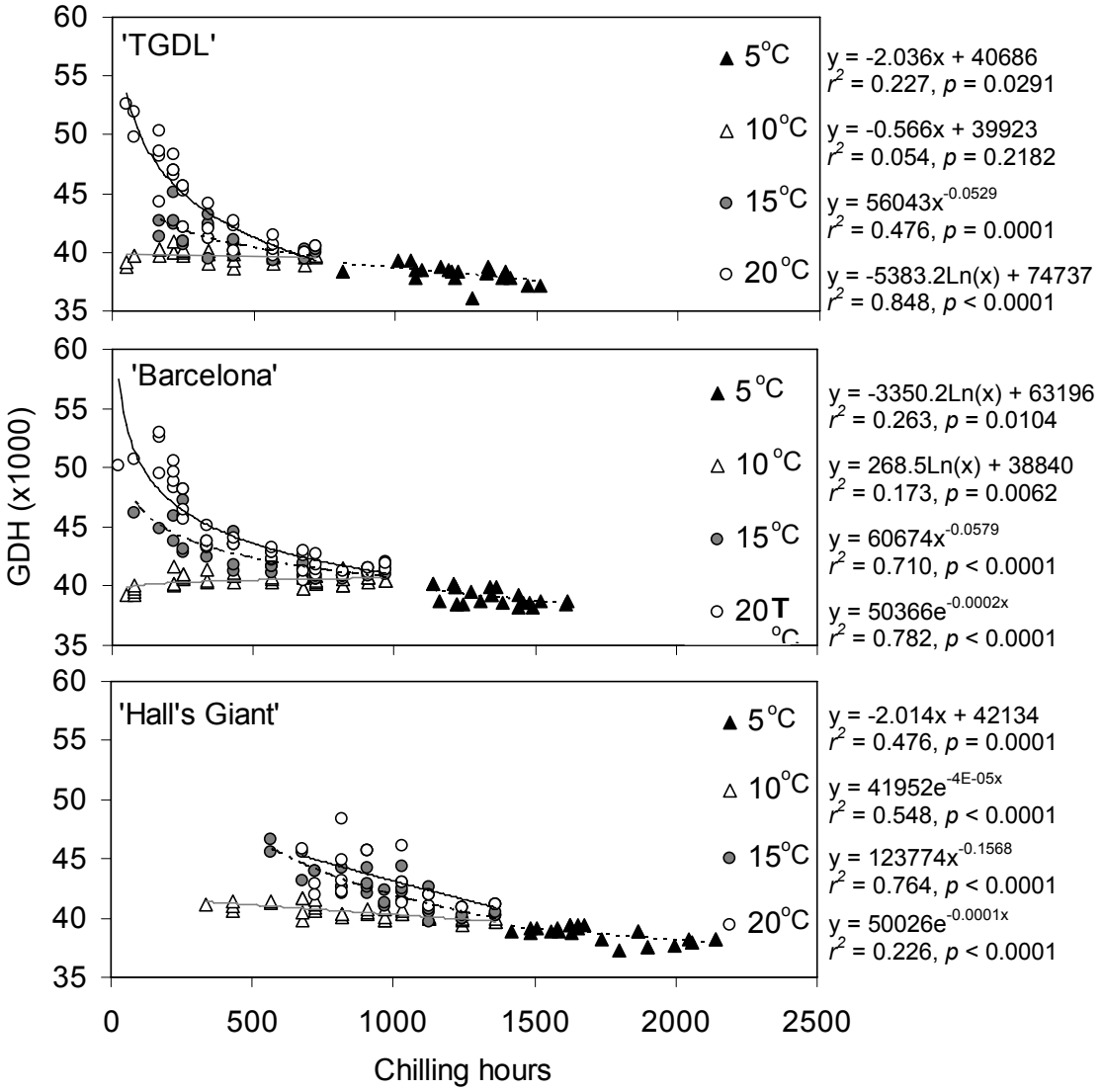

Fig. 3.3a. Regression between chilling hours (cumulative chill hours at 0-7 °C) and cumulative growing degree hours (GDH) for catkins to reach anthesis of three hazelnut cultivars that were held at 5, 10, 15, and 20 °C.

