ISSN: 0394-6169
ISNN: 1592-1573

ADVANCES IN HORTICULTURAL SCIENCE

n. 1-2 **2013**

formerly
«Rivista dell'Ortoflorofrutticoltura Italiana»
founded in 1876

FIRENZE UNIVERSITY PRESS

Advances in Horticultural Science
Published by **Firenze University Press** - University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/ahs

Direttore Responsabile: **Franco Scaramuzzi**, University of Florence, Italy.

ADVANCES IN HORTICULTURAL SCIENCE

Formerly Rivista dell'Ortoflorofrutticoltura Italiana
founded in 1876 and issued by University of Florence, Italy

supported by ENTE CASSA DI RISPARMIO DI FIRENZE

Advances in Horticultural Science is covered in the following indexing and abstracting services: BIOBASE - Biological Abstracts - BIOSIS Previews - Horticultural Abstracts - Ornamental Horticulture - Plant Breeding Abstract

Advances in Horticultural Science is published by the Department of Agri-Food Production and Environmental Sciences, University of Florence, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.
Phone +39-055-4574021-22, Fax +39-055-4574078-17, E-mail: advances@dipsa.unifi.it, Homepage: http://www.dipsa.unifi.it.

SUBSCRIPTIONS - The subscription price of volume 27, 2013 is € 60. 00 in Italy and € 70.00 in other countries. Mailing costs: € 3 for Italy, € 6.50 for Europe and € 10.00 for the rest of the world. The subscription price of an issue is €17.00 in Italy and € 20.00 in other countries.

CONTENTS

Adv. Hort. Sci., 2013 27(1-2): 5-17

Apricot flower bud dormancy: main morphological, anatomical and physiological features related to winter climate influence

R. Viti*, S. Bartolini, L. Andreini***

* *Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Via del Borghetto, 80, 56124 Pisa, Italy.*

** *Istituto di Scienze delle Vita, Scuola Superiore Sant'Anna, Piazza Martiri della Liberta, 33, 56127 Pisa, Italy.*

Key words: budbreak, climate, endodormancy process, *Prunus armeniaca* L.

Abstract: This review examines recent advances regarding flower bud dormancy in apricot, focusing on biological, anatomical, and physiological processes which occur during the induction and depth of dormancy. In a scenario of global climate change, the relationship between endodormancy and winter climate influence is discussed. Dormancy regulation is a complex process necessary for plant survival and development. In fruit species, the knowledge of mechanisms controlling dormancy and establishing its release appears crucial for successful yields. Specific studies have suggested that, when the flower buds are apparently inactive, slow and gradual changes occur in the whorls: organogenesis, such as microsporogenesis processes and vascular connections take place during the entire dormancy period. It has been indicated that an asynchronism between biological (i.e. endodormancy release, microsporogenesis evolution), anatomical (i.e. xylem vessel differentiation) and biochemical (i.e. changes in metabolic compounds and enzymes) events could represent further causes determining an inconstant rate of blooming. Temperature is the main factor involved in dormancy triggering and releasing. In the perspective of global warming, mild winter temperatures could greatly impact apricot ecological cropping systems. Phenological process-based models are considered to be the best tool to study the climatic changes and subsequent expected phenology variation (dormancy and flowering). A new model, calibrated and validated on apricot cultivars, is proposed to predict the dormancy release date in a future scenario.

1. Introduction

In stone fruit trees, the cycle of shoot growth is marked by the development of two bud types: the vegetative and reproductive buds. Vegetative buds comprise bud scales, leaf primordia, and the shoot apical meristem. Reproductive buds have a more complex structure made up of bud scales and flower primordia represented by pistil and stamens. The flower buds of temperate-zone fruit trees are initiated during the previous growing season and several critical steps during bud morphogenesis were identified, from floral induction up to complete bud differentiation (Ryugo, 1990).

During the autumn-winter season, the plant elaborates mechanisms for survival under unfavorable growing conditions by adopting a dormancy strategy to cold temperature acclimation. Meristem activity becomes insensitive to growth-promoting signals (Rohde and Bhalerao, 2007) preserving the buds in a quiescent state under potentially damaging environmental conditions (Ćechová *et al.*, 2012). Bud dormancy starts with the perception by the plant of rest-signals under the influence of short and cool days; this process finishes after an accumulation of chilling temperatures.

The conventional terminology identifies dormancy evolution as: *paradormancy*, when growth is inhibited by endogenous factors outside the dormant structure; *endodormancy*, when growth is regulated by physiological factors within the dormant structure; and *ecodormancy*, when the inability to grow is imposed by environmental factors (Lang *et al.*, 1987). In trees, winter dormancy means endodormancy: during this phase the chilling temperatures are accumulated, and flower buds are unable to respond to warm temperatures required for dormancy breaking (Fig. 1). When the chilling requirement (CR) is fulfilled, buds are in ecodormancy and can react to warm temperatures, but remain in an apparent quiescent state due to persistent low temperatures (Horvath *et al.*, 2003). The release of dormancy is genetically controlled and regulated by complex phenomena affected by various integrated elements whose interaction(s) determine the point in time when release of bud dormancy occurs (Faust *et al.,* 1997). En-

The authors equally contributed to the manuscript.

Received for publication 21 December 2012
Accepted for publication 2 April 2013

vironmental factors (temperature, light, relative humidity, etc.) are directly related to biochemical changes involved in bud dormancy release. In fruit species, understanding the mechanisms controlling dormancy and establishing its release appear crucial for successful yields.

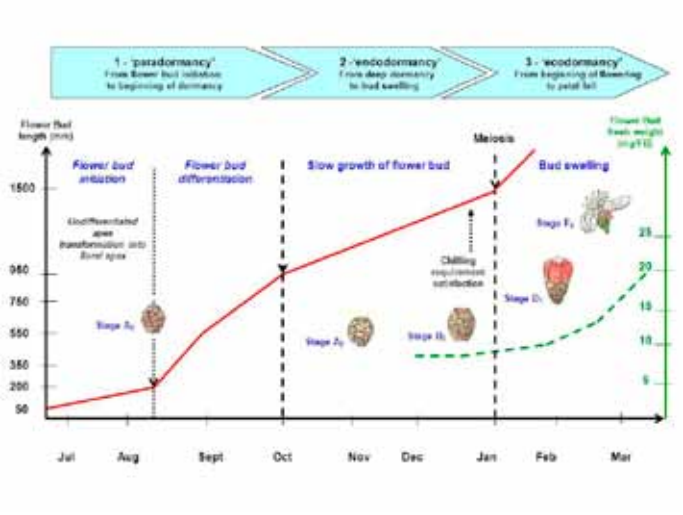


Fig. 1 - Schematic representation of apricot flower bud growth during the annual cycle**.** 1) Paradormancy: from flower bud initiation to beginning of dormancy; 2) Endodormancy: from deep dormancy to bud swelling; 3) Ecodormancy: from beginning of flowering to petal fall. Solid and dotted lines represent the flower bud length (mm) and the flower bud fresh weight (mg/FB), respectively. Evolution of the main phenological stages of flower buds are illustrated.

Apricot *(Prunus armeniaca* L.) is characterized by a restricted spread in the Mediterranean basin and its adaptability to specific environments is strongly influenced by the ability of certain cultivars to overcome flower bud dormancy (Viti *et al.,* 2010). Climatic-environmental factors may heavily influence flower bud dormancy breaking, affecting the entity of appearance of floral anomalies and determining irregular and/or insufficient fruit bearing (Clanet and Salles, 1972; Legave *et al.,* 1984; Viti and Monteleone, 1995). Cold winter temperatures influence the time of bud dormancy release and, consequently, the CR of a genotype (Garcia *et al*., 1999). Most of the known apricot cultivars are characterized by a high CR (Table 1); more than 1000 Chill Units (CU) are required to overcome bud dormancy (Guerriero *et al*., 2006). In Mediterranean apricot growing areas, characterized by a mild-winter climate, many cultivars have an inadequate satisfaction of CR. During a short or mild rest season (Garcia *et al.,* 1999), the unsatisfactory CR can determine an incomplete release of flower bud dormancy, a late bud break with scanty blooming and a high flower bud drop (Viti and Monteleone, 1991; Erez, 2000). Thus, CR is a key factor for breaking dormancy and knowledge about it has practical significance and economic impact on the control, maintenance and production of woody plants (Fennell, 1999). This statement appears crucial for the crop management of apricot cultivars in most cultivation areas.

Table 1 - Chilling Units (CU) required to induce break of endo-dormancy in flower buds of several apricot cultivars. Starting date and amount (%) of flowering are reported

Cultivars	CU	Flowering	
		Starting date	%
San Castrese	870	1 Mar	57
Goldrich	950	7 Mar	18
Sarritzu I°	950	7 Mar	51
Alessandrino	1000	7 Mar	10
Baracca	1000	14 Mar	21
D'Alessandria	1000	10 Mar	5
Bebeco	1030	7 Mar	15
Canino	1030	2 Mar	34
Moniqui	1125	5 Mar	2
Aurora	1140	29 Feb	2
Amabile Vecchioni	1140	2 Mar	30
S.Nicola Grosso	1140	9 Mar	25
Bergeron	1225	17 Mar	1
Rapareddu	1250	7 Mar	23
Polonais	1300	9 Mar	5
Orange Red	1450	14 Mar	1
Stark Early Orange	not defined	not defined	0
Mean	1100		
SD	151		

The present paper examines the dormancy process of apricot flower buds, focusing on biological, anatomical, and physiological changes that occur during the endodormancy phase in relation to winter temperature influence within the context of global climate change.

2. Morphological and anatomical features

The pattern of apricot floral organogenesis after initiation is typical of other Prunoideae. The first floral organs to appear are sepals and petals followed by the stamen and pistil; the most internal whorls of the pistil derive from the gradual evolution of the receptacle (Monet and Bastard, 1968; Bartolini *et al.,* 2013). This process requires about two months and is normally completed before leaf fall but not all buds reach the stage of pistil appearance (Legave, 1975).

Apricot flower bud evolution was described for the first time by Baggiolini (1952). The classification has been revised to describe accurately the progress of flower bud phenological development by the addition of sub-phases within each phenological stage (Bartolini *et al.,* 2004). Figure 2 depicts the evolution of flower bud phenological stages from deep dormancy (stage A) until petal fall (stage H). The stages related to the dormancy process are referred to as: 1) stage A_0-A_2 during endodormancy; 2) stage B_1- E_2 from ecodormancy to bud swelling; and 3) stage F_1-H from beginning of flowering to petal fall. Dormant buds (stages A_0, A_1, A_2) are characterised by a conical shape, rounded at the base, with the brown bud scales tightly closed (A_0) or

Fig. 2 - Evolution of flower bud phenological stages in apricot: deep dormancy (stage A), pink sepal tips appearance (stage B), sepals clearly visible (stage C), achievement of bud swelling (stage D), petals clearly visible (stage E), anthesis (stage F), end of flowering (stage G) and petal fall (stage H). From Bartolini *et al.* (2004).

more enlarged (A_1 and A_2); at the beginning of bud swelling (stages B_1 and B_2) the pink sepal tips appear; at stages C_1 and C_2 sepals are clearly visible; at stages D_1 and D_2 full bud swelling takes place with the appearance of the white petal tips; at stages E_1 and E_2 petals are clearly visible; at stages F_1 and F_2 full flowering occurs; at stages G and H petal fall begins with the end of flowering.

During endodormancy, the accumulation of chilling temperatures occurs and it is usually believed that flower bud development is arrested because no clear signs of growth are evident. However, specific studies have shown that bud growth is hardly perceptible, although constant (Alonso *et al.,* 2005). The buds are metabolically active and continue their development during the entire winter, leading to bud break. In peach flower buds continuous anatomical development during the late autumn and winter dormancy period were observed, without macroscopic changes (Reinoso *et al.,* 2002). Apricot flower buds also have a gradual and prolonged development during morphogenesis since their organogenesis is not generally completed until just before anthesis (Erez and Couvillon, 1987). During the first stage (A) a minimal variation in bud size and weight was detected. Indeed, during December and January, when buds are apparently still dormant, it was possible to detect a continuous growth in weight and size. The parameters of weight increase and height/width ratio have revealed a statistically appreciable slow and progressive buds growth from the middle of December (Guerriero *et al.,* 1986; Scalabrelli *et al.,* 1991).

During this apparent rest period, anatomical observations revealed that processes of microsporogenesis, macrosporogenesis and xylogenesis take place. As regards microsporogenesis, the following phases have been identified (Fig. 3) in relation to flower bud development (Nyújtó and Banai, 1975; Viti and Scalabrelli, 1988; Viti and Monteleone, 1991): a) *Sporigen Cells Differentiation* (sticked cells), during the paradormancy period; b) *Pollen Mother Cells* (diploid microsporocytes differentiated from sporogenous cells that become spherical and separated; c) *Tetrads appearance* (four haploid microspores produced by meiosis of the pollen mother cells and surrounded by a callose wall) during the endodormancy period; d) *Young Pollen Grains* (isolated microspores produced when the callose wall disappears) at the overcoming of endodormancy; e) *Mature Pollen Grains* (full development of the wall made up of two layers, exine and intine) after the resumption of growth. The tetrad stage has been considered a signal marking the end of endodormancy (Bordeianu *et al.,* 1962; Szabò *et al.,* 2002). Nevertheless, other studies have shown that tetrads occur at a late stage of morphological bud growth and they are not closely linked to chilling accumulation, because the meiosis process took place also in buds exposed to high temperature (Martìnez-Tellez *et al.,* 1982; Felker *et al.,* 1983; Viti and Scalabrelli, 1988). Contradictory studies have shown that winter dormancy sets a boundary between the development of the sporogenous tissue and further microspore development (Julian *et al.,* 2011). These authors observed that, in autumn, stamens develop until the differentiation of sporogenous tissue, remaining in this anatomically quiescent stage during the three months of winter; microspore development took place only after dormancy.

Fig. 3 - Main microsporogenesis phases (x 400). **a**) Pollen Mother Cells (PMC); **b), b$_1$**) Tetrads (T) and **c**) Young Pollen Grains (YPG).

Concerning gyneceum development and the related macrosporogenesis, a later evolution than stamen and microsporogenesis process was observed. Morphologically, the pistil has a lengthwise growth that can be divided into different phases: intensive growth during paradormancy; slow growth during endodormancy; and increased growth during ecodormancy, at first very slowly then followed by a significant rapid rise a few weeks before flowering (Szalay and Nemeth, 2010).

Anatomical observations showed that the first signs of the ovary can be detected in October. Several studies have found a well-developed embryo sac at anthesis; Egea and Burgos (1995) found a frequent presence at anthesis of ovules without the sac being formed. In apricot, two to three ovules/ovary are usually noted, but at anthesis they are frequently malformed and quickly degenerate. Ovule

number seems to be related to agronomic and climatic conditions, rather than genetics (Egea and Burgos, 1995; Burgos and Egea, 1993; Albunquerque *et al.,* 2002).