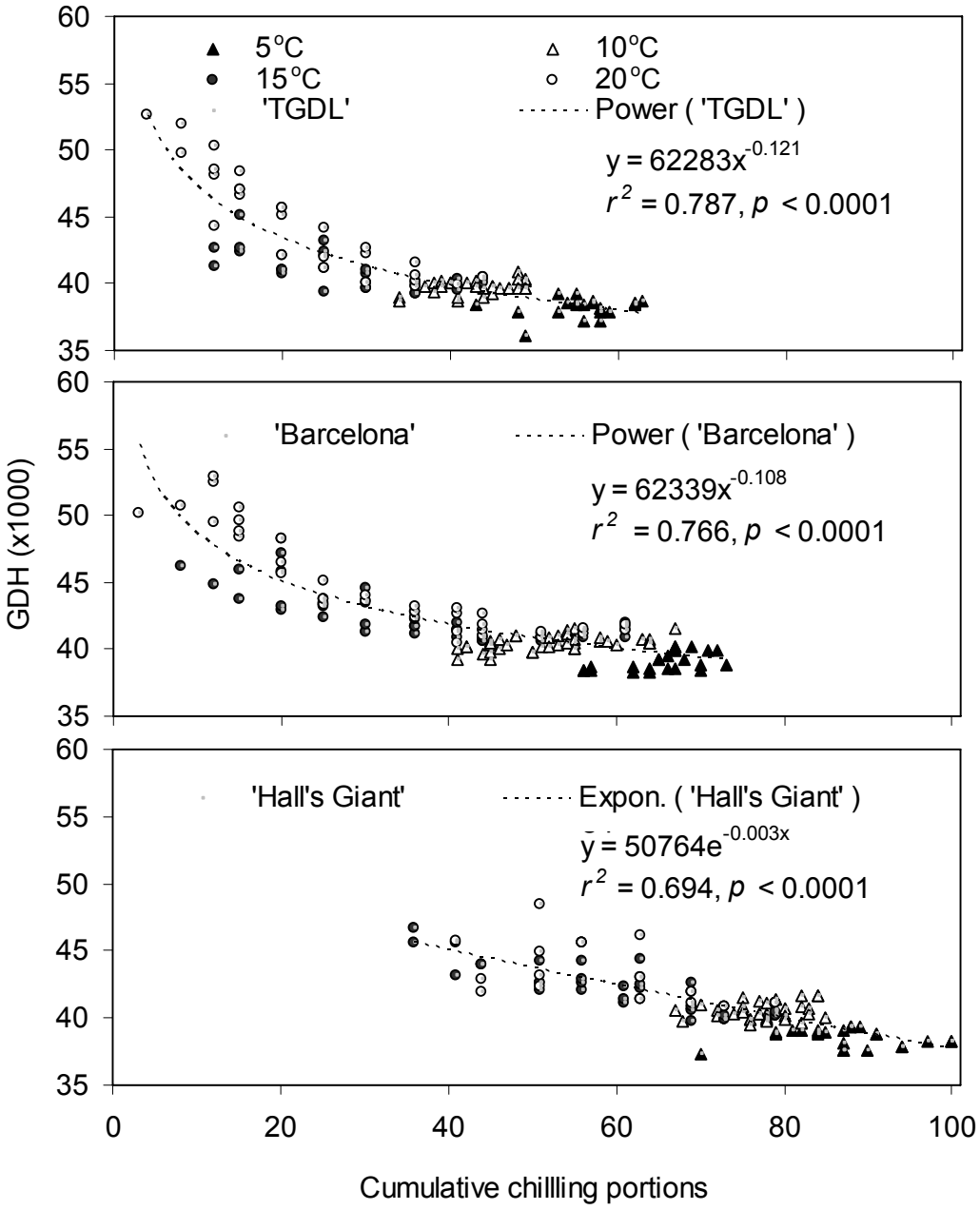

Fig. 3.3b. Regression between cumulative chilling portions and cumulative growing degree hours (GDH) for catkins to reach anthesis of three hazelnut cultivars that were held at 5, 10, 15, and 20 °C.

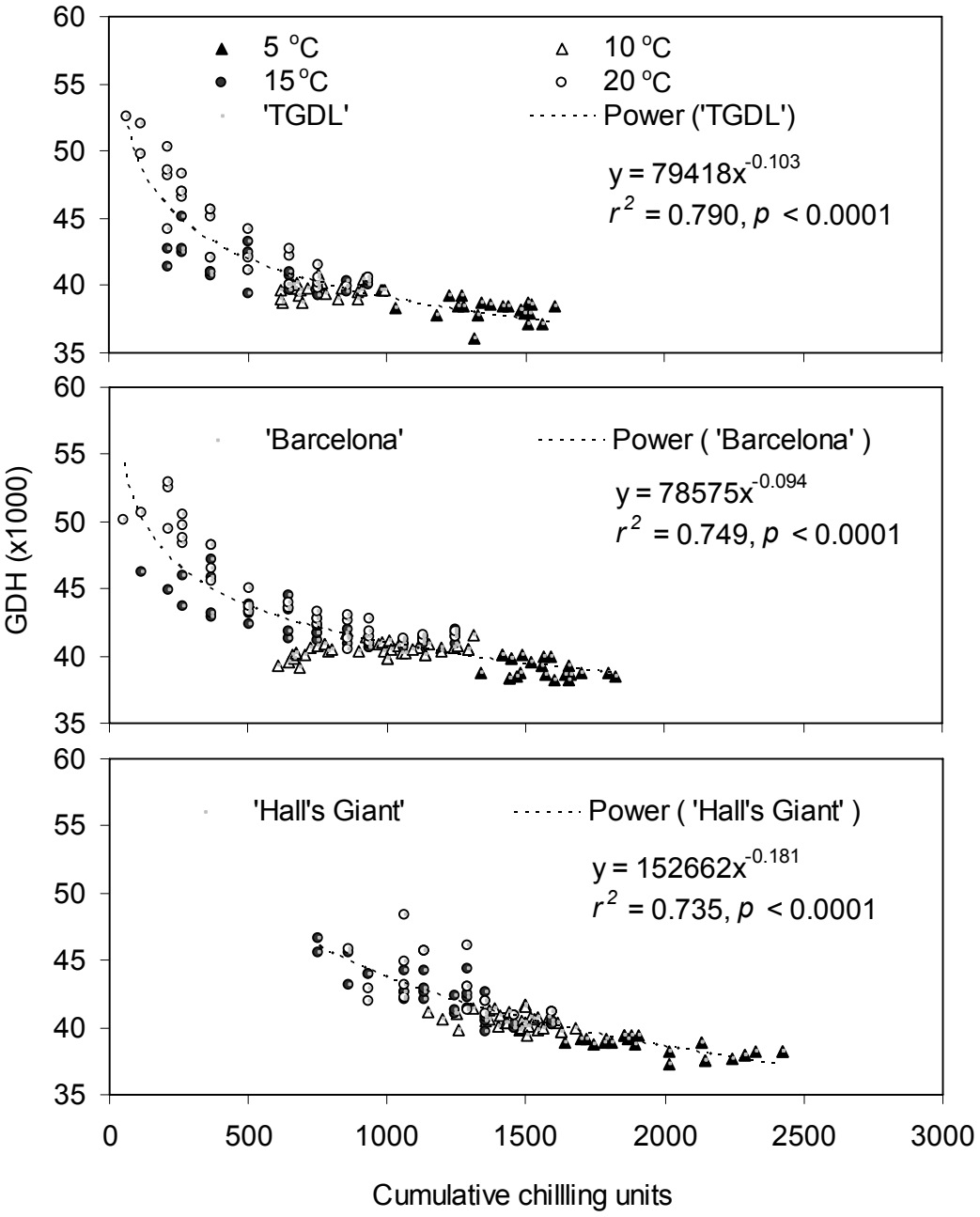

Fig. 3.3c. Regression between cumulative chilling units, according to the Utah model, and cumulative growing degree hours (GDH) for catkins to reach anthesis of three hazelnut cultivars that were held at 5, 10, 15, and 20 °C.

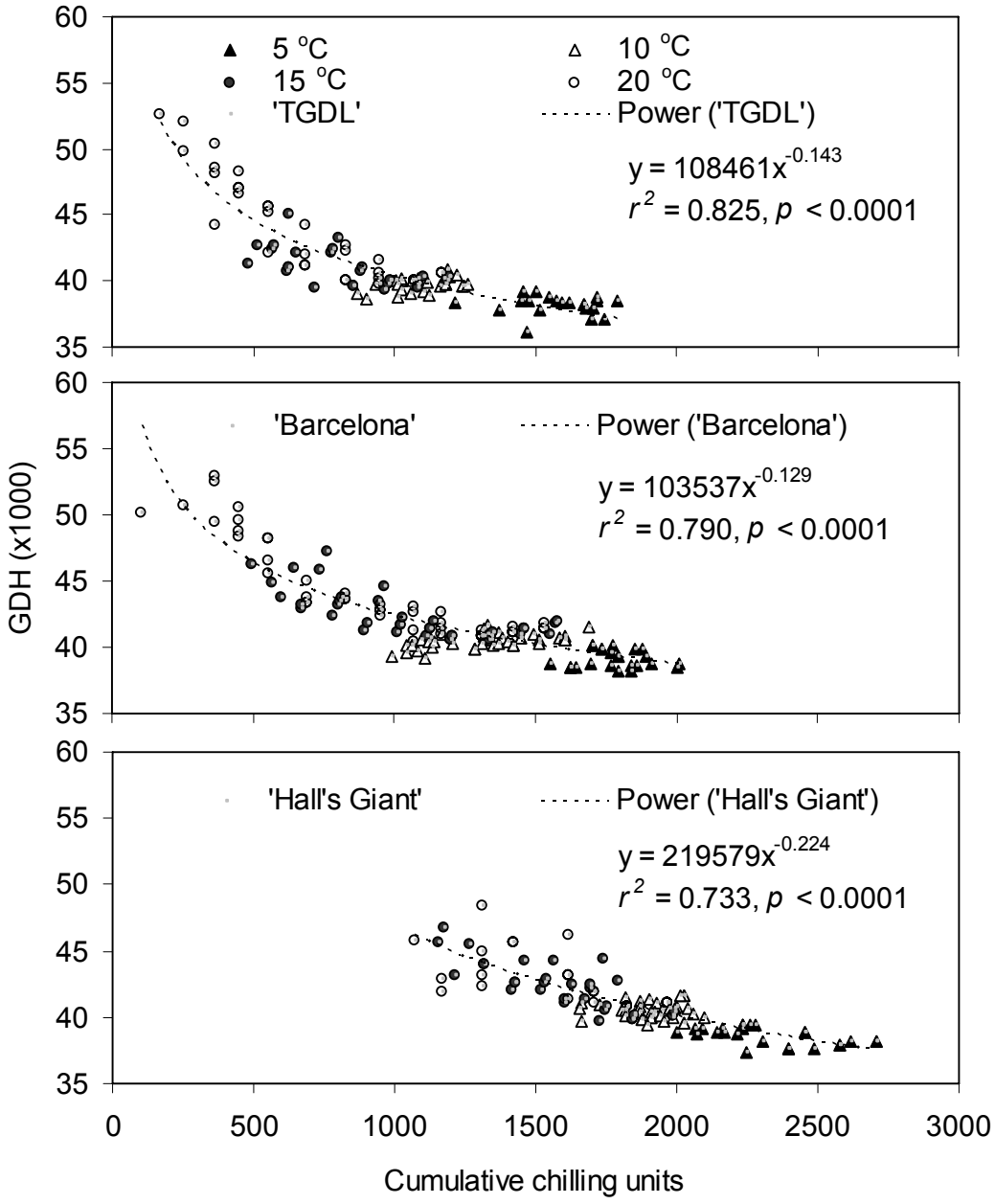

Fig. 3.3d. Regression between cumulative chilling units, according to this study, and growing degree hours (GDH) for catkins to reach anthesis of three hazelnut cultivars that were held at 5, 10, 15, and 20 °C.