At the end of the endodormancy process (end of winter-beginning of spring), a rapid weight increase and the complete development of floral verticils at the transition from phenological stage B (visual beginning of bud swelling) up to stage D (appearance of the white petal tip) have been observed. In this period, elongation of pistil and stamen filaments occurs while mature pollen grains and complete gynaecium development take place only few days before blossoming (Luna *et al*., 1990). In concomitance with the effective warm temperatures, female and male gametophytes become functional.

During the temporary delay of visible bud growth, the process of xylem development within the flower bud axis occurs. The bud, during early growth, is connected to the stem through a parenchymatous zone, traversed by a procambial strand. In several *Prunus* species, it was observed that, during winter, vascular tissues are not completely differentiated and the connection between the flower primordium and the bud axis is formed only by the procambium (Ashworth and Rowse, 1982). This tissue, which constitutes the vascular strands, is made up of elongated cells and contains densely stained cytoplasm and lacks lignified secondary wall thickenings (Esau, 1965). The differentiation process consists of the transition from this meristematic tissue to xylem cells, i.e. dead cells with lignified walls producing an empty conduit through which water flows. In apricot, gradual xylem development was observed during winter, when no bud growth changes were visible. The acropetal progression of primary xylem differentiation along the flower bud axis was defined by five stages according to Bartolini and Giorgelli (1994): *stage 1* = at the base of the axis; *stage 2* = at ½ of the axis; *stage 3* = at ¾ of the axis; *stage 4* = at the base of the ovary; *stage 5* = inside the pistil (Fig. 4). Stage 3 appears to be the most significant with regard to breaking dormancy because it is in concomitance with the first morphological sign of bud growth resumption. A good relationship between an advanced xylem differentiation ('stage 3') and endodormancy release was observed in cultivars with a low chilling requirement (i.e. 'San Castrese'). The availability of nutritional elements throughout xylem supply in correspondence with bud growth reactivation could be a factor determining flowering regularity. A correlation between an increase of certain elements (i.e. potassium and boron) by the xylem acropetal transport and bud swelling has been observed (Hanson and Breen, 1985; Essiamah and Eschrich, 1986; Bartolini and Giorgelli, 1995). On the other hand, in cultivars with a high chilling requirement (i.e. 'Orange Red'), xylem 'stage 3' occurs when flower buds are still in endodormancy. This early anatomical trait of flower buds does not coincide with the reactivation of bud growth; it could be hypothesized that the newly formed vessels might preserve the ability to function in water transport. This feature was confirmed by a recent research where a good syncronism between overcoming

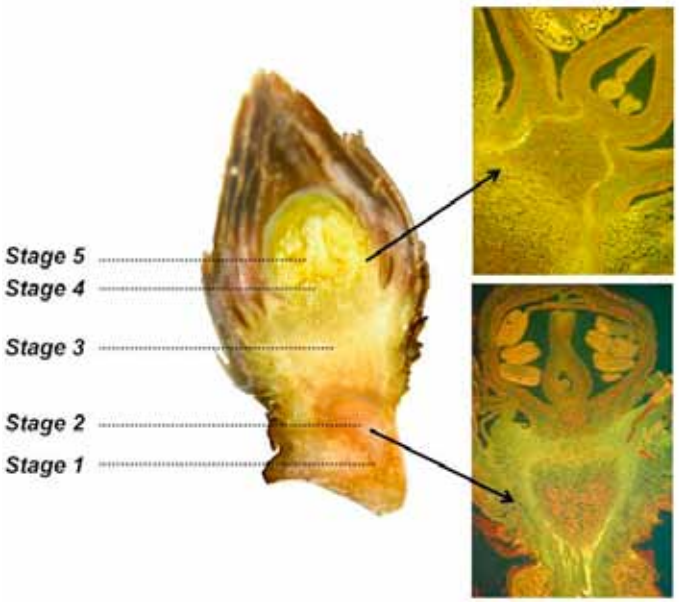


Fig. 4 - Representation of xylem vessel differentiation along the flower bud axis, from Bartolini *et al*., 2006: *stage 1* (at the base of the axis); *stage 2* (at ½ of the axis); *stage 3* (at ¾ of the axis); *stage 4* (at the base of the ovary); *stage 5* (inside the pistil).

dormancy, xylogenesis and microsporogenesis, was found in cultivars with a low CR, while in cultivars with a medium or high CR an asynchrony between such processes was observed (Bartolini *et al.*, 2006 a), leading to inconstant blooming and fruit yield.

3. Physiological features

Control of plant dormancy is the result of multifactorial regulatory networks in which nutrients, phytohormones, genes, proteins and climatic factors (namely temperature and photoperiod) are involved at some point in time (Chao and Anderson, 2010). Light and temperature climate parameters are important determinants in several aspects of dormancy. In northern temperate regions, the stimulus for induction of growth cessation and dormancy has been considered to be primarily controlled by short photoperiod (Allona *et al.,* 2008). Increasingly, several authors have shown that temperature may replace or strongly mediate this short photoperiod dormancy response in woody species (Kalcsits *et al*., 2009; Tanino *et al.,* 2010).

The onset and release of dormancy state might begin with the perception of a signal by the plant upon exposure to chilling temperatures (Or *et al.*, 2002). Then, it would be followed by transduction of this signal via a cascade of biochemical events to the stage where it imposes or releases repression of bud meristematic activity (Faust *et al.*, 1997). Many cellular activities leading to morphological, physiological and biochemical changes take place inside the bud during the transition period from endodormancy to active bud growth, including respiratory rate, reserve carbohydrate mobilisation, water content increase, energy transport, and gene expression.

'Sink strength' and carbohydrates

Considering that the bud of a woody plant is morphologically complex and constituted by organs differing in structure and physiology, the bud anabolic potential is partially regulated by the "sink strength" of dormant and non-dormant tissues influencing the subsequent capacity to accumulate metabolites (Crabbé and Barnola, 1996). During early bud growth, the parenchymatous zone, which represents the connection between bud and stem, is fed through the symplasm of this region (Pétel and Gendraud, 1996). Thus, plant dormancy and dormancy breaking appear to depend on peculiar short distance relationships between the bud and its underlying tissues (Champagnat, 1973; Gendraud and Pétel, 1990). In peach, during the dormant period, the parenchyma shows a strong ATPase activity driving a powerful proton extrusion, with a consequent pH cytoplasmatic alkalisation linked to enzyme activity (Pétel *et al.,* 1992; Petél and Gendraud, 1996). A relationship between changes in intracellular pH (pHi) and bud dormancy release has been observed, first, in Jerusalem artichoke and peach vegetative buds (Marquat *et al*., 1996; Aue *et al.*, 2000), and subsequently in apricot buds (Zanol and Bartolini, 2003), suggesting the involvement of pHi changes as signalled by growing evidence (Zimmermann *et al.*, 1999). In particular, when apricot flower buds were still in deep dormancy, an increase of pHi in the flower primordia tissues was found, just before the first sign of growth reactivation, usually denoted by a significant bud weight increase (Fig. 5). The changes in pHi values might be useful to detect in advance bud growth capacity, showing the potential competitive sink for nutrients between the different bud tissues from dormancy to growth resumption (Zanol and Bartolini, 2003). The pHi measurement is a good parameter to estimate 'sink strength' determining the nutrient fluxes (Gendraud and Pétel, 1990; Bonhomme *et al*., 1999; Robert *et al*., 1999).

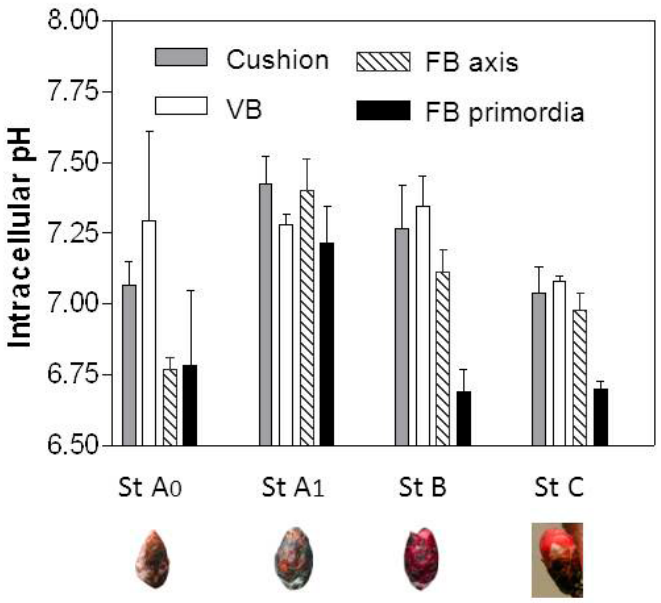


Fig. 5 - Intracellular pH at different phenological stages (from St A_0 to St C). Changes in different tissues: Cushion, Vegetative Bud (VB), Flower Bud (FB) axis, Flower Bud (FB) primordial.

The energy source for budbreak comes mainly from the mobilization of products stored in the perennial parts of the tree. Carbohydrates are the main source of energy for the metabolic changes that occur during the dormant period and for spring sprouting and blooming (Flore and Layne, 1996; Sherson *et al.,* 2003). Changes in the content of carbohydrates in different tissues from vegetative and reproductive structures were found during dormancy, associated with chilling temperatures (Wang and Faust, 1987; Valentini *et al.,* 2006).

There was a significant decrease in starch concentration in the bark tissue of *Prunus* sp. due to exposure to chilling temperatures (González-Rossia *et al.,* 2008). The effect of low temperatures on starch and sugar concentration during the rest period can be explained: amylase activity is induced by cold temperature, increasing starch hydrolysis and, consequently, sugar concentration (Elle and Sauter, 2000; Bonhomme *et al.,* 2005).

Soluble sugars, important signalling molecules involved in many processes in the life-cycle of plants, are also related to the dormancy period and involved in increased frost resistance (Tabuenca, 1975; Sheen *et al.,* 1999; Smeekens, 2000). In particular, starch levels were negatively correlated with hardiness but most soluble sugars were positively correlated (Jones *et al.,* 1999).

Glucose, fructose, and sorbitol were the main sugars in the bark tissues of peach, nectarine, plum and apricot; sorbitol concentrations varied significantly with chilling accumulation (Bonhome *et al*., 2005; González-Rossia *et al.,* 2008). In other sorbitol-synthesizing plants, such as sweet cherry (*Prunus avium* L.), sucrose is the most predominant soluble carbohydrate during dormancy (Keller and Loescher, 1989). In peach primordia, especially the floral ones, very high concentrations of transport forms of carbohydrates (sucrose and sorbitol), imported during growth capacity recovery, were found (Bonhomme *et al.,* 2005). From autumn to mid-winter, a significant relationship between total sugars and starch concentrations, with a marked increase in amounts of sorbitol, fructose, glucose and sucrose, was found in the bark tissue of stone and pome fruits, coinciding with a decrease in starch content (Wang and Faust, 1987; González-Rossia *et al.,* 2008).

Free radicals and antioxidant mechanisms

Studies have shown that free radicals, activated oxygen species (AOS), implicated in a number of biological phenomena, are produced in dormant buds of some fruit species, where their removal seems to be associated with bud break as a result of changes in antioxidant systems (Wang *et al*., 1991). The generation of AOS, particularly H_2O_2, during stress has been proposed as part of the signalling cascade leading to plant response (Anderson *et al.*, 1998). The plant's antioxidant defence system, via enzymatic and non-enzymatic mechanisms (i.e. amino acids, glutathione, acid ascorbic, carotenoids, α-tocopherol), provides protection against high levels of free radicals responsible for the

peroxidation of membrane lipids and the destruction of proteins (De Kok and Stulen, 1993).

Antioxidative enzymes, individually or cooperatively, have been viewed as a defensive team that protect cells from active oxygen damage performing a detoxifying function (Kranner and Grill, 1996). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) are the main antioxidative enzymes involved in the reduction of H_2O_2 to H_2O (Schmidt and Kunert, 1986; Noctor and Foyer, 1998; Rojas-Beltran *et al*., 2000). Maximum H_2O_2 accumulation could act as a signaling molecule to trigger the sequence of reactions to break endodormancy (Kuroda *et al.*, 2002). During the dormancy process, biochemical analysis conducted on apricot flower buds revealed CAT and GPX as the most involved enzymes, with significant increases of activity at the release of endodormancy, particularly in cultivars with a low or medium CR (Viti *et al.,* 2013). Other metabolites such as glutathione and antioxidant proteins were associated with increased capacity for free radical scavenging (Siller-Cepeda *et al*., 1991). Glutathione (γ-glutamyl-cysteinyl-glycine) is generally considered to be a ubiquitous sulfhydryl-containing tripeptide in living cells (Fahey *et al*., 1975), being the main reserve and long-distance transport form of reduced sulfhydryls which are indispensable for protein synthesis (Rennemberg, 1982). It is an important metabolite in stabilizing the cell redox state during the cold hardening process (De Kok and Stulen, 1993). In particular, in stressed plants, reduced glutathione (GSH) protects protein thiol groups from auto-oxidation (Kranner and Grill, 1996). GSH is oxidized to glutathione disulfide (GSSG) and, under normal conditions, is reduced efficiently back to GSH by the action of glutathione reductase (Foyer *et al.,* 2001). The major portion of the glutathione in the cell is maintained in the reduced state, and a high reduced/oxidized ratio (GSH/GSSG) is necessary for numerous physiological functions. Glutathione was suggested as one of the strong factors in controlling bud dormancy, first, in grape (Tohbe *et al.,* 1998); later, in apricot flower buds a significant relationship between the end of endodormancy and the increase of GSH/GSSG ratio was found (Bartolini *et al.*, 2004). In cultivars with low-medium CR the reduced and oxidized glutathione ratio (GSH/GSSG) increased in accordance with the end of endodormancy, while in cultivars with high CR the GSH/GSSG ratio was kept low during the rest period. This could suggest that a minimum threshold in antioxidant activities could be crucial for scavenging free radicals during the rest season.