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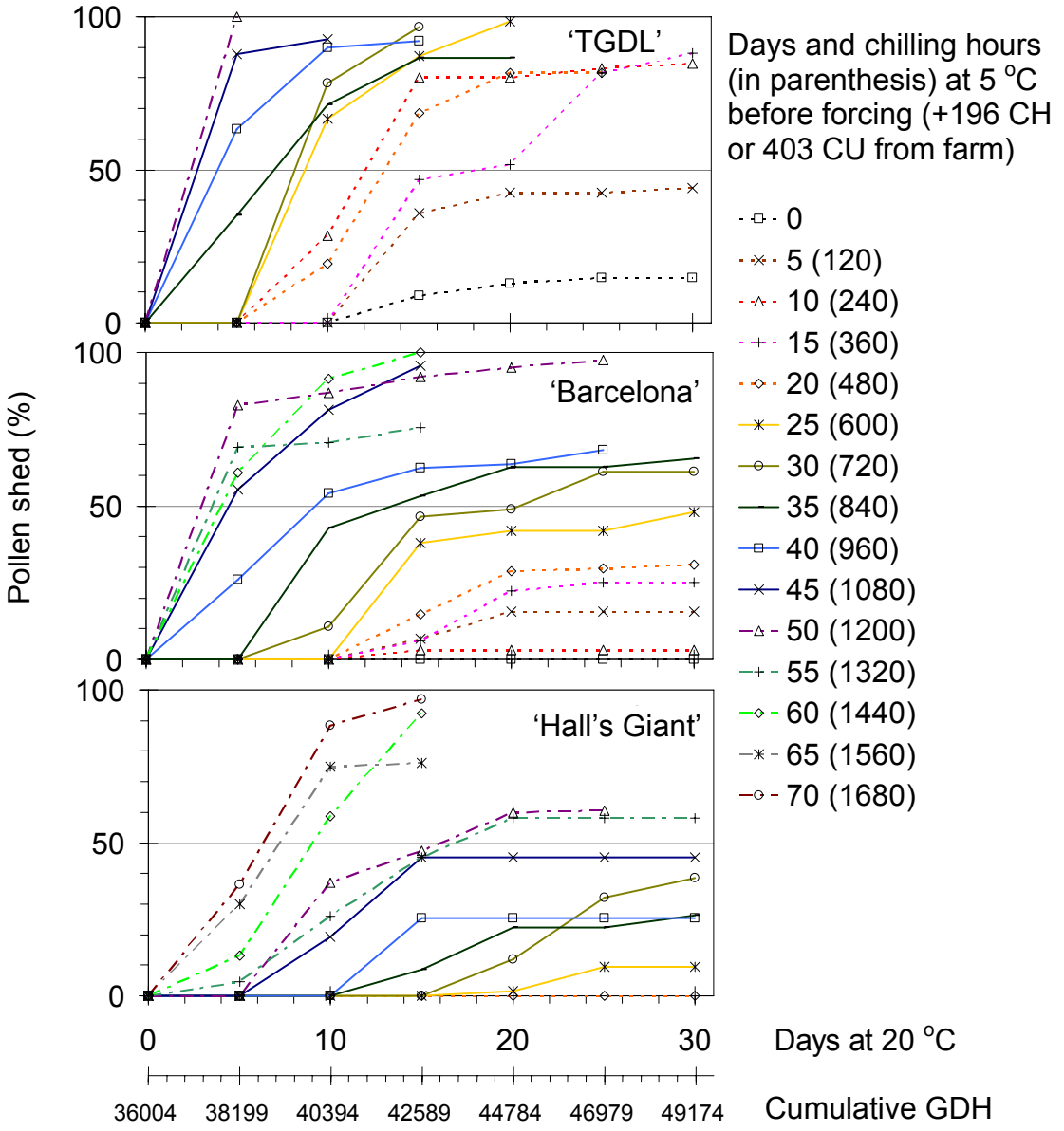

Fig. 3.4. Percent pollen shed of three hazelnut cultivars cut on 1 Nov. 2006 and held at 5 °C for up to 70 days, then forced at 20 °C for up to 30 days (n= 5).