Hormonal involvement

The endogenous system of hormonal regulation mediates the annual transition from growth to dormancy due to the shortened length of autumn days and reduction of daily mean temperatures (Thimann, 1985). Knowledge of hormonal regulation of dormancy processes has become increasingly more complex, particularly with recent findings of auxin-and ethylene-triggered abscisic acid induction revealing more responses mediated by abscisic acid (ABA) than originally considered (Tanino, 2004). ABA has long been studied as a potential mediator of short-induced cessation of growth and initiation of bud dormancy in trees (Guak and Fuchigami, 2001). An increase in its activity or its accumulation in the fall is an indispensable condition for the onset of apical growth inhibition, and an essential prerequisite for emergence of the dormant state and the ability to adapt to winter frosts in apricot tress (Kuzina and Kalinina, 1993). Kawamata *et al.* (2002) found that free ABA levels in buds increased suddenly at onset of dormancy and decreased afterward. Chen *et al.* (2002) suggested that changes in dormancy status are more closely related to changes in ABA receptivity than to changes in ABA levels. ABA affects dormancy progression through its action on dehydrins or membrane permeability (Jacobsen and Shaw, 1989). Moreover, the regulatory effect of ABA in growth inhibition, dormancy, and thermoregulation in woody plants is realized only with the action of other phytohormones: auxins, gibberellins, and cytokinins (Back and Richmond, 1971). Gibberellin GA_3 was tested in peach buds to promote bud burst under conditions of prolonged dormancy (Erez *et al*., 1971). The comparatively high levels of GA_3 found in mid-winter could be one of the factors that control the process of anther and gynoecium development (Basconsuelo *et al.,* 1995).

Molecular features

Population and quantitative genetics studies indicate that phenological traits such as time to bud set, chilling requirement and time to bud flush show significant genetic variation, and that such traits are often controlled by multiple genes exhibiting small effects (Howe *et al*., 2003; Rohde *et al*., 2011). Dormancy signals, impacting numerous physiological processes, involve changes in the expression patterns of numerous regulatory genes that could play a key role in dormancy transition (Horvath *et al.,* 2008). In contrast to paradormancy, the molecular aspects of endodormancy are poorly understood. The molecular biology of endodormancy has been analysed in several recent studies by global approaches where an initial set of candidate genes involved in cold- or light-induced dormancy in tree species were described (Bielenberg *et al.,* 2008). Gyllenstrand *et al*., (2007) found a significant and close correlation between growth rhythm (both bud set and bud burst) and the expression pattern of an FT (flowering locus T) homologue, suggesting that FT is a key integrator of photoperiodic and thermal signals in the control of growth rhythms in gymnosperms. Additionally, the substitution of a single amino acid can transform an FT protein from an activator into a suppressor of flowering. Thus, the limited but tantalizing linkage between the floral regulatory machinery and seasonal growth cessation and bud set, through regulation of FT and FT-like genes, suggests a general model for endodormancy regulation (Horvath, 2009).

Flowering locus C (FLC)-like genes have been shown to be regulated differentially during the satisfaction of

CR in vegetative buds of poplar (Chen and Coleman, 2006). Furthermore, Bielenberg *et al.* (2008) revealed a cluster of six MADS-box transcription factors (named dormancy-associated MADS-BOX or DAM genes) as candidate genes for the regulation of terminal bud formation in evergrowing peach. The expression of two of these genes, DAM5 and DAM6, is suppressed by chilling temperatures and inversely correlated with bud break rate in peach (Jimenez *et al.,* 2010), whereas DAM4 and DAM6 expression is promoted by short photoperiods (Li *et al.,* 2009). Similar genes were expressed differentially during dormancy induction, maintenance and release also in apricot. In *Prunus mume*, Yamane *et al.* (2008) have generated two SSH/MOS (subtractive hybridization supplemented with mirror orientation selection) libraries containing gene pools that are expressed preferentially in endodormant buds in comparison with paradormant or ecodormant buds to search for the genes that are up-regulated by endodormancy induction or down-regulated by endodormancy release. Differential screening and sequencing indicated that genes involved in gibberellin metabolism, stress resistance, cell wall modification, and signal transduction, such as transcription factors, are up-regulated in endodormant buds. At transition from dormancy to active bud growth, genes related to carbohydrate and energy metabolism have been specifically identified in *Prunus armeniaca* L. Céchová *et al*., (2012) have observed strong expression of xyloglucan endotransglycosylase/hydrolase (XTH) and EXGTA1 (endoxyloglucan transferase) in the week before, and during, the exit of apricot flower buds from endogenous dormancy.

Research is still in progress to study changes in the expression in regulatory genes involved in numerous physiological signals related to the dormancy process.

4. Influence of mild temperatures during the dormancy process

The complex process of dormancy is affected by a close interaction between genotype and environment, where photoperiod and temperature are the main factors involved in triggering and releasing. Their individual and combined effects change during the transition from a dormant to a non-dormant state (Caffarra *et al*., 2011).

Apricot culture is greatly restricted by climatic conditions, with a decisive influence on development and productivity (Quamme *et al.,* 1982; Guerriero and Bartolini, 1991). It is well known that some cultivars are closely linked to their geographical origin and, consequently, have a low adaptability to other climatic conditions (Bassi *et al.,* 2006). Autumn-winter temperature trends seem to be the main cause of this low plasticity related to the need for adequate and specific satisfaction of CR for dormancy breaking (Viti *et al.,* 2010). A warm climate may prevent or delay this process. In fact, during winter mild temperatures have a negative impact on endodormancy release due to an unfulfilled CR. In this situation, apricot is frequently affected by the appearance of floral anomalies, e.g. pistil abortion and/or browning and/or necrosis of flower buds, which are usually attributed to unfulfilled CR. However, a correlation between flower anomalies and CR satisfaction was not always achieved (Guerriero and Bartolini, 1991; Viti and Monteleone, 1993; Legave, 2002). At present, knowledge about the mechanisms and nature of anomalies is still scant but, on several genotypes, a genetic determinism has been found through analysis of different apricot progenies (Legave *et al.,* 2006).

From a physiological point of view, climatic conditions affect inductive signals regarding metabolism and phytohormones involved in the control of several events such as primary vascular differentiation micro- and macrosporogenesis (Aloni, 1980; Creber and Chaloner 1984; Fukuda, 1996). In apricot flower buds, xylem differentiation was found to be slow when the winter minimum mean temperatures were predominantly below zero (Bartolini *et al*., 2006 a). Moreover, during the autumn-winter season, after a minimum threshold of chilling amount, the supply of constant warm temperatures stimulated the development of vascular elements, at least in cultivars with a low-medium CR (Bartolini and Giorgelli, 1995). As regards the microsporogenesis process, the post-meiotic phase is crucial because temperature may influence the transition from tetrads to pollen grains (Viti and Scalabrelli, 1988). A lack of synchronism between dormancy release, xylem differentiation and microsporogenesis evolution has recently been demonstrated under warm winter conditions (Bartolini *et al.,* 2006 b; Andreini *et al.,* 2012).

The negative role of certain temperatures is confirmed also during the ecodormancy stage. In particular, temperature fluctuations affect different stages of reproductive development and this stress might lead to developmental asynchrony in pollen–pistil–ovule functioning, leading to reduced fertilization levels (Hedhly, 2011).

Considering such events, determining the effectiveness of temperature regimes on dormancy release has, for a long time, been a focus of many studies. Several models have been proposed to predict the response of buds to chilling, establishing the CR of each specific genotype. The most simple one was introduced by Weinberger (1950) who simply defined the 'chilling hours' as the number of hours at or below 7°C. The method of Bidabé (1965) calculates the effect of given temperatures either for chilling or heat requirements by exponential models. The Utah model weights the efficiency of different temperatures for CR fulfillment (Richardson *et al.,* 1974 and 1975). This model is the best tailored for cool and temperate regions (Seeley, 1996), while under warm conditions (i.e. the subtropical regions) Erez *et al.* (1990) proposed the 'dynamic model' as a better indicator for peach CR estimation. The model assigned negative values to high temperatures (negation of rest) during endodormancy (Allan *et al.,* 1993). Bonhomme *et al.* (2010), indicate a simplified smoothed Utah model as the best for French conditions, introducing a more broad range of effective temperatures to break dormancy.

…and what about dormancy processes with regard to climatic changes?

Global warming of the climate system is unequivocal from observations of increases in average air temperatures in many parts of the world (Legave *et al.,* 2009). Since the end of the 1980s, this temperature change has influenced plant phenology, and in the future further climate changes will probably have an impact on crop yields (Chuine and Cour, 1999; Chmielewski *et al.,* 2004). Mean temperatures will probably rise between 1.8 and 4.0°C by the end of the 21st century, according to IPCC reports.

Over the period 1910-2003, climate warming was already in motion with a minimum temperature increase of 0.25°C per decade, as reported for the Californian San Joaquin Valley (Baldocchi and Wong, 2007). The most striking feature of climate change in eastern Asian countries during the past century may be the remarkable winter-season warming (Kwon *et al.,* 2008). As a consequence, significant impact can be expected on winter dormancy and spring bud-burst for crops and natural vegetation in this region, as has been observed in Europe (Chmielewski *et al.,* 2004). In the Mediterranean area, the winter climate shows a tendency to become progressively milder (Guerriero *et al.,* 2010; Menzel *et al*., 2011). In this context, certain years (i.e. 2006-2007) were characterized by constantly very mild autumn and winter temperatures: the minimum values occasionally went below 0°C, while the maximum values often exceeded 15°C (Luterbacher *et al*., 2007; Viti *et al.*, 2010). Thus, over the past twenty years a progressive reduction of chilling amount was recorded (Fig. 6).

In the global warming scenario, phenological process-based models are considered the best tool to study the climatic changes and subsequent expected phenology alteration (Chuine *et al.,* 2003). BRIN is a recent phenological model, calibrated for grapevine, able to predict, simultaneously, timing of budburst and flowering (García de Cortázar-Atauri *et al.,* 2009). This model computes the dormancy period using Bidabe's Cold Action model (Bidabé, 1965), and the post-dormancy period by the sum of hourly temperatures (growing degree hours-GDH, method of Richardson *et al*., 1974). Recently, the BRIN model has been calibrated and validated also for apricot cultivars under the climatic conditions of southern France allowing an effective prediction of dormancy release date (Andreini *et al.,* 2013). As a consequence, BRIN model could be applied in a "A1B" future scenario taking into account two aspects valuable for all cultivars: a delayed dormancy break and an early flowering. Advances in modeling will be made using the experimental data of dormancy release to calibrate forecast models that can reproduce the physiological behaviour of the three. According to Bonhomme *et al.* (2010) further study will consist in testing a wide panel of data and also assessing the introduction of the optimized endodormancy release model as sub-model into bud break/bloom phenological model. This will likely improve them and could be very interesting for phenological predictions in the global change context.

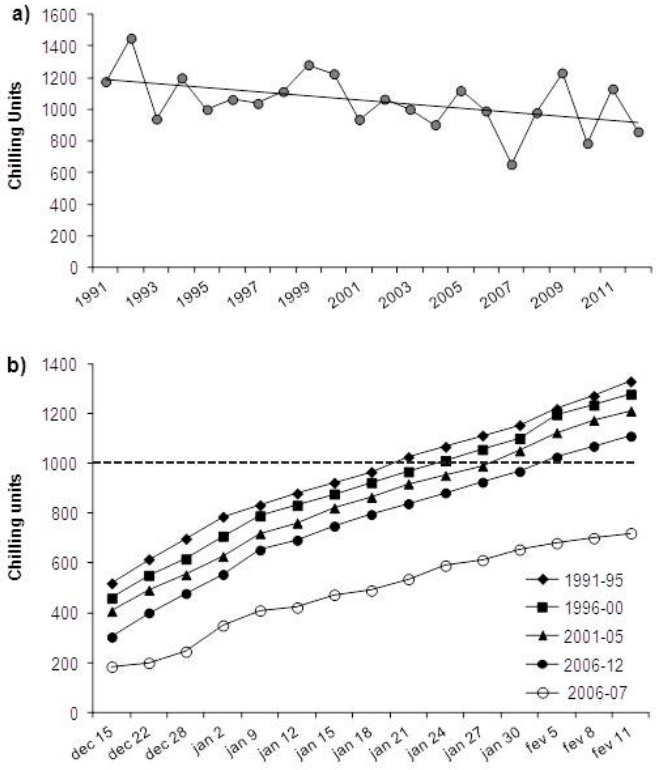


Fig. 6 - Chilling Units accumulation from 1991 to 2012 : a) during winter season, b) on 31 January. Data recorded under the climatic conditions of the Tuscany coastal area.

Concluding remarks and perspectives

Survival and competitive success of woody plants depend on a precise timing of growth, winter dormancy in synchrony with seasonal changes in temperature. In particular, apricot is a species sensitive to satisfying the CR and thus achieving the release of dormancy and bud break. Several cultivars of different geographical origins are heavily affected by the problem of dormancy release, which is one of the main causes of inconstant yields. The effect of temperature on the induction and depth of dormancy may explain some of the observed annual variation in dormancy and CR for its release.

The picture emerging from this work points to a complex relationship between winter temperatures and several biological processes in response to overcoming dormancy in flower buds. In particular, it has been demonstrated that climatic conditions characterized by mild autumn-winter seasons can affect the regular development of flower bud organs, leading to the appearance of anomalies. Moreover, several studies have indicated that an asynchronism among biological (i.e. endodormancy release, microsporogenesis evolution), anatomical (i.e. xylem vessel differentiation) and biochemical (i.e. changes in metabolic compounds and enzymes) events could represent further causes for an inconstant rate of blooming. At this time, knowledge of the mechanisms involved in dormancy is still fragmentary although recent studies of global gene expression in dif-

ferent species have carried us several steps forward, providing an excellent basis for elucidating function of genes and components thought to be involved in temperature and other environmental signaling.

In a context of global warming, mild winter temperatures could greatly impact apricot cropping systems. Forthcoming research on this particular topic will be crucial and of great economic importance. A new model, calibrated and validated on apricot cultivars, is proposed to predict the dormancy release date in a future scenario. Specific breeding programs, focusing on rustic cultivars more appropriate for particular environmental conditions, should provide additional improvements in apricot culture in the Mediterranean basin.

References

ALBURQUERQUE N., BURGOS L., EGEA J., 2002 - *Variability in the development stage of apricot ovules at anthesis and its relationship with fruit set.* - Annals of Applied Biology, 141: 147-152.

ALLAN P.G., RUFUS G., LINSLEY-NOAKES G.C., MATTHEE G., 1993 - *Winter chill models in a mild subtropical area and effects of constant 6°C chilling on peach budbreak*. - Acta Horticolturae, 409: 9-17.

ALLONA I., RAMNOS A., IBANEZ C., CONTRERAS A., CASADO R., ARAGONCILLO C., 2008 - *Molecular control of dormancy establishment in trees.* - Span J. Agric. Res, 6: 201-210.

ALONI R., 1980 - *Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures.* - Planta, 150: 255-263.

ALONSO J.M., ANSON J.M., ESPIAU M.T., SOCIAS I., COMPANY R., 2005 - *Determination of endodormancy break in almond flower buds by correlation model using the average temperature of different day intervals and its application to estimation of Chill and heat requirements and blooming date.* - J. Amer. Soc. Hort. Sci., 30(3): 308-318.

ANDERSON M.C., CHEN Z., KLESSIG F.D., 1998 - *Possible involvement of lipid peroxidation in salicylic acid mediated induction of PR-1 gene expression.* - Phytochem, 47: 555-566.