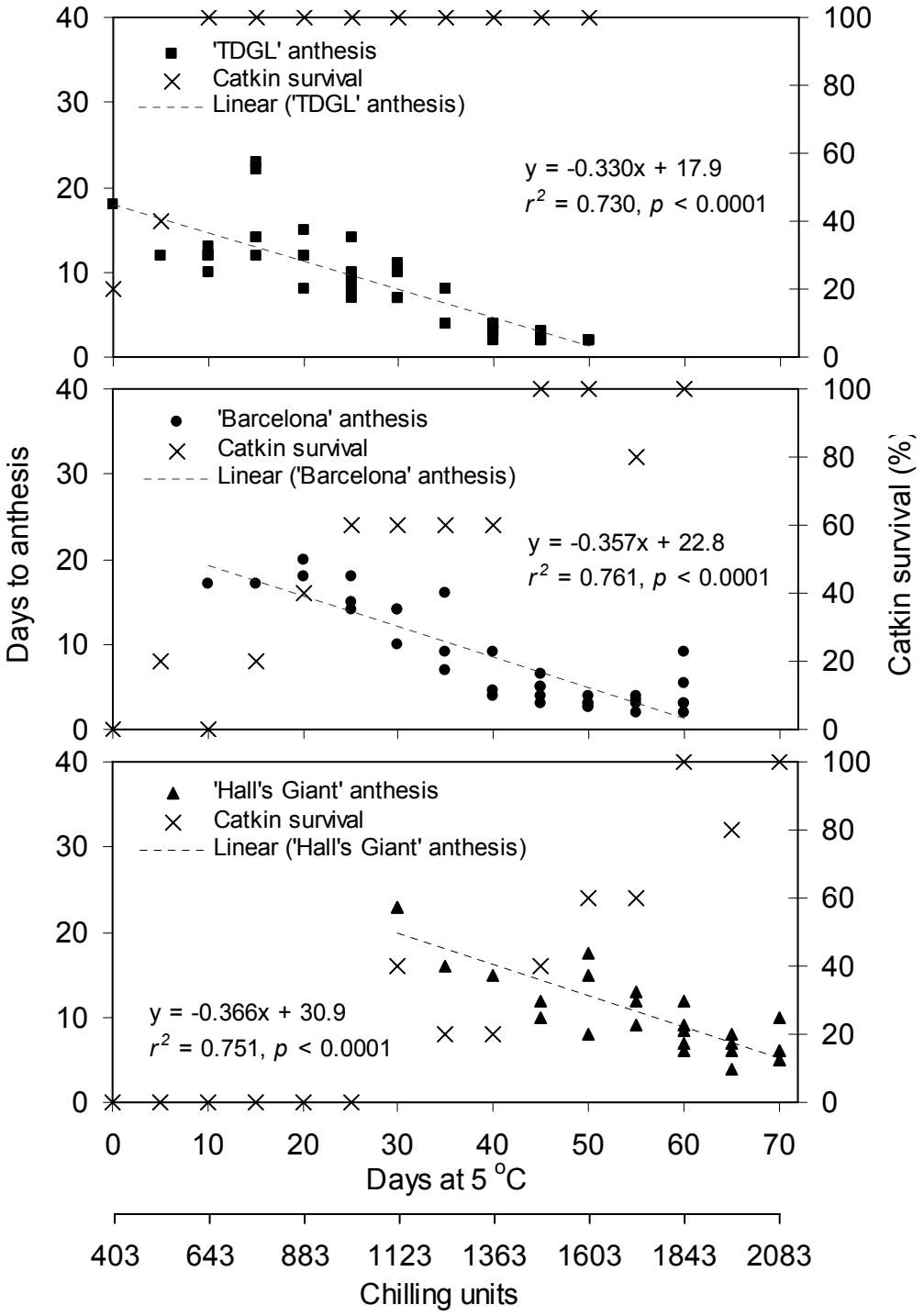

Fig. 3.5. Catkin survival percentage and regression between days at 5 °C and days to reach anthesis of three hazelnut cultivars cut on 1 Nov. 2006 and held at 5 °C for up to 70 days, then forced at 20 °C for up to 30 days (n = 5 on start forcing date).

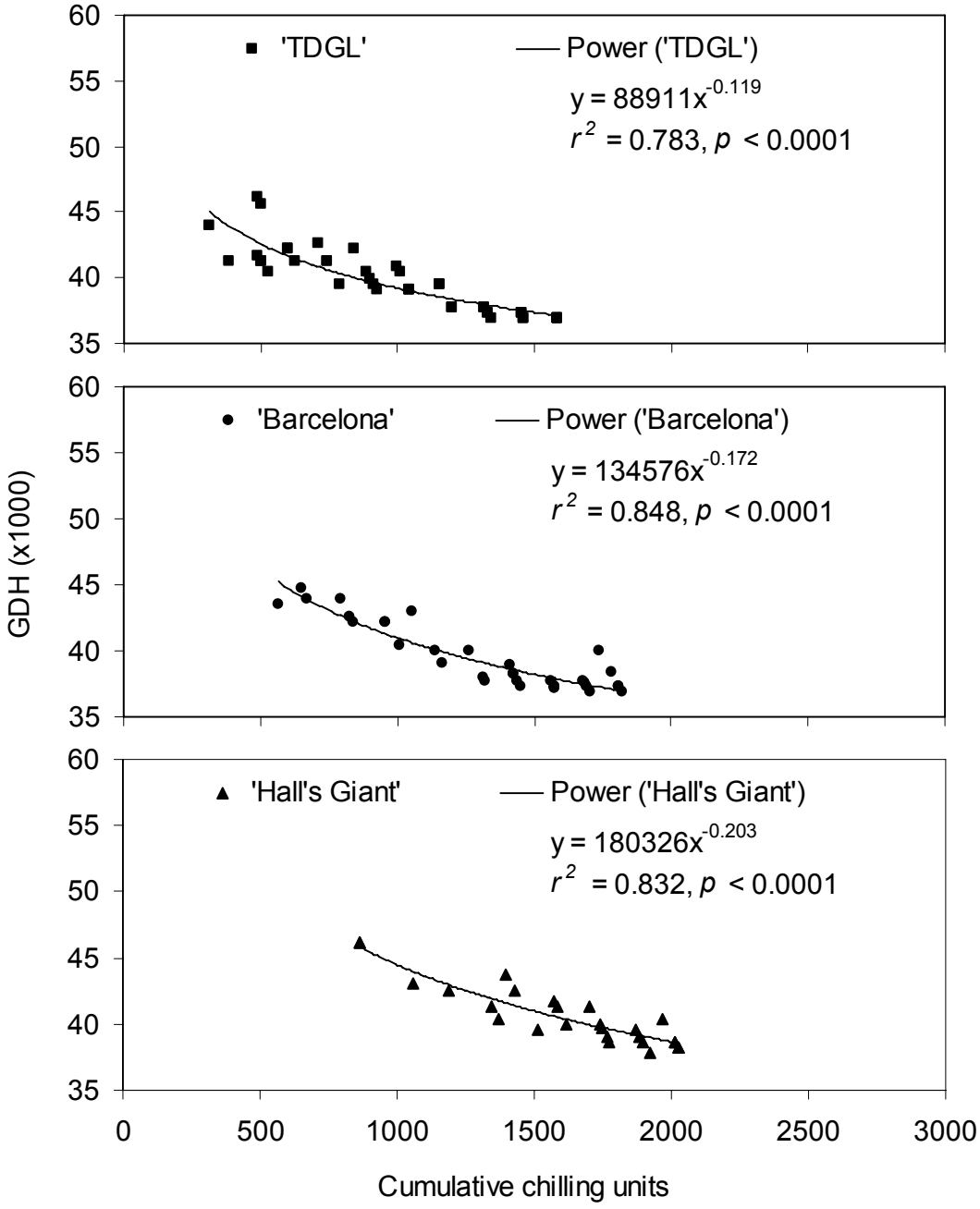

Fig. 3.6. Regression between cumulative chilling units, according to this study, and cumulative growing degree hours (GDH) of three hazelnut cultivars cut on 1 Nov. 2006 and held at 5 ^{o}C for up to 70 days, then forced at 20 ^{o}C for up to 30 days.

Table 3.1. Regression between number of days sampling from 1 Oct. 2006 (x) and number of days to reach anthesis (y) of three hazelnut cultivars after weekly cutting interval which combined four temperatures (5, 10, 15, and 20 ^{o}C), date to reach anthesis estimated from the equations, and actual period that anthesis occurred in the field.

Cultivars	Regression $y = b_1x + b_2$	Coefficients	95% Confidence interval		Estimated anthesis date	Actual anthesis period
			Lower bound	Upper bound		
'TGDL'	y = -0.632x + 51.973	b_1	-0.753	- 0.510	21 Dec.	18-26 Dec.
	r^2 = 0.502, df = 1 and 105	b_2	45.549	58.397		2006
	p < 0.0001					
'Barcelona'	y = -0.453x + 48.543	b_1	-0.513	-0.393	15 Jan.	8-16 Jan.
	r^2 = 0.603, df = 1 and 146	b_2	44.370	52.717		2007
	p < 0.0001					
'Hall's Giant'	y = -0.595x + 80.314	b_1	-0.685	-0.506	12 Feb.	6-12 Feb.
	r^2 = 0.596, df = 1 and 117	b_2	71.172	89.456		2007
	p < 0.0001					

Table 3.2. Regression between days at 5 ^{o}C (x) and number of days to reach anthesis (y) of three hazelnut cultivars, and estimated days at 5 ^{o}C and chilling units that anthesis may occur without forcing (where y = 0).

Cultivars	Regression $y = b_1x + b_2$	Coefficients	95% Confidence interval		Estimated days at 5 ^{o}C	Estimated CU
			Lower bound	Upper bound		
'TGDL'	y = -0.330x + 17.865	b_1	-0.391	-0.269	54	1699
	r^2 = 0.730, df = 1 and 44	b_2	15.904	19.825		
	p < 0.0001					
'Barcelona'	y = -0.357x + 22.773	b_1	-0.427	-0.285	64	1939
	r^2 = 0.761, df = 1 and 33	b_2	19.681	25.864		
	p < 0.0001					
'Hall's Giant'	y = -0.366x + 30.909	b_1	-0.454	-0.277	84	2419
	r^2 = 0.751, df = 1 and 24	b_2	25.842	35.976		
	p < 0.0001					

Table 3.3. Coefficients and their 95% confidence intervals of linear-equations transformed from power-equations in Figure 3.3d and 3.6

Figure no. and varieties	Power-equation $y = b_2x^{b1}$	Linear-equation $Log(y) = b_1 Log(x) + Log(b_2)$ $= b_1 Log(x) + b_3$	Coefficients	95% Confidence interval	
				Lower bound	Upper bound
Fig. 3.3d					
'TGDL'	$y = 108461x^{-0.143}$	$Log(y) = -0.143Log(x) + 5.035$	B_1	-0.156	-0.130
			B_3	4.998	5.073
'Barcelona'	$y = 103537x^{-0.129}$	$Log(y) = -0.129Log(x) + 5.015$	B_1	-0.140	-0.119
			B_3	4.982	5.049
'Hall's Giant'	$y = 219579x^{-0.224}$	$Log(y) = -0.224Log(x) + 5.342$	B_1	-0.248	-0.199
			B_3	5.261	5.422
Fig. 3.6					
'TGDL'	$y = 88911x^{-0.119}$	$Log(y) = -0.119Log(x) + 4.949$	B_1	-0.138	-0.100
			B_3	4.893	5.005
'Barcelona'	$y = 105868x^{-0.139}$	$Log(y) = -0.139Log(x) + 5.025$	B_1	-0.166	-0.112
			B_3	4.942	5.108
'Hall's Giant'	$y = 180326x^{-0.203}$	$Log(y) = -0.203Log(x) + 5.256$	B_1	-0.241	-0.164
			B_3	5.133	5.379

CHAPTER 4

GENERAL CONCLUSION

CHAPTER 4 GENERAL CONCLUSION

Pollen development was studied in nine cultivars of hazelnut that had a range in bloom time from late November until early February. Pollen development was compared at ten sampling dates in order to determine if developmental differences occurred across the genotypes. Early cultivars began differentiation earlier. The total time from the differentiation to anthesis of early cultivars was shorter than late cultivars. The onset of anthesis is possible as soon as the minimum chilling requirement has been met or once the mature pollen stage is reached. A limiting factor for complete anthesis is that an adequate amount of heat in order to result in catkin elongation which will enable pollen shed.

The model of catkin development and temperature is proposed in Fig. 2.4. The catkin length increases and is concomitant with microsporogenesis or stage 1-5. During this period, the young catkins with archesporial cells go through several developmental stages as explained in Chapter 2 up to the point where free microspores are formed (Stage 5). After this period catkin growth goes through a lag phase where no external development and elongation is visible. We think chilling is needed to complete development from the microspore stage to the mature pollen grain stage (Stage 5-7). The chilling requirement is considered adequate when catkins reach the pollen maturation stage (Stage 7). The catkins stay at this stage until a required

amount of heat, accumulated as growing degree hours (GDH), is received. Then the rachis will elongate rapidly, allowing exposure of anthers to the air, the septum to separate and release pollen.