ANDREINI L., BERTUZZI P., AUDERGON J.M., HUARD F., SATGER S., LIENNARD M.E., VITI R., BARTOLINI S., 2013 - *Performance of a model to predict the flowering date of Apricot in three different regions of South France.* - Acta Horticolturae (in press).

ANDREINI L., VITI R., BARTOLINI S., RUIZ D., EGEA J., CAMPOY J.A., 2012 - *The relationship between xylem differentiation and dormancy evolution in apricot flower buds (*Prunus armeniaca *L.): the influence of environmental conditions in two Mediterranean areas.* - Trees, 26(3): 919-928.

ASHWORTH E.N., ROWSE J.D., 1982 - *Vascular development in dormant Prunus flower buds and its relationship to supercooling.* - HortScience, 17(5): 790-791.

AUE H.L., LECOMTE I., PÉTEL G., 2000 - *Changes in parameters of the plasmalemma ATPase during peach vegetative bud dormancy*. - Biol. Plant., 43: 25-29.

BACK A., RICHMOND A.E., 1971 - *Interaction between gibberellic acid, cytokinins, and absisic acid in retarding leaf senescence.* - Physiol. Plant, 24: 76-80.

BAGGIOLINI M., 1952 - *Stades repéres de l'abrocotier.* - Revue d'Agr. Vitic. et Arboric, 8(4): 28-29.

BALDOCCHI D., WONG S., 2007 - *Accumulated winter chill is decreasing in the fruit growing regions of California.* - Climatic Change, 87(S1): 153-166.

BARTOLINI S., GIORGELLI F., 1994 - *Observations on development of vascular connections in two apricot cultivars.* - Adv. Hort. Sci., 8(2): 97-100.

BARTOLINI S., GIORGELLI F., 1995 - *Boron accumulation and xylem differentiation in apricot flower buds.* - Acta Horticulturae, 38: 297-302.

BARTOLINI S., VITI R., ANDREINI L., 2013 - *The effect of summer shading on flower bud morphogenesis in apricot (*Prunus armeniaca *L.).* - Cent. Eur. J. Biol, 8(1): 54-63.

BARTOLINI S., VITI R., GUERRIERO R., 2006 a - *Xylem differentiation and microsporogenesis during dormancy of apricot flower bud*. - European Journal of Horticultural Science, 71: 84-90.

BARTOLINI S., VITI R., LAGHEZALI M., OLMEZ H.A., 2006 b - *Xylem vessel differentiation and microsporogenesis evolution in 'Canino' cultivar growing in three different climatic areas: Italy, Morocco and Turkey.* - Acta Horticulturae, 701: 135-140.

BARTOLINI S., VITI R., ZANOL G., 2004 - *The involvement of glutathione in flower bud dormancy overcoming in apricot (*Prunus armeniaca *L.).* - Recent Research Developments in Agronomy and Horticulture, Research Signpost Press, Kerala, India, 1: 11-28.

BASCONSUELO S., REINOSO H., LORENZO E., BOTTINI R., 1995 - *Dormancy in peach (*Prunus persica *L.) flower buds. IV. Morphogenesis of excised buds as influenced by chilling and gibberellin* A_3. - Plant Growth Regul., 16: 113-119.

BASSI D., BARTOLINI S., VITI R., 2006 - *Recent advances on environmental and physiological challenges in apricot growing.* - Acta Horticulturae, 717: 23-31

BIDABÉ B., 1965 - *Contrôle de l'époque de la floraison du pommier par une nouvelle conception de l'action de températures.* - C.R. Acad. Agric. Fr., 49: 934-945.

BIELENBERG D.G., WANG Y., LI Z.G., ZHEBENTYAYEVA T., FAN S.H., REIGHARD G.L., SCORZA R., ABBOTT A.G., 2008 - *Sequencing and annotation of the evergrowing locus in peach* Prunus persica *(L.) Batsch reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation.* - Tree Genet. Genomes, 4: 495-507.

BONHOMME M., RAGEAU R., LACOINTE A., 2010 - *Optimization of endodormancy release models, using series of endodormancy release data collected in France.* - Acta Horticulturae, 872: 51-60.

BONHOMME M., RAGEAU R., LACOINTE A., GENDRAUD M., 2005 - *Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica *L. Batch).* - Scientia Horticulturae, 105: 223-240.

BONHOMME M., RAGEAU R., RICHARD J.P., EREZ A., GENDRAUD M., 1999 - *Influence of three contrasted climatic conditions on endodormant vegetative and floral peach buds: analyses of their intrinsic growth capacity and*

their potential sink strength compared with adjacent tissues. - Scientia Horticulturae, 80(3-4): 157-171.

BORDEIANU T., TARNAVSCHI I., RADU I.F., BUMBAC E., BOTEZ M, MARIN A., 1962 - *Etude concernant le repos d'hiver et le seuil biologique chez les bourgeons floraux d'abricotier.* - XVIth International Congress of Horticulture, pp. 238-239.

BURGOS L., EGEA J., 1993 - *Apricot embryo-sac development in relation to fruit set.* - J. Hort. Sci., 68: 203-208.

CAFFARRA A., DONNELLY A., CHUINE I., JONES M.B., 2011 - *Modelling the timing of* Betula pubescens *budburst. I. Temperature and photoperiod: a conceptual model.* - Clim. Res., 46: 147-157.

ĆECHOVÁ J., BARÁNEK M., KRŠKA B., PIDRA M., 2012 - *Screening of differentially expressed genes during the end of endogenous dormancy of flower buds in* Prunus armeniaca *L.* - Plant Growth Regul., 67(2): 141-150.

CHAMPAGNAT P., 1973 - *Quelques aspects des dormancies chez les végétau*x. - Bull. Groupe Etude Rythmes Biol., 4(2): 47-59.

CHAO W.S., ANDERSON J.V., 2010 - *Plant dormancy, a mechanism involving assorted molecular, physiological, and cellular processes.* - Plant Mol. Biol., 73: 1-2.

CHEN K.Y., COLEMAN G.D., 2006 - *Type-II MADS-box genes associated with poplar apical bud development and dormancy.* - Amer. Soc. Plant Biolists Meeting, Boston, MA, USA, 5-9 Aug., 2006.

CHEN T.H.H., HOWE G.T., BRADSHAW H.D., 2002. - *Molecular genetic analysis of dormancy-related traits in poplars.* - Weed Sci., 50: 232-240.

CHMIELEWSKI F.M., MÜLLER A., BRUNS E., 2004 - *Climate changes and trends in phenology of fruit trees and field crops in Germany, 1961-2000.* - Agricultural and Forest Meteorology, 121(1-2): 69-78.

CHUINE I., COUR P., 1999 - *Climatic determinants of budburst seasonality in four temperate-zone tree species.* - New Phytologist, 143(2): 339-349.

CHUINE I., KRAMER K., HANNINEN H., 2003 - *Plant development models.* - In: SCHWARTZ, M.D. (ed.) *Phenology. An integrative environmental science*. Kluwer, pp. 217-235.

CLANET H., SALLES JC., 1972 - *Contribution à l'étude de la fructification de l'abricotier dans des conditions climatiques différentes.* - Annals Am. des Plantes, 24(2): 97-127.

CRABBÉ J., BARNOLA P., 1996 - *A new conceptual approach to bud dormancy in woody plants*, pp. 83-113. - In: LANG G.A. (ed.) *Plant dormancy: physiology, biochemistry and molecular biology.* - CAB International, New York, USA.

CREBER G.T., CHALONER W.G., 1984 - *Influence of environmental factors on the wood structure of living and fossil trees.* - Bot. Rev., 50: 357-448.

DE KOK I.J., STULEN I., 1993 - *Role of glutathione in plants under oxidative stress*, pp. 125-138. - In: *Sulfur nutrition and assimilation in higher plants.* - APB Academic Publishing, The Hague, The Netherlands.

EGEA J., BURGOS L., 1995 - *Supernumerary ovules in flowers of apricot.* - Acta Horticulturae, 384: 373-377.

ELLE D., SAUTER J.J., 2000 - *Seasonal changes of activity of a starch granule bound endoamylase and a tarch phosphorylase in poplar wood (*Populus x Canadensis Moench *"robusta") and their possible regulation by temperature and phytohormones*. - J. Plant Physiol., 156: 731-740.

EREZ A., 2000 - *Bud dormancy: phenomenon, problems and solutions in the tropics and subtropics*, pp. 17-48. - In: EREZ A (ed.) *Temperature fruit crops in warm climates*. Kluwer Academic Publishers, The Netherland.

EREZ A., COUVILLON G., 1987 - *Characterization of the influence of moderate temperature on rest completion in peach.* - J. Amer. Hort. Sci., 112(4): 677-680.

EREZ A., FISHMAN S., LINSLEY-NOAKES G.C., ALLAN P., 1990 - *The dynamic model for rest completion in peach buds.* - Acta Horticulturae, 276: 165-173.

EREZ A., LAVEE S., SAMISH R.M., 1971 - *Improving methods for breaking rest in the peach and other deciduous fruit species.* - J. Amer. Soc. Hort. Sci., 96: 519-522.

ESAU K., 1965 - *Vascular differentiation in plant.* - Holt, Rinehart and Winston, New York.

ESSIAMAH S., ESCHRICH W., 1986 - *Water uptake in deciduous trees during winter and the role of conducting tissues in spring reactivation.* - IAWA Bulletin, 7(1): 31-38.

FAHEY R.C., BRODY S., MIKOLAJCZYK S.D., 1975 - *Changes in the Glutathione Thiol-Disulfide Status of Neurospora crassa Conidia During Germination and Aging.* - Journal of Bacteriology, 121(1): 144-151.

FAUST M., EREZ A., ROWLAND L.J., WANG S.Y., NORMAN H.A., 1997 - *Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release.* - HortScience, 32: 623-629.

FELKER F.C., ROBITAILLE E., HESS E.D., 1983 - *Morphological and ultrastructural development and starch accumulation during chilling of sour cherry flower buds.* - American Journal of Botany, 70: 376-386.

FENNELL A., 1999 - *Systems and approaches to studying dormancy: Introduction to the workshop.* - HortScience, 34: 1172-1173.

FLORE J.A., LAYNE D.R., 1996 - *Prunus*, pp. 797-823. - In: ZAMSKI E., and A.S. SCHAFFER (eds.) *Photoassimilatem distribution in plants and crops. Source-sink relationships.* Marcel Dekker, Inc., NY, USA.

FOYER C.H., THEODOULOU F.L., DELROT S., 2001 - *The functions of inter and intracellular glutathione transport systems in plants*. - Trends in Plant Sci., 6: 486-492.

FUKUDA H., 1996 - *Xylogenesis: initiation, progression, and cell death*. - Annu. Rev. Plant Physiol. Plant Mol. Biol, 47: 299-325.

GARCIA DE CORTÁZAR-ATAURI I., BRISSON N., GAUDILLERE J.P., 2009 - *Performance of several models for predicting budburst date of grapevine (*Vitis vinifera *L.)* - Int. J. Biometeorol., 53: 317-326.

GARCIA G.I., GUERRIERO R., MONTELEONE P., 1999 - *Apricot bud chilling and heat requirement in two different climatic areas: Murcia and Tuscan Maremma.* - Acta Horticulturae, 488: 289-294.

GENDRAUD M., PETÉL G., 1990 - *Modification in intercellular communications, cellular characteristics and change in morphogenetic potentialities of Jerusalem artichoke tubers (*Helianthus tuberosus *L.)*, 171-175. - In : MILLET B., and H. GREPPIN (eds.) *"Intra- and extracellular communication in plants: reception, transmission, storage and expression of messages"*. INRA, Paris.

GONZÁLEZ-ROSSIA D., REIG C., DOVIS V., GARIGLIO N., AGUSTÍ M., 2008 - *Changes on carbohydrates and nitrogen*

content in the bark tissues induced by artificial chilling and its relationship with dormancy bud break in Prunus *sp.* - Scientia Horticulturae, 118(4): 275-281.

GUAK S., FUCHIGAMI L.H., 2001 - *Effects of applied ABA on growth cessation, bud dormancy, cold acclimation, leaf senescence and N mobilization in apple nursery plants.* - J. Hort. Sci. Biothech., 74: 459-464.

GUERRIERO R., BARTOLINI S., 1991 - *Main factors influencing cropping behaviour of some apricot cultivars in coastal areas.* - Acta Horticulturae, 293: 229-243.

GUERRIERO R., BARTOLINI S., VITI R., 1986 - *Confronto fra metodi diversi per stabilire l'epoca di uscita di dormienza delle gemme a fiore della cultivar di albicocco "Reale d'Imola".* - Riv. Ortoflorofrutt. Ital., 70: 257-266.

GUERRIERO R., MONTELEONE P., VITI R., 2006 - *Evaluation of end of dormancy in several apricot cultivars according to different methodological approaches.* - Acta Horticulturae, 701: 99-103.

GUERRIERO R., VITI R., IACONA C., BARTOLINI S., 2010 - *Is apricot germplasm capable of withstanding warmer winters? This is what we learned from last winter.* - Acta Horticulturae, 862: 265-272.

GYLLENSTRAND N., CLAPHAM D., KÄLLMAN T., LAGERCRANTZ U., 2007 - *A Norway spruce FLOWERING LOCUS T homolog is implicated in control of growth rhythm in conifers*. - Plant Physiol., 144(1): 248-257.

HANSON E.J., BREEN P.J., 1985 - *Xylem differentiation and boron accumulation in 'Italian' prune flower buds.* - J. Amer. Soc. Hort. Sci., 110(4): 566-570.

HEDHLY A., 2011 - *Sensitivity of flowering plant gametophytes to temperature fluctuations.* - Environmental and Experimental Botany, 74: 9-16.

HORVATH D., 2009 - *Common mechanisms regulate flowering and dormancy.* - Plant Sci., 177: 523-531.

HORVATH D.P., ANDERSON J.V., CHAO W.S, FOLEY M.E., 2003 - *Knowing when to grow: signals regulating bud dormancy.* - Trends in Plant Science, 8: 534-540.

HORVATH D.P., CHAOW S., SUTTLE J.C., THIMMAPURAM J., ANDERSON J.V., 2008 - *Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula *L.).* - BMC Genomics, 9: 536.

HOWE G.T., AITKEN S.N., NEALE D.B., JERMSTAD K.D., WHEELER N.C., CHEN T.H.H., 2003 - *From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees.* - Canadian Journal of Botany, 81: 1247-1266.

JACOBSEN J.V., SHAW D.C., 1989 - *Heat-stable proteins and abscisic acid action in barley aleurone cells.* - Plant Physiol., 91: 1520-1526.

JIMENEZ S., REIGHARD G.L., BIELENBERG D.G., 2010 - *Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate.* - Plant Mol. Biol., 73: 157-167.