In addition to the above microscope study, the cultivar-related chilling (calculated in chill hours; CH) and heat (calculated in GDH) requirements were studied. Early cultivars appeared to need less CH than the later cultivars. In our calculations, CH was accumulated between 0-7 ^{o}C and GDH was accumulated between 4-25 ^{o}C. With temperatures between 4-7 ^{o}C, plants were able to accumulate both CH and GDH.

In order to determine the effects of chilling and GDH requirements, we studied the responses of different cultivars under semi-controlled field studies and more controlled laboratory studies. For the three cultivars studied, when the minimum chilling requirement was met, catkins required more GDH to elongate and shed pollen. When twigs were exposed to more chilling than the minimum requirement, catkins responded to heat at a faster rate. However, after a certain point, additional chilling did not cause any changes in response time to reach anthesis.

Based on our results, a model of the role of CU and GDH in anthesis of hazelnut staminate flowers is proposed (Fig. 4.1). Two forms of dormancy are hypothesized in our model. Endodormancy, which is affected by physiological factors inside the plant, is insinuated to start when catkin length reaches a plateau (approximately Stages 5) until pollen grains reach maturity (Stage 7).

During this period a specific amount of chilling (minimum) is needed for each cultivar. At the end of the endodormant phase, the catkins can be induced to elongate and anthers to shed pollen with a certain amount of GDH (nearly the same for the three hazelnut cultivars in this study). Ecodormancy, which is affected by environmental factors, is purported to occur during this heat requirement period. Less GDH are required when more CU's accumulate over the minimum required amount. These values differ for different cultivars with early blooming cultivars needing less CU's compared to late blooming cultivars.

In late cultivars, for example, 'Hall's Giant', when the minimum chilling requirement was barely met or in cases where the minimum chilling requirement was not met, and plants were subjected to heat, high rates of catkin abscission were observed. An estimate of the minimum chilling requirement for each of the studied cultivars were approximately 640 CU for 'TGDL', 1,000 CU for 'Barcelona' and 1600 CU for 'Hall's Giant'. These values were obtained by using our improved method to calculate chilling. In cases where adequate CU's were accumulated but where heat accumulation was suboptimal (in our trials 0°C) catkin development and pollen shed took place at dramatically slower rates than more optimal ecodormant conditions (ranging between 10-20°C).

By using the amount of CU's calculated for 1989-1990 (Mehlenbacher study) and matching them with the 2002-2003 calendar dates of the

microscopy study (Chapter 2), the chilling requirement was considered complete around 10 Oct for ‘TGDL’, 13-16 Nov for ‘Barcelona’, and 18-22 Nov for ‘Hall’s Giant’. These dates are generally correlated with the mature pollen stage on 10 Oct for ‘TGDL’, 24 Oct for ‘Barcelona’, and 22 Nov for ‘Hall’s Giant’. Moreover, CU’s calculated from 1989-1990 of each cultivar fall into the period when hazelnut twigs in the controlled condition study (Fig. 3.5) have at least 50% catkin survival after being moved from the cold room and forced at room temperature.

We propose a model for staminate flower dormancy and growth (Fig. 4.1). We conclude that a minimum amount of chilling must be accumulated, and is coincident with completion of pollen maturation (endodormancy). Once the pollen is mature, then catkins can be induced to shed pollen with adequate GDH (ecodormancy). However, continued chilling reduces the GDH requirement for pollen shed and catkin elongation. Parameters have been established for three important hazelnut cultivars. More research is needed for a better understanding of the period of dormancy, minimum and optimum chilling, and threshold temperature for GDH in order to establish more accurate models to predict staminate bloom. However, the information from this study can be useful to growers planning to establish new hazelnut orchards in areas where hazelnuts have not previously been cultivated. We believe that these parameters will allow growers to plan cultural practices and spray programs that may affect the normal pollen development. In addition,

this knowledge will be useful in breeding program to assess chilling requirements of breeding selections.