JONES K.S., PAROSCHY J., MCKERSIE B.D., BOWLEY S.R., 1999 - *Carbohydrate composition and freezing tolerance of canes and buds in* Vitis vinifera*.* - Journal of Plant Physiology, 155(1): 101-106.

JULIAN C., RODRIGO J., HERRERO M., 2011 - *Stamen development and winter dormancy in apricot (*Prunus armeniaca*).* - Annals of Botany, 108: 617-625.

KALCSITS L., SILIM S., TANINO K., 2009 - *The influence of temperature on dormancy induction and plant survival in woody plants,* pp. 108-118. - In: GUSTA L., M. WISNIEWSKI, and K. TANINO (eds.) *Plant cold hardiness: from the laboratory to the field*. CABI International, London, UK.

KAWAMATA M., NISHIDA E., OHARA H., OHKAWA K., MATSUI H., 2002 - *Changes in the intensity of bud dormancy and internal compositions of current shoot in fig.* - J. Japan. Soc. Hort. Sci., 71: 177-182.

KELLER J.D., LOESCHER W.H., 1989 - *Nonstructural carbohydrate partitioning in perennial parts of sweet cherry.* - J. Amer. Soc. Hort. Sci., 114: 969-975.

KRANNER I., GRILL D., 1996 - *Significance of thiol-disulfide exchanges in resting stages of plant development.* - Bot. Acta, 109: 8-14.

KURODA H., SUGIURA T., ITO D., 2002 - *Changes in hydrogen peroxide content in flower buds of Japanese pear (*Pyrus pirifolia *Nakai) in relation to breaking of endodormancy.* - J. Japan. Soc. of Hort. Sci., 71: 610-616.

KUZINA G.V., KALININA G.A., 1993 - *Abscisic acid content in relation to passage of the autumn photopheriodic response, induction of deep dormancy and frost resistance of apricot.* - Russ. Plant. Physiol., 40(3): 360-367.

KWON E.Y., JUNG J.E., CHUNG U., YUN J.I., PARK H.S., 2008 - *Using thermal time to simulate dormancy depth and bud-burst of vneyards in Korea for the twentieth century.* - Journal of Applied Meteorology and Climatology, 47(6): 1792-1801.

LANG G.A., EARLY J.D., MARTIN C.G., DARNEL R.L., 1987 - *Endo-para-, and ecodormancy: physiological terminology and classification for dormancy research.* - Hortic. Sci., 22: 371-377.

LEGAVE J.M., 1975 - *La différentiation du burgeon à fleur et le repos invernal chez l'abricotier.* - Pomologie Française, 17: 150-168.

LEGAVE J.M., 2002 - *Fertility and regular production.* - Final report ERBIC18CT980310, Bruxelles, Luxemburg, pp. 17-23.

LEGAVE J.M., CHRISTEN D., GIOVANNINI D., OGER R., 2009 - *Global warming in Europe and its impact on floral bud phenology in fruit species.* - Acta Horticulturae, 838: 21-26.

LEGAVE J.M., GARCIA G., MARCO F., 1984 - *Interférence des conditions de température et des besoins variétaux en froid et en chaleur sur la détermination de la fin de dormance puis de la floraison des diverses variétés d'abricotier dans l'aire de culture française.* - Fruits, 39: 399-410.

LEGAVE J.M., RICHARD J.C., VITI R., 2006 - *Inheritance of floral abortion in progenies in 'Stark Early Orange'.* - Acta Horticulturae, 701: 127-130.

LI Z., REIGHARD G.L., ABBOTT A.G., BIELENBERG D.G., 2009 - *Dormancy-associated MADS genes from the EVG locus of peach [*Prunus persica *(L.) Batsch] have distinct seasonal and photoperiodic expression patterns.* - J. Exp. Bot., 60: 3521-3530.

LUNA V., LORENZO E., REINOSO H., TORDABLE M.C., ABDALA G., PHARIS R.P., BOTTINI R., 1990 - *Dormancy in peach (*Prunus persica *L.) flower buds. I. Floral morphogenesis and endogenous gibberellins at the end of the dormancy period.* - Plant Physiology, 93: 20-25.

LUTERBACHER J., LINIGER M.A., MENZEL A., ESTRELLA N., DELLA-MARTA P.M., PFISTER C., RUTISHAUSER T., XOPLAKI E., 2007 - *Exceptional European warmth of autumn 2006 and winter 2007: Historical context, the underlying dynamics, and its phenological impacts.* - Geophysical Research Letters, 34(12): 1-6.

MARQUAT C., PÉTEL G., GENDRAUD M., 1996 - *Study of H^+-nutrients cotransport in peach-tree and the approach to their involvement in the expression of vegetative bud growth capability.* - J. Plant Physiol., 149: 102-108.

MARTÌNEZ-TELLEZ J.J., MONET R., CROSSA-RAYNAUD P., 1982 - *Contribution a une meilleure connaissance de la biologie florale et de la fécondation chez le pêcher.* - Arboriculture Fruitière, 338: 39-45.

MENZEL A., SEIFERT H., ESTRELLA N., 2011 - *Effects of recent warm and cold spells on European plant phenology.* - International Journal of Biometeorology, 55(6): 921-932.

MONET R., BASTARD Y., 1968 - *Morphologie végétale, morphogènes et croissance des ébauches chez le pêcher* (Prunus persica *L. Batsch*). - Centre Recherche Académie Science, Paris, pp. 1845-1848.

NOCTOR G., FOYER C.H., 1998 - *Ascorbate and glutathione: keeping active oxygen under control.* - Annual Rev. Plant Physiol. Plant Mol. Biol., 49: 249-279.

NYÚJTÓ F., BANAI B., 1975 - *Preliminary report upon winter morphogenesis of flower buds by some apricot varieties.* - Gyümölcstermesztes, 2: 15-21.

OR E., VOLOZNY I., FENNEL A., EYAL Y., OGRODOVITCH A., 2002 - *Dormancy in grape buds: isolation and characterization of catalase cDNA and analysis of its expression following chemical induction of bud release.* - Plant Sci., 162: 121-130.

PÉTEL G., GENDRAUD M., 1996 - *Processes at the plasma membrane and plasmalemma ATPase during dormancy*, 233-243. - In: LANG G.A. (ed.) *Plant dormancy: physiology, biochemistry and molecular biology*. CAB International, New York.

PÉTEL G., LAFLEURIEL J., DAUPHIN G., GENDRAUD M., 1992 - *Cytoplasmic pH and plasmalemma ATPase activity of parenchyma of cells during the release of dormancy of Jerusalem artichoke tubers.* - Plant Physiol. Biochem., 35: 161-167.

QUAMME H.A., LAYNE R.E.C., RONALD W.G., 1982 - *Relationship of supercooling to cold hardiness and the northern distribution of several cultivated and native* Prunus *species and hybrids.* - Canadian Journal of Plant Science, 62(1): 137-148.

REINOSO H., LUNA V., PHARIS R.P., BOTTINI R., 2002 - *Dormancy in peach flower buds, Anatomy of bud development in relation to phenological stage.* - Canadian Journal of Botany, 80(6): 656-663.

RENNENBERG H., 1982 - *Glutathione metabolism and possible biological roles in higher plants.* - Phytochem., 21: 2771-2781.

RICHARDSON E.A., SEELEY S.D., WALKER D.R., 1974 - *A model for estimating the completion of rest for "Redhaven" and "Elberta" Peach Trees.* - HortScience, 9: 331-332.

RICHARDSON E.A., SEELEY S.D., WALKER R.D., ANDERSON J., ASHCROFT G., 1975 - *Pheno-climatography of spring peach bud development.* - HortScience, 10: 236-237.

ROBERT F., GENDRAUD M., PÉTEL G., 1999 - *Using intracellular pH to evaluate growth inhibition of strawberry plants.* - Plant Physiol. Biochem., 37: 155-166.

ROHDE A., BHALERAO RP., 2007 - *Plant dormancy in the perennial context.* - Trends in Plant Science, 12(5): 217-223.

ROHDE A., STORME V., JOERGE V., GAUDET M., ITACOLONNA N., FABBRINI F., RUTTINK T., ZAINA G., MARRON N., DILLEN S., STEENACKERS M., SABATTI M., MORGANTE M., BOERJAN W., BASTIEN C., 2011 - *Bud set in poplar - genetic dissection of a complex trait in natural and hybrid populations.* - The New Phytologist, 189(1): 106-121.

ROJAS-BELTRAN J.A., DEJAEHERE F., ABD ALLA KOTB M., DU JARDIN P., 2000 - *Expression and activity of antioxidant enzymes during potato tuber dormancy.* - Potato Research, 43: 383-393.

RYUGO K., 1990 - *Fattori di regolazione della fioritura e della allegagione nelle specie frutticole temperate.* - Riv. Fruttic., 11: 27-31.

SCALABRELLI G., VITI R., CINELLI F., 1991 - *Change in catalane activity and dormancy of apricot in responce to chilling.* - Acta Horticulturae, 293: 267-274.

SCHMIDT A., KUNERT K.J., 1986 - *Lipid peroxidation in higher plants.* - Plant Physiol., 82: 700-702.

SEELEY S.D., 1996 - *Modeling climatic regulation of bud dormancy,* pp. 361-376. - In: LANG G.A. (ed.) *Plant dormancy: Physiology, biochemistry and molecular biology.* CABI, Wallingford, Oxon, UK, pp. 408.

SHEEN J., ZHOU L., JANG J.C., 1999 - *Sugars as signalling molecules.* - Curr. Opin. Plant Biol., 2: 410-418.

SHERSON S.M., ALFORD H.L., FORBES S.M., WALLACE G., SMITH S.M., 2003 - *Roles of cell wall invertases and monosaccharide transporters in the growth and development of Arabidopsis.* - J. Exp. Bot., 54: 525-531.

SILLER-CEPEDA J.H., CHEN T.H.H., FUCHIGAMI L.H., 1991 - *High performance liquid chromatography of reduced and oxidized glutathione in woody plant tissues.* - Plant Cell Physiol., 32: 1179-1185.

SMEEKENS S., 2000 - *Sugar-induced signal transduction in plants.* - Annual Rev. Plant Physiol. Plant Mol. Biol., 51: 49-81.

SZABÒ Z., SZALAY L., PAPP J., 2002 - *Connection between the developmental stage and the cold hardiness of peach cultivars.* - Acta Horticulturae, 592: 549-552.

SZLAY L., NEMETH S., 2010 - *Phenological processes of dormancy in apricot genotypes in the central part of Carpathian basin.* - Acta Horticulturae, 862: 251-255.

TABUENCA M.C., 1975 - *Relacion entre caida de yemas de flor en melocotonero y concentracion de hidratos de carbono y de compuestos nitrogenados.* - An. Aula Dei, 13: 150-166.

TANINO K.K., 2004 - *Hormones and endodormancy induction in woody plants.* - Journal of Crop Improvement, 10(1-2): 157-199.

TANINO K.K., KALCSITS L., SILIM S., KENDALL E., GRAY G.R., 2010 - *Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction.* - Plant Mol. Biol., 73: 49-65.

THIMANN K.V., 1985 - *The interaction of the hormonal and environmental factors ion leaf senescence.* - Biol. Plant., 27: 83-89.

TOHBE M., MOCHIOKA R., HORIUCHI S., OGATA T., SHIOZAKI S., KUROOKA H., 1998 - *The role of glutathione-on the onset of endodromancy of grape buds.* - J. of Japan. Soc. for Hort. Sci., 67(6): 912-916.

VALENTINI N., RUFFA E., ME G., SPANNA F., LOVISETTO M., 2006 - *Chilling, thermal time and metabolic changes in five apricot varieties.* - Acta Horticulturae, 701: 147-150.

VITI R., ANDREINI L., RUIZ D., EGEA J., BARTOLINI S., CAMPOY J.A., 2010 - *Effect of climatic condition on the overcoming of dormancy in apricot flower buds in two Mediterranean areas: Murcia (Spain) and Tuscany (Italy).* - Scientia Horticulturae, 124: 217-224.

VITI R., BARTOLINI S., ZANOL G.C., 2013 - *Biological changes and active oxygen-scavenging enzymes activities in apricot (*Prunus armeniaca *L.) flower buds during dormancy transitions.* - Acta Horticulturae (in press).

VITI R., MONTELEONE P., 1991 - *Observations on flower bud growth in some low yield varieties of apricot.* - Acta Horticulturae, 293: 319-326.

VITI R., MONTELEONE P., 1993 - *Etude et caractérisation des anomalies de développement des bourgeons à fleur de l'abricotier.* - Rapport EUR15009 FR Programme de Recherche AGRIMED, Bruxelles, Luxemburg, pp. 31-41.

VITI R., MONTELEONE P., 1995 - *High temperature influence on the presence of flower bud anomalies in two apricot varieties characterized by different productivity.* - Acta Horticulturae, 384: 283-289.

VITI R., SCALABRELLI G., 1988 - *Influenza delle condizioni climatiche invernali sulla sporogenesi in due cultivar di albicocco.* - Frutticoltura, 6: 88-91.

WANG S.Y., FAUST M., 1987 - *Metabolic activities during dormancy and blooming of deciduous fruit trees.* - Isr. J. Bot., 37: 227-243.

WANG S.Y., JIAO H.J., FAUST M., 1991 - *Changes in ascorbate, glutathione, and related enzymes activities during thidiazuron-induced bud break of apple.* - Physiol. Plant., 82: 231-236.

WEINBERGER J.H., 1950 - *Chilling requirements of peach varieties.* - Proc. Am. Soc. Hort. Sci., 56: 122-128.

YAMANE H., KASHIWA Y., OOKA T., TAO R., YONEMORI K.J., 2008 - *Suppression subtractive hybridization and differential screening reveals endodormancy-associated expression of an* SVP/AGL24 *-type MADS-box gene in lateral vegetative buds of japanese apricot.* - J. Amer. Soc. for Hort. Sci., 133(5): 708-716.

ZANOL G., BARTOLINI S., 2003 - *Changes in intracellular pH in apricot buds during the winter season.* - Adv. Hort. Sci., 17(2): 97-101.

ZIMMERMANN S., EHRHARDT T., PLESCH G., MUELLER-ROEBER B., 1999 - *Ion channels in plant signaling*. - Cell. Mol. Life Sci., 55: 183-203.

Adv. Hort. Sci., 2013 27(1-2): 18-24

Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications

Issam M. Qrunfleh*, Paul E. Read**

* *Department of Plant Production and Protection, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt 19117, Jordan.*

** *Department of Agronomy and Horticulture, University of Nebraska-Lincoln, 377J PLSH, Lincoln, 68583-0724, Nebraska, USA.*

Key words: Amigo oil, bud break delay, frost, NAA.

Abstract: Delaying bud break is an approach to avoid spring frost damage. Field experiments were conducted during the winters of 2009 and 2010 at James Arthur Vineyards in Raymond, Nebraska to study the effect of spraying naphthaleneacetic acid (NAA) and Amigo Oil to delay bud break in 'Edelweiss' grapevines to avoid such damage. In 2009, the experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l), oil applied at 10%, and a non-sprayed control. There were four application dates: 6 January, 3 February, 3 March, and 1 April. In 2010, treatments were NAA at 500, 1000, and 1500 mg/l, 10% oil, and the control; application dates were 28 January, 25 February, and 25 March. Bud break was evaluated throughout spring. During harvest, number and weight of clusters were recorded. Berry samples were analyzed for pH, °Brix, and titratable acidity (TA). Pruning weights and number of clusters of the 2009 treated vines were recorded in March and August 2010, respectively. In the 2009 field experiment, oil and NAA at 1000 mg/l significantly delayed bud break by two to six days, compared to the control. Pruning weights were not significantly affected by the treatments. In 2010, oil applications significantly delayed bud break by eight to 12 days compared to the control and no significant differences were found between NAA at 1500 and 1000 mg/l. In both years, treatments had no significant effects on yields, cluster weights, berry weights, °Brix, pH or TA.

1. Introduction

Grapes are considered one of the world's major fruit crops. Grapes in the Midwest states of the USA are greatly influenced by frost injury. Particularly in Nebraska, spring frost is one of the major limitations to grape production. In 2007, grapevines were severely damaged because extraordinarily warm temperatures at the end of March were followed by extremely cold temperatures during the first week of April. Losses in affected areas in the Midwest states due to that particular freeze event were estimated to exceed one billion dollars (Guinan, 2007).

Heaters, wind machines, and sprinkler irrigation have been employed to minimize frost impact. These methods help reduce frost injury but are very costly. Due to their expense many grape growers do not utilize them, hoping that frost injury will affect only the primary bud and that secondary buds will recover growth after primary bud damage. Protecting the primary bud is essential because, for example in 'Concord', they produce 300 to 400% more fruit with 135 to 190% larger clusters compared to secondary buds (Wiggans, 1926). Some grape cultivars are not productive on secondary buds, as is the case for 'Edelweiss' (Smiley *et al*., 2008).

A different approach is to delay bud break until the period of frost risk passes, thus reducing frost injury damage. Growers have used many methods to delay bud break. Late or delayed pruning was shown to delay bud break and bloom date (Loomis, 1939). Call and Seeley (1989) reported a five day delay in bud break by using dormant oils on peach trees. Dami and Beam (2004) obtained a 20-day delay compared to the control after applying Amigo Oil on 'Chancellor' grapevines. Nigond (1960) applied NAA in the 500 to 1000 ppm range on 'Aramon' grapevines on various dates from October until March and noticed retarded bud break by 16 to 27 days. Many American hybrids such as 'Edelweiss' are known to exhibit cold hardiness. Nevertheless, these hybrids can remain under frost threat until frost period passes.

The objectives of the present study were to compare NAA and vegetable "Amigo Oil" applications on 'Edelweiss' vines to determine the best treatment for delaying bud break and to determine the effect of delaying bud

Received for publication 4 January 2013
Accepted for publication 30 April 2013

break on fruit yield and characteristics such as juice pH, °Brix, and titratable acidity (TA).

2. Materials and Methods

Field experiment 2009

The research was conducted during the 2009 winter season at James Arthur Vineyards located in Raymond, Nebraska within Lancaster County. Treatments were applied on 12-year-old vines. The vines are trained according to the Geneva Double Curtain trellis system. Planting distances are 2.44 m between plants and 3.66 m between rows. Rows are oriented north to south. The experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l) purchased from Phyto Technology Laboratories (Shawnee Mision, KS), Amigo Oil (Loveland Industries, Greely, CO) applied at 10% v/v which consisted of 9.3% oil and 0.7% emulsifier, and the control which was not sprayed. A randomized complete block design was used with three blocks of 20 vines each. There were four application dates: 6 January, 3 February, 3 March, and 1 April 2009. Most canes were pruned to leave the five proximal buds before applying treatments. The remaining distal portion of the canes was removed by the normal pruning practices employed at James Arthur Vineyards. Thus, the canes studied in the first year all had the same number of buds (i.e. five) and the total number of buds on vines were approximately the same. The whole vine was sprayed using a hand sprayer with each vine receiving approximately 0.33 l. After spraying, two canes per vine, each having five buds, were randomly selected and labeled. In the spring of 2009, vines were visually evaluated for bud break. Bud break was determined as stage five of the Eichhorn and Lorenz (1977) scale of grapevine development, where bud scales have expanded to the point at which a green shoot is visible. Bud break was evaluated day by day throughout the spring until each cane reached 60% bud break (three buds opened out of the five left after pruning). The number of Julian Days starting from 1 January 2009 until achieving 60% bud break was used as the basis of calculating number of days for bud break.

Harvest date was determined by taking random samples of berries and measuring their °Brix with a refractometer. On 14 August 2009 the number and weight of clusters from the two selected canes were recorded. From the total clusters, 50 berries were randomly counted, placed in a plastic storage bag, and placed at 0°C until berry sample analysis could be conducted. On 14 September 2009 berry samples were analyzed for pH, °Brix, and titratable acidity (TA). The 50 berries/vine were weighed, allowed to thaw to reach room temperature, wrapped in cheese cloth, and crushed manually using a mortar and pestle. The extracted juice was poured into test tubes to conduct the analyses. Juice pH was measured with a Pope pH/ion meter model 1501. Soluble solids (°Brix) content was measured using an Atago PR-101 digital refractometer purchased from Nova-Tech International, Inc. (Houston, TX). TA was determined by titration with NaOH, using the procedure of Dharmadhikari and Wilker (2001). On 18, 22, and 25 March 2010 pruning weights were recorded using an upright balance scale. In order to obtain data regarding cumulative effects of the treatments on fruiting the following year, the total number of clusters/vine were counted on 10 August 2010. Temperatures for Raymond, Nebraska throughout 2009 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln. All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

Field experiment 2010

Treatments were applied on 13-year-old vines. These vines were not the ones sprayed in 2009. The experiment consisted of five treatments: NAA (500, 1000, and 1500 mg/l), Amigo Oil applied at 10% v/v, and the control which was not sprayed. A randomized complete block design was used with three blocks of 15 vines each. There were three application dates: 28 January, 25 February, and 25 March 2010. The entire unpruned vines were sprayed and each vine received approximately 1l of treatment solution. The treated vines were then pruned on 30 March 2010. The total number of buds/vine was recorded to determine when 50% of the total buds showed bud break and not 60% as in 2009 because of the differences in treatment procedures. In the spring of 2010, vines were visually evaluated for bud break, which was determined as previously mentioned. The total number of buds/vine was counted and bud break was evaluated day by day throughout the spring until each vine reached 50% bud break of the total number of buds that were recorded in March. The number of Julian Days starting from 1 January 2010 until 50% bud break was achieved was used as the basis to calculate the number of days for bud break. On 11 August 2010 the number of clusters and weight of clusters/vine were recorded. From the clusters, 30 berries were randomly counted, placed in a plastic storage bag, and placed in the freezer until berry sample analysis could be conducted. On 18 August 2010 berry samples were analyzed for pH, °Brix, and titratable acidity (TA) using the same procedures as described for the 2009 field experiment. Similarly to 2009, temperatures for Raymond, Nebraska throughout 2010 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln. All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

3. Results and Discussion

Field experiment 2009

As revealed from the analysis of variance, there was a significant treatment by month interaction for bud break

and pH at ($P \leq 0.05$) (Table 1). Similar interactions were found by Dami and Beam (2004) in 'Chancellor' and 'Chambourcin' but not in 'Chardonel'. Due to interaction effects, month effects within treatments and treatment effects within the month are presented in Table 2.

Delaying pruning until March had a significant effect in delaying bud break by two days compared to vines pruned in February (Table 2). While a difference between the pruning carried out in February and March was detected, that difference does not exist when considering pruning in January and April. Therefore, the treated vines in 2010 study were pruned on the same date (30 March 2010) to avoid pruning effects. The idea of pruning, then applying treatments was to maintain apical dominance since pruning can signal vine bud growth, increase the effectiveness of spraying since 'Edelweiss' exhibits a vigorous growth, and reduce chemical applications since the canes will eventually be headed back to a certain number of buds in the pruning season. From the results of NAA applications, it seems that auxin applications failed to maintain apical dominance and inhibit lateral bud growth because grapevines exhibit such a strong dominance (Friend *et al*., 2001). No significant differences were found among NAA 500, 750, and 1000 ppm between the months (Table 2). Regarding oil, no significant differences were found within months except in March (Table 2). This was due to the improper mixing of the oil with water because the oil was mistakenly frozen on the day of spraying.

With regard to the effects of treatments within months, it appeared that desirable results were achieved by oil and NAA at 1000 ppm treatments in all months (Table 2). Except in March, oil applications significantly delayed bud break by five days compared to the control (Table 2). Oil and NAA at 1000 ppm were only significantly different in January and April (Table 2). Overall, there were no significant differences between the control, NAA at 500, and 750 ppm.

Table 1 reveals neither a treatment by month interaction nor a treatment effect, but it does show a month effect at ($P \leq 0.05$) regarding the number of clusters in 2009. The largest average number of clusters per cane was found in April-treated vines but they were not significantly different from averages for vines treated in March and January (Table 3). The average number of clusters was lowest in February-treated vines and significantly different from the other three treatment dates (Table 3). Most importantly, no treatment effect was detected. The number of clusters per shoot ranged from 5.5 to 8.2 (data not shown).

Five buds were retained after pruning. Usually, each bud can produce one to two clusters (personal observation), hence ten clusters would have been an optimum production in this case. Nevertheless, the above averages were acceptable to James Arthur Vineyards and the average difference between April- and February- treated vines, although statistically different, is only 1.5 clusters (Table 3). This difference could be explained by cluster characteristics of 'Edelweiss' which are known to be very loose and many clusters can simply fall down on the ground by any

Table 3 - Least significant difference test (LSD) for average number of clusters per cane of 12-year-old 'Edelweiss' grapevines

Pruning time	Average number of clusters per cane
April	7.3 a
March	7.2 a
January	7.0 a
February	5.8 b

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's protected LSD.

Table 1 - Analysis of variance table for the experiment conducted in 2009

Source of variance	DF	Bud break	No. of clusters produced in summer 2009	CW	BW	°Brix	pH	TA	PW	No. of clusters produced in summer 2010
Treatment	4	< 0.0001	0.49	0.18	0.68	0.60	0.29	0.16	0.88	0.98
Month	3	0.60	0.02	0.32	0.44	0.51	0.38	0.63	0.53	0.43
Interaction	12	0.02	0.63	0.68	0.47	0.63	0.0039	0.38	0.34	0.47

CW=Cluster weight, BW=Berry weight, TA=Titratable acidity, PW=Pruning weight.

Table 2 - Pruning time and spray treatment effects on average days to show 60% bud break in 12-year-old 'Edelweiss' grapevines

Pruning time	Spray treatments				
	Control	NAA 500 ppm	NAA 750 ppm	NAA 1000 ppm	Oil
January	125.0 ab (c)	126.0 a (c)	126.5 a (bc)	128.0 a (b)	130.0 a (a)
February	124.0 b (c)	124.5 a (bc)	126.0 a (b)	129.0 a (a)	129.0 a (a)
March	126.0 a (b)	126.0 a (b)	127.0 a (ab)	128.3 a (a)	126.5 b (ab)
April	125.5 ab (c)	125.0 a (c)	126.0 a (bc)	127.7 a (b)	129.7 a (a)

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD. The lower case letters in parenthesis are related to treatment effects within months.

means of physical contact (Swenson *et al*., 1980; Brooks and Olmo, 1997; Smiley *et al*., 2008).

No significant interaction, month, or treatment effect was found in average weights of 'Edelweiss' clusters at ($P \leq$ 0.05) (Table 1). Cluster weights of the two selected canes ranged from 1.33 to 2.22 kg (data not shown). This supports the lack of differences found regarding the average number of clusters per cane since cluster weights were recorded as averages of the two canes selected and the differences found in average number of clusters per cane is attributed to the looseness characteristic of 'Edelweiss' clusters.

Also, no significant interaction, month, or treatment effect was found in weights of 50 samples of 'Edelweiss' berries ($P \leq$ 0.05) (Table 1). Once again, this will supports neglecting the differences detected in the average number of clusters per cane. The 50 berry sample weights ranged from 116.0 to 125.2 g (data not shown).

A treatment by month interaction was only found in pH analysis at ($P \leq 0.05$) (Table 1). Due to interaction effects in pH analysis, month effects within treatments and treatment effects within the month are presented in Table 4.

NAA at 750 and 1000 ppm showed no differences in pH values for all four pruning times (Table 4). Meanwhile, oil treatment gave a significantly lower pH for April pruning, as did the control in January, although the latter was not significantly different from February's result (Table 4). More obvious differences were observed with NAA at 500 ppm for the four pruning times (Table 4).

Regarding treatment effects within months, no significant differences were found among treatments within March (Table 4). The control was significantly different from all other treatments in January (Table 4) and the oil treatment was significantly different from the control and NAA at 750 ppm in April. From the results in Table 4 and Table 1, it seems that differences in pH values are not due to the delay in bud break but to environmental conditions. In this regard, Creasy and Creasy (2009) mentioned that berry characteristics are totally dependent on environmental conditions, especially the microclimate (climate within canopy). This was also confirmed by Huck (2009). In her study, training systems influenced sunlight penetration, canopy structure and thus fruit composition of 'Frontenac' was totally dependent on climate within the canopy. The vines showed vigorous vegetative growth in 2010 with a low number of clusters. Weaver (1976) noted that calyptras may not fall in cold rainy weather and this will reduce the amount of fruit set.

The °Brix ranged from 12.3 to 13.2, pH values were 3.14 to 3.27, and TA values were 0.83 to 1.13 g/100 ml (data not shown). Regarding harvest parameters, Dharmadhikari and Wilker (2001) mentioned that optimum ranges for white wine would be 21-22%, 3.2-3.4, and 0.7-0.9 g/100 ml for the total soluble solids, pH, and the TA, respectively. 'Edelweiss' is purposely harvested at an earlier stage regarding °Brix and then chaptalized. Swenson *et al*. (1980) mentioned that 'Edelweiss' juice is relatively low in acidity (0.6-0.8%) and has moderate soluble solids (14-16%), and recommended that for wine making it should be picked at an early mature stage (14°Brix).

Harvest parameter results of the 2009 study were in the recommended ranges, except for soluble solids. Lower °Brix values than the preferable ranges (14-16%) of the samples harvested in 2009 were due to the cooler July temperatures (Fig. 1). Higher temperatures during that month would have been preferable for the vines to produce more photosynthates and accumulate more sugar. Weaver (1976) and Winkler *et al*. (1974) mentioned that an optimum temperature for photosynthesis ranges from 25 to 30°C. In addition, lower nighttime temperatures would have been preferable to reduce respiration rates and breakdown the accumulated sugars. Winkler *et al*. (1974) mentioned that 4.4°C halves the respiration rate and is an advantage to suppress fungal disease.