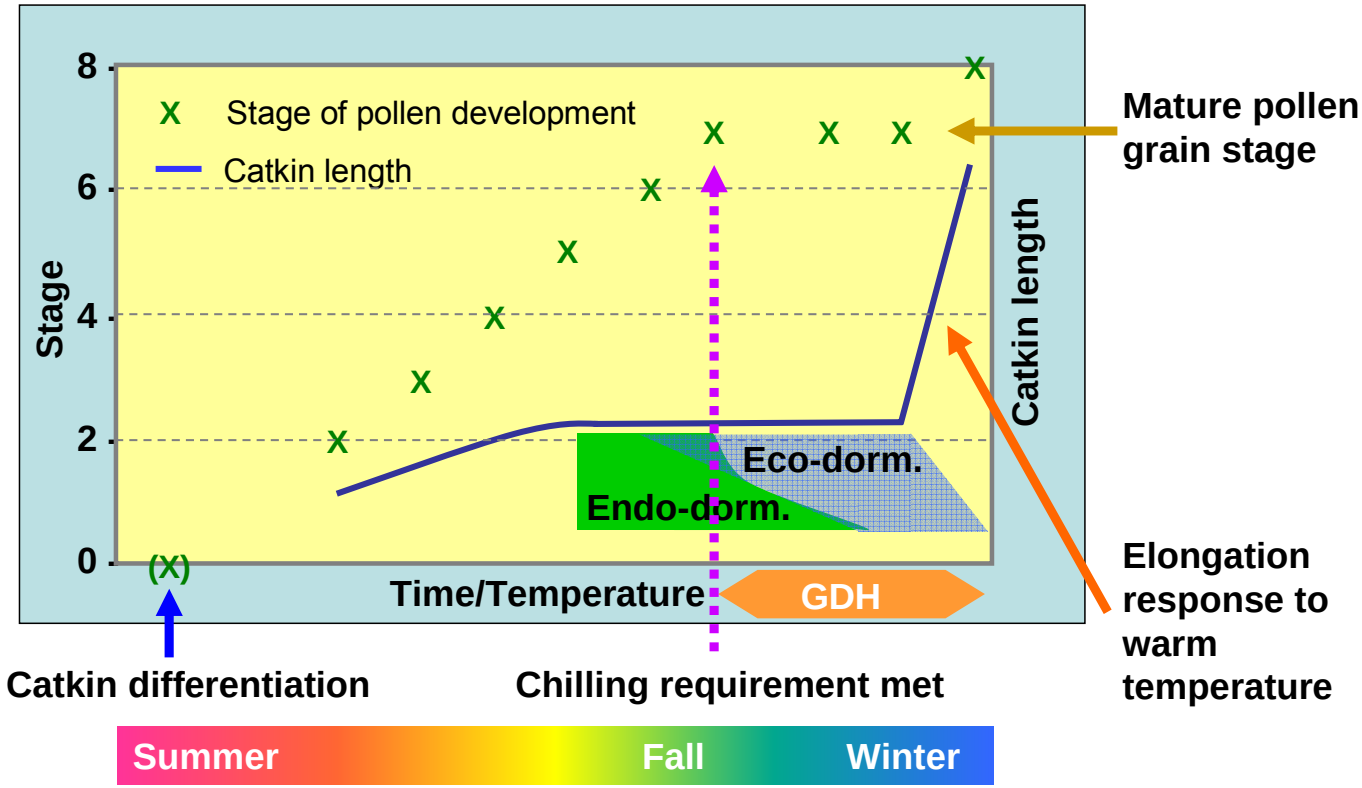

Fig. 4.1. The proposed model of catkin length and stages of pollen development in relation to the chilling requirement and growing degree hour accumulation. Types of dormancy are defined within the developmental period.

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APPENDICES

Appendix A. Stages of pollen development of collected cultivars from August 2002 to January 2003 (n=2)

Cultivars	Stages of pollen development																								
	4-Aug			22-Aug			5-Sep				19-Sep			26-Sep		10-Oct			24-Oct		22-Nov		6-Dec	17-Jan	
	1	2	3	2	3	4	2	3	4	5	4	5	6	5	6	5	6	7	6	7	6	7	7	7	8
'TGDL'			X			X			X	X		X	X		X			X		X		X		X		X
'TDG'		x	X		x	X			X	X		X			X		X		X	X		X		X	X	
'Barcelona'			X		x				X			X			X		X		X	X		X		X	X	
'Creswell'		x	X	x	x			x				X		x			X		X			X		X	X	
'Hall's Giant'		x			x			x	X			X			X		X		X			X		X	X	
'Brixnut'		x		x	x			x			X			x			X		X			X		X	X	
'Gem'		x		x	x			x			X			x		x	X		X			X		X	X	
'Gasaway'	x			x	x			x			X	X		x			X		X		X	X		X	X	
'Contorta'	x			x			x	x			X			x		x	X		X		X	X		X	X	

Stages: 1 = archesporial cells and epidermis, 2 = sporogenous cells, 3 = pollen mother cells, 4 = meiosis/tetrad, 5 = free microspores, 6 = young pollen grains, 7 = mature pollen grain, and 8 = anthesis

Appendix B Chilling hour (CH) and growing degree hour (GDH) to reach tetrad stage (Stage 4), mature pollen stage (Stage 7), and anthesis (Stage 8) of nine hazelnut cultivars collected from August 2002 to January 2003.

Cultivars	Chilling requirement (SAM)[z]	Date which developmental stage occurred				CH			GDH[y]				
						From 1 Oct. to Stage 7	Anthesis		Differentiation to Stage 4	End of microgameto-genesis		Anthesis	
		Differen tiation	Stage 4	Stage 7	Stage 8		Stage 7-8	Stage 1-8		Stage 4-7	Stage 1-7	Stage 7-8	Stage 1-8
'TGDL'	<100	15-Jun	22-Aug	10-Oct	10-Jan	31	869	900	20571	13486	34057	7281	41338
'TDG'	170-240	15-Jun	22-Aug	24-Oct	24-Jan	93	995	1088	20571	16323	36894	4941	41835
'Barcelona'	240-290	15-Jun	5-Sep	24-Oct	24-Jan	93	995	1088	25006	11888	36894	4941	41835
'Creswell'	365-480	22-Jun	19-Sep	22-Nov	31-Jan	294	814	1108	26970	10460	37430	3537	40966
'Hall's Giant'	290-365	22-Jun	5-Sep	22-Nov	31-Jan	294	814	1108	23211	14219	37430	3537	40966
'Brixnut'	365-480	22-Jun	19-Sep	22-Nov	31-Jan	294	814	1108	26970	10460	37430	3537	40966
'Gem'	600-680	22-Jun	19-Sep	22-Nov	31-Jan	294	814	1108	26970	10460	37430	3537	40966
'Gasaway'	600-680	30-Jun	19-Sep	6-Dec	6-Feb	476	705	1181	24587	10459	35046	3755	38801
'Contorta'	990-1040	7-Jul	19-Sep	6-Dec	2-Mar	476	998	1474	22457	10460	32917	4881	37798

[z] chilling hours (CH) according to study of chilling requirement by Mehlenbacher, 1991
[y] cumulative GDH from estimated catkin differentiation dates to specific stages of pollen development

Appendix C. Comparison of cumulative chilling hour (CH), chilling portion, chilling unit (CU) according to Utah model, chilling unit according to this study, and cumulative growing degree hour (GDH) in 2006-2007 growing season.