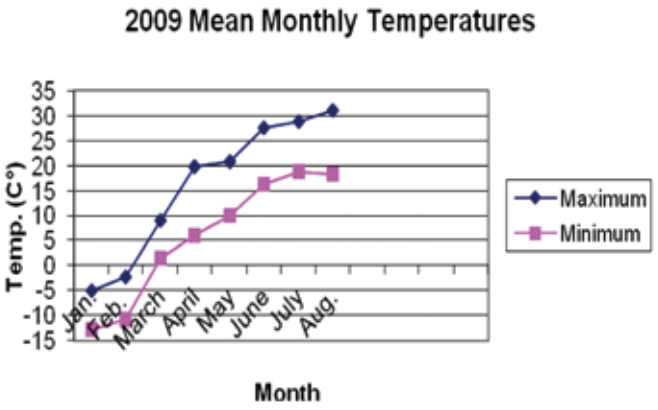


Fig. 1 - The maximum and minimum monthly average temperatures for Raymond, Nebraska in 2009. Source: High Plains Regional Climate Center.

Table 4 - Pruning time and spray treatment effects within month on pH of fruit harvested in 2009 from 12-year-old 'Edelweiss' grapevines treated with NAA and "Amigo Oil"

Pruning Time	Spray Treatments				
	Control	NAA 500 ppm	NAA 750 ppm	NAA 1000 ppm	Oil
January	3.14 b (b)	3.26 ab (a)	3.26 a (a)	3.22 a (a)	3.26 a (a)
February	3.21 ab (ab)	3.27 a (a)	3.23 a (ab)	3.17 a (b)	3.25 a (a)
March	3.23 a (a)	3.18 c (a)	3.20 a (a)	3.21 a (a)	3.22 a (a)
April	3.25 a (a)	3.20 bc (ab)	3.24 a (a)	3.20 a (ab)	3.14 b (b)

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD. The lower case letters in parenthesis are related to treatment effects within months.

The treatments had no effect on pruning weights taken in winter 2010 as shown in Table 1 at ($P \leq 0.05$). This shows that NAA and oil applications had no negative effect on vegetative growth during spring, summer, and fall seasons after bud break.

Pruning weights ranged from 1.05 to 1.43 kg (data not shown). Cultural practices such as mounding that tend to increase cold hardiness can result in higher pruning weights (Gu, 2003). In his study, mounding protected 'Gewürztraminer' vines from the cold winter and significantly increased pruning weights. Although cold hardiness was not measured in this study, the experimental applications had no negative effects on grapevine vegetative growth and such delays in bud break should be of no concern.

Regarding the concerns of grape growers on cumulative effects, especially with applications of plant growth regulators, the number of clusters per vine ranged from 12 to 16 (data not shown). Analysis of variance in Table 1 showed that there were no such effects regarding the number of clusters produced in the following harvest year 2010 ($P \leq 0.05$).

Field experiment 2010

Unlike the study in 2009, there was no significant treatment by month interaction, but a significant pruning time and spray treatment effect was present ($P \leq 0.05$) (Table 5).

The oil treatment significantly delayed bud break by eight days compared to the control. Furthermore, it significantly delayed bud break by nearly four and five days compared to NAA 1500 ppm and 1000 ppm, respectively (Table 6).

Delaying bud break up to 12 days can encourage grape growers to use oil as an effective method to delay bud break and avoid spring frost injury. "The probability of freezing temperatures occurring decreases as spring progresses. Therefore, cultural methods that delay the onset of bud break will decrease the risk of frost damage" (Friend *et al.*, 2001). Furthermore, delaying pruning until March was very effective in improving results compared to the study in 2009 regarding delaying bud break. It is suggested that grape growers should delay pruning as much as possible and if this strategy is to be adopted to reduce frost risk, pruning should be done late in the pruning season especially for cultivars that exhibit early bud break. Although there was no month effect on bud break, overall March applications delayed by one to two days more than February and January (data not shown).

Table 5 shows no effects on the total number of clusters per vine in 2010 (it was cluster per cane in 2009) ($P \leq 0.05$). The total number of clusters per vine ranged from 12 to 19 (data not shown), which is similar to the range found in 2009. In addition, similar analysis of variance results were obtained compared to the 2009 study regarding treatment by month interaction and treatment effects. The only difference was a month effect present in the 2009 study which was not present in 2010. Totally different weather conditions prevailed in 2009 compared to 2010 (Fig. 2).

Table 6 - Least Significant Difference Test (LSD) for average number days of bud break for 13-year-old 'Edelweiss' grapevines in 2010

Treatment	Average number days to bud break
Oil	122 a
NAA 1500 ppm	118 b
NAA 1000 ppm	117 b
NAA 500 ppm	115 c
Control	114 c

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD.

According to the High Plains Regional Climate Center, normal precipitation for Raymond, Nebraska is 121 mm and 95 mm for May and June, respectively. In 2010, monthly precipitation in June was 249 mm, almost three times the monthly average. This had a negative impact on 'Edelweiss' fruit set. In fact, 'Edelweiss' yields harvested at James Arthur Vineyards were 8 tons/acre in 2009 and

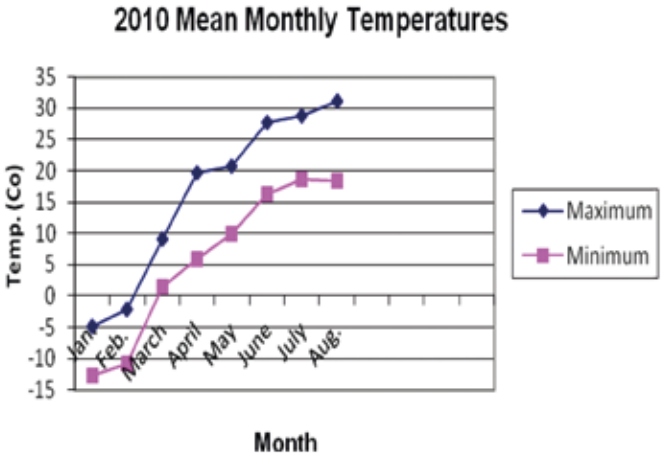


Fig. 2 - The maximum and minimum monthly average temperatures for Raymond, Nebraska in 2010. Source: High Plains Regional Climate Center.

Table 5 - Analysis of variance table for the experiment conducted in 2010

Source of variance	DF	Bud break	No. of clusters produced in summer 2010	CW	BW	°Brix	pH	TA
Treatment	4	< 0.0001	0.90	0.91	0.76	0.89	0.81	0.64
Month	2	0.26	0.26	0.28	0.34	0.84	0.03	0.07
Interaction	8	0.20	0.94	0.92	0.10	0.81	0.23	0.82

CW=Cluster weight, BW=Berry weight, TA=Titratable acidity, PW=Pruning weight.

only 3 tons/acre in 2010.

No significant effects ($P \leq 0.05$) were found in 'Edelweiss' cluster weights (Table 5), which ranged from 2.26 to 3.65 kg (data not shown).

Analysis of variance for berry weights showed a similar trend in 2010 as in 2009. No significant effects were present at $P \leq 0.05$ (Table 5). Berry sample weights ranged from 97.94 to 104.45 g (data not shown).

Similar trends were present in 2010 °Brix and TA results with no significant effects at $P \leq 0.05$. Regarding pH, unlike the results of 2009 where a treatment by month interaction was present, the 2010 study showed a month effect at ($P \leq 0.05$). °Brix ranged from 12.7 to 13.5, pH values were 3.26 to 3.41, and TA values were 1.1 to 1.4 g/100 ml (data not shown). °Brix ranges were higher in 2010, which was expected since average July temperatures were 27.2°C and 28.8°C in 2009 and 2010, respectively. Regarding pH and TA, the 2010 results for the former are in the same range as recommended (3.2-3.4) by Dharmadhikari and Wilker (2001), but TA ranges were a little higher than the recommendation of 0.7-0.9%.

The analysis of variance table for pH results showed a significant month but not a treatment effect at ($P \leq 0.05$). Absence of a treatment effect on berry characteristics is important for recommendation purposes. However, only slight differences were found between months (Table 7).

4. Conclusions

Based on the 2010 results, delaying pruning until March, especially for cultivars that show early bud break such as 'Edelweiss', will delay bud break. Amigo Oil did not exhibit the 20-day delay reported by Dami and Beam (2004) in French-American hybrids and NAA did not exhibit the 16- to 27-day delay that was obtained in the study with cut stems taken from 'Aramon' (*Vitis vinifera*) vines (Nigond, 1960). Amigo Oil gave better performance compared to NAA in both years, even at higher NAA concentrations. It delayed bud break slightly longer (four to five days) and did not affect either the quantity or quality of fruit produced. NAA at 1000 and 1500 ppm showed a potential bud break delay similar to that of Amigo Oil. Oil applications and NAA at 1000 to 1500 ppm in March and up to early April could provide grape growers with an acceptable delay of bud break. This is based on the performance of both oil and NAA applications in the field experiments. Delaying bud break shows no negative impact on berry characteristics.

As a result of this research, it can be recommended to use Amigo Oil at 10% or NAA at 1000 to 1500 ppm from March to April for sites that are prone to frost events such as in southeastern Nebraska and on cultivars that show early bud break such as 'Edelweiss'. Any resulting delay in bud break will decrease the possibility of frost injury. Furthermore, this study opens the door to future studies regarding the value of repeated spraying (Qrunfleh, 2010) or mixing Amigo Oil with NAA. Furthermore, investigation of any phytotoxicity damage to buds caused by oil applications that could possibly occur under vineyard conditions is warranted.

Table 7 - Least Significant Difference Test (LSD) for pH of berry samples in 2010

Month	Average pH
February	3.37 a
January	3.34 ab
March	3.29 b

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD.

References

BROOKS R., OLMO H., 1997 - *The Brooks and Olmo Register of fruit and nut varieties*. - Third Edition. ASHS Press, Alexandria, VA, USA, pp. 744.

CALL R., SEELEY S., 1989 - *Flower bud coatings of spray oils delay dehardening and bloom in peach trees*. - HortScience, 24(6): 914-915.

CREASY G., CREASY L., 2009 - *Grapes. Crop production science in horticulture. 16.* - CABI, Cambridge, UK, pp. 312.

DAMI I., BEAM B., 2004 - *Response of grapevines to soybean oil application*. - Am. J. Enol. Vitic., 55: 269-275.

DHARMADHIKARI M., WILKER K., 2001 - *Micro vinification: A practical guide to small-scale wine production*. - Missouri State Fruit Experiment Station, Mountain Grove, Missouri, USA, pp. 145.

EICHHORN K., LORENZ D., 1977 - *Phänologische Entwicklungsstadien der Rebe*. - Nachrichtenbl. Deut. Pflanzenschutz, 29: 119-120.

FRIEND A., STUSHNOFF C., CREASY G., TROUGHT M., 2001 - *Manipulating bud break date in grapevines*. - Proceedings of the ASEV 52nd Anniversary Annual Meeting, pp. 16.

GU S., 2003 - *Rootstock and mounding effect on growth and cold hardiness of 'Gewürztraminer' (*Vitis vinifera*) and bud dormancy of 'Lacrosse' and 'Chambourcin' (*Vitis *spp.)*. - Ph D. Dissertation, University of Nebraska, Lincoln, USA.

GUINAN P., 2007 - *Understanding and preventing freeze damage in vineyards*. - Workshop Proceedings, University of Missouri Extension, pp. 7-12.

HUCK C., 2009 - *Training system effects on sunlight penetration, canopy structure and fruit composition of 'Frontenac' grape (*Vitis *spp.)*. - M.Sc. Dissertation, University of Nebraska, Lincoln, USA.

LOOMIS N., 1939 - *Note on grape foliation as affected by time of pruning*. - Proc. Amer. Soc. for Hort. Sci., 37: 653-654.

NIGOND J., 1960 - *Delaying bud break in vines by the use of α-naphthaleneacetic acid and defense against frost*. - Compt. Rend. Acad. Agr. France, 46: 452-457.

QRUNFLEH I., 2010 - *Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications*. - Ph D. Dissertation, University of Nebraska, Lincoln, USA.

SMILEY L., DOMOTO P., NONNECKE G., MILLER W., 2008

- *Cold climate cultivar. A review of cold climate grape cultivars*. - Iowa State University, Ames, IA, USA.
SWENSON E., PIERQUET P., STUSHNOFF C., 1980 - *'Edelweiss' and 'Swenson Red' grapes*. - HortScience, 15(1): 100.
WEAVER R., 1976 - *Grape growing*. - Wiley-Intescience, A John Wiley & Sons, Inc. Publication, Ames, IA, USA, pp. 374
WIGGANS C., 1926 - *A study of the relative value of fruiting shoots arising from primary and secondary buds of the 'Concord' grape*. - Proc. Amer. Soc. for Hort. Sci., 23: 293-296.
WINKLER A.J., COOK J.A., KLIEWER W.M., LIDER L.A., 1974 - *General viticulture*. - University of California Press, USA, pp. 710.

Adv. Hort. Sci., 2013 27(1-2): 25-32

Bud dormancy in Japanese pear

Y. Takemura, F. Tamura
Faculty of Agriculture, Tottori University, Koyama, Tottori 680-8553, Japan.

Key words: budbreak, chill units, chilling requirements, climatic change, global warming, Japanese pear, phytohormone.

Abstract: In this paper, after reviewed recent advances in research of endodormancy, research on Japanese pear were reported. In the case of Japanese pear, endodormancy was induced by low temperature of 5°C, without effect of day length. Chilling requirement (CR) of Japanese pear for completion of leaf bud endodormancy shows wide range from below 800 to 1800 chilling units (CU). We investigated the budbreak percent in Taiwanese pear Yokoyama, in Japanese pear strain TH3 and their F1 for 3 years. The percentage of budbreak in TH3 was lower than Yokoyama on any observational days and it gradually increased from early December to early January. The percentage of budbreak in F1 plants was widely distributed between that of Yokoyama and TH3 on all observational days. From results of chi-square test, it was suggested that pear plant had quantitative trait loci (QTL) as genetic factor to decide chilling requirement (CR) for breaking endodormancy. Expression levels of gene encoding GAST-like gibberellin (GA) regulated protein increased with development of endodormancy in Japanese pear cultivars. ABA concentrations in bud of Japanese pear in open-field were increased with the induction of endodormancy by chilling. However, the ABA concentrations in un-chilled plants were lower.

1. Introduction

Bud dormancy in temperate-zone deciduous fruit trees is an adaptive mechanism to survive unfavourable conditions during the winter (Faust *et al*., 1997) and it is classified into three different stages: paradormancy, endodormancy and ecodormancy (Lang, 1987). In autumn, buds enter a dormant state known as endodormancy after the trees stop growing and the leaves fall. During this state the trees cannot start bud growth even if the environmental conditions are favourable. Endodormancy is broken by accumulation of low temperatures, known as chilling requirement (CR), and it depends on the species and cultivar (Westwood, 1978; Saure, 1985). However, a lack of adequate chilling to satisfy the CR to break endodormancy due to climate change (especially global warming) in recent years inhibits the normal growth of new organs in spring (Sugiura *et al*., 2007).

Japanese pear [*Pyrus pyrifolia* (Burm. f.) Nakai] is one of the most important fruits for the Japanese and the earliest records of pear cultivation in Japan date back 1300 years. Commercial Japanese pear cultivation increased with the development of a consumer society during the Edo period (1600-1868). Since then, Japanese pear production underwent from 1920 to 1930, then again from 1950 to a peak in 1970; cultivation is now stable (Tamura, 2006). Figure 1 and Table 1 show the main pear growing areas in Japan. The average annual temperatures of these areas range from 12 to 17°C, and the average growing season temperature (April to September) is 19 to 23°C.

In recent years, Japanese pear cultivation has significantly increased in warm-winter regions (Chen *et al*., 1995). In countries such as New Zealand (Kingston *et al*., 1990; Klinac and Geddes, 1995) and Brazil (Petri and Herter, 2002; Petri *et al*., 2002), a lack of winter chilling has caused problems in promoting bud break in pear during spring. Several recent studies have focused on the breaking of dormancy in grapes (Or *et al*., 2000, 2002; Pang *et al*., 2007; Halaly *et al*., 2008) by using hydrogen cyanamide (HC) as a tool to modify the breaking of endodormancy. In grapes, treatment of buds with HC increased

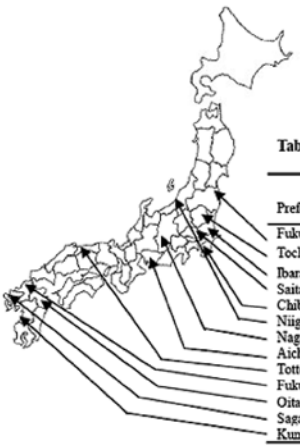


Table 1. Planted area and climate of the main producing prefectures in Japan (2003).

Prefecture	Area (ha)	Average temp (°C)		Rainfall (mm)	
		Annual	Apr.-Sept.	Annual	Apr.-Sept.
Fukushima	1160	12.8	19.5	1105	743
Tochigi	882	13.4	20.0	1443	1076
Ibaraki	1600	14.2	20.3	1154	742
Saitama	678	14.6	21.0	1338	917
Chiba	1660	15.4	21.2	1294	790
Niigata	546	12.9	19.7	1828	820
Nagano	1010	12.5	19.4	1607	1094
Aichi	421	14.5	21.1	1448	1002
Tottori	1390	14.4	20.3	1754	962
Fukuoka	559	16.0	22.3	1919	1450
Oita	450	15.1	21.8	1843	1365
Saga	413	15.5	21.6	2207	1640
Kumamoto	629	16.5	22.9	1993	1514

Fig. 1 - Main producing prefectures in Japan.

Corresponding author: takemura_yoshihiro67@yahoo.co.jp

Received for publication 28 November 2012
Accepted for publication 19 February 2013

hydrogen peroxide concentration and inhibited catalase activity (Perez *et al*., 2008). This is the first step in a cascade that up-regulates several signaling proteins such as transcription factors, protein phosphatases, and protein kinases (Neill *et al*., 2002). Application of HC has also been shown to result in transcriptional up-regulation of grape dormancy-breaking related protein kinase (GDBRPK), a sucrose non-fermenting protein kinase (SNF-like protein), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), thioredoxin h (Trxh), glutathione S-transferase (GST), ascorbate peroxidase (APX), glutathione reductase (GR), and sucrose synthase (SuSy) (Or *et al*., 2000; Pérez and Lira, 2005; Keilin *et al*., 2007; Halaly *et al*., 2008; Pérez *et al*., 2008). However, the mechanism regulating induction and breakage of endodormancy in Japanese pear remains unknown.

In this paper, we review the progress of our research on endodormancy related problems, e.g. CR of cultivars, genetic factors of CR, endodormancy induction, gene expression in Japanese pear over the past 10 years, and future trends.

2. Materials and Methods

Environmental factors of endodormancy induction

The effects of temperature and day length during autumn on induction of endodormancy in Japanese pear were examined. The experiment was conducted using two Japanese pear cultivars [(*Pyrus pyrifolia* (Burm.f.) Nakai)], 'Nijisseiki', grafted onto *P. betulaefolia* Bunge seedlings planted at Tottori University, Tottori, Japan (35.5°N, 134.2°E). Potted 'Nijisseiki' pear trees were placed before endodormancy induction in a greenhouse kept at a minimum temperature above 18°C in September 2004 or in an open field; the photoperiod was controlled for 16 hrs or kept under natural conditions in each place, respectively. Then, one year old shoot was collected and the percentage of budbreak was defined. The apical flower bud was cut, and shoots were divided into five-node cuttings containing five continuous lateral leaf or floral buds. The basal part of the cuttings was submerged in 0.03% (v/v) aluminum sulfate and 0.3% (v/v) 8-hydroxyquinoline. The cuttings were then maintained in a growth chamber at 23±1°C and 24-h photoperiod for four weeks. Bud break is defined as a developmental stage characterized by swelling of the buds and the emergence of a green tip between scales (Tamura *et al*., 1992). The incidence of bud break in each bud type was determined on five shingle shoots having five buds for 28 days.

In addition to this experiment, 'Nijisseiki' pear shoots were collected in late September 2005. Shoots were submerged in 0.003% (v/v) aluminum sulfate and 0.3% (v/v) 8-hydroxyquinoline and treated at 5 and 15°C for 5, 7 and 14 days. After that bubreak percentage was defined.

CRs and forecasting model for endodormancy breaking

In order to estimate the CR for breaking endodormancy in wild pear species and pear cultivars, cultivars grown in the orchard of Tottori University and Tottori Horticultural Experiment Station (35.5°N, 133.7°E) during the 2008-2009 season were used. Leaf bud break on the cuttings prepared from these trees was determined as previously described. The CR was calculated as chill unit (CU) values using the Saitama method (Asano and Okuno, 1990) as described by Tamura *et al*. (1997). Effective chilling hours were calculated after October 31 when the largest negative accumulation was attained.

In addition to this experiment, we also investigated the budbreak percentage in Taiwanese pear Yokoyama, Japanese pear strain TH3 which is a selected strain from the seedlings of self-pollinated 'Osa-Nijisseiki' having homozygote of S4sm gene and their F1 for three years (2008, 2009 and 2010 seasons).

Mechanisms involved in induction and breaking of endodormancy

We isolated the candidate gene related to endodormancy breaking by suppression subtractive hybridization (SSH) method. Total RNA was isolated from 'Nijisseiki' pear buds on 12 November 2005 (deepest period of endodormancy: DP) and 12 January 2006 (breaking period of endodormancy: BP). SSH was carried out between bud in BP ('tester') and bud in DP ('driver') using the PCR-Select Subtractive Hybridization kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions, except for the modification of the first- and second-round PCR conditions. Genes isolated as candidate genes related to bud endodormancy were analyzed by Northern blot analysis using total RNA isolated from buds of 'Nijisseiki' and 'Kousui' pear.

3. Results and Discussion

Endodormancy is induced in buds of deciduous fruit trees in autumn, and then it is broken by the accumulation of low temperature in winter. The report focused on endodormancy induction are few, but the necessary environment condition to induce endodormancy is reported to be short day condition (Kawase, 1961) or low temperature (Tohbe *et al*., 1998). In 'Delaware' grape before endodormancy induction, buds treated long-days inhibited the induction of endodormancy than buds on natural condition (Horiuchi *et al*., 1981). On the other hands, Tamura *et al*. (1997) showed that the induction period of endodormancy in Japanese pear varies greatly as calendar days from year to year.

Thus, the effects of temperature and day length during autumn on induction of endodormancy in Japanese pear were tested. As a result, green house grown 'Gold Nijisseiki' trees kept high level of budbreak even though on mid-December, with no effect of photoperiod (Table 2) (Takemura *et al*., 2011). In contrast, the percentage of budbreak of trees decreased in both open field. Thus, a lack of chilling by the heating treatment above 18°C inhibited endodormancy induction, but a 16 hours photoperiod did not prevent it. In addition to this experiment, we collected 'Nijisseiki' pear shoots on late September, and then were

exposed at 5 and 15 °C for 5, 7 and 14 days. Then percentage of budbreak was detected. As a result in the experiment, it was cleared that temperature of 5°C was effective for inducing bud endodormancy in the cuttings, whereas a temperature of 15°C was ineffective (Fig. 2) (Takemura *et al*., 2011). From these results, the endodermancy of the Japanese pear was induced by low temperatures in autumn rather than short-day.

The CU and Developmental Index (DVI) models were earlier developed to predict the day of breaking bud endodormancy in the Japanese pear (Asano and Okuno, 1990; Sugiura and Honjo, 1997; Tamura *et al*., 1997). The start day of the calculation and the low temperature accumulation on both models are decided as the day perceived low temperature or calendar day. To decide environmental factors of endodormancy induction is important to reduce the gap of start day in both models by climate change in the future.

Next, the effects of temperature and day length during autumn on induction of endodormancy in Japanese pear was tested. From these results, the chilling requirement for completion of leaf bud endodormancy in pear plants ranged from below 400 to 1800 CU (Table 3) (Takemura *et al*., 2013). Among the pear plants examined, 'Yokoyama', which is originated in Taiwan, had the lowest chilling requirement (below 400 CU). In wild pear species, *P. fauriei* showed the lowest chilling requirement (400-800 CU), followed by *P. calleryana* and *P. dimorphophylla*. In addition, Akibae cultivar had the lowest CR among the Japanese pear cultivars examined, and their CR was between 800 and 1000 CU (Table 3) (Takemura *et al*., 2013). This cultivar has the same level of CR of 'Hosui' and 'Ninomiya', the lowest CU cultivar observed among Japanese pear varieties (Tamura *et al*., 2001). Therefore, these three cultivars may serve as suitable cultivars for propagation in warm-winter areas.

On the other hand, there is no Japanese pear which required low-chilling as shown in 'Anna' apple, having 300 CU as chilling requirement. From these results, 'Yokoyama' was selected as gene resource for breeding Japanese pear requiring low-chilling. In 2003, 'Yokoyama' pollen was pollinated with the flower of Japanese pear strain 'TH3' just after castration. 'TH3' is a S1 of 'Osa-Nijisseiki', which has homozygous of S4sm as the gene of self-compatibility haplotype. Using the seedlings obtained, the budbreak percentage in the parents, 'Yokoyama' and 'TH3', and their F1 was investigated for 3 years.

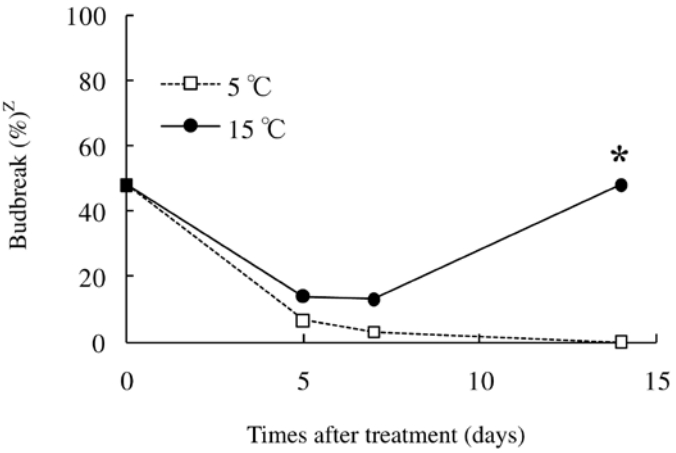


Fig. 2 - Effect of temperature on endodormancy induction of leaf bud in 'Gorld-Nijisseiki' pear (Takemura *et al*., 2011).
(z) 28 days after forcing at 23°C.

Table 3 - Chilling requirement for breaking leaf bud endodormancy in *Pyrus* plants evaluated by the seasonal changes in percent leaf budbreak (Takemura *et al*., 2013)

Chilling requirement (CU)	Registered name
-400	'Yokoyama'
400-800	*P. fauriei*
800-1000	*P. calleryana*, *P. dimorphophylla*, 'Ci Li', 'Akibae', 'Hosui', 'Ninomiya'
1000-1200	*P. betulaefolia*, 'Kosui', 'Wasekouzo'
1200-1400	*P. pyrifolia*, 'Qui Bai Li', 'Ya Li', 'Chojuro', 'Hakko', 'Niitaka', 'Nijisseiki', 'Taihaku', 'Yakumo'
1400-1600	*P. longipes*, 'Choju', 'Hattastu', 'Imamuraaki', 'Kikusui', 'Kumoi',
1600-1800	*P. aromatica*, *P. communis*, 'Bai Li', 'Beijing Bai Li', 'Akaho', 'Amanogawa', 'Doitsu', 'Kimitsukawase', 'Okusankichi', 'Shinsetsu', 'Shinsui'

Table 2 - Effect of temperature and photoperiod on endodormancy induction of leaf bud in 'Gorld-Nijisseiki' pear (areas in Japan) (Takemura *et al*., 2011)

Temperature conditions	Photoperiod conditions	Bud break (%)					
		Sampling date/Days of forcing					
		24 Oct.			10 Dec.		
		14 days	21 days	28 days	14 days	21 days	28 days
Greenhouse (18°C)	16 hr.	18.0 a[z]	40.0 a	60.0 a	36.0 a	50.0 a	60.0 a
	Natural	18.0 a	34.0 a	44.0 b	22.0 a	50.0 a	64.0 a
Open field	16 hr.	0.0 b	14.0 b	16.0 c	0.0 b	8.0 b	8.0 b
	Natural	0.0 b	18.0 b	18.0 c	0.0 b	6.0 b	16.0 b

[z] Different letters within the same column show a significant difference at $P < 0.05$ by *t*-test.

The data obtained showed that the percentage of budbreak in 'Yokoyama' was higher than 60% on all observational days. The percentage of budbreak in 'TH3' was lower than in 'Yokoyama' on all observational days, and it gradually increased from early December to early January. The percentage of budbreak in F1 plants was widely distributed between that of 'Yokoyama' and 'TH3'. On 8 of all observational days, the average percentage of budbreak in F_1 plants was near to that of 'TH3' rather than 'Yokoyama' (Fig. 3) (Takemura *et al*., 2012). Thus, we formulated the hypothesis that 'TH3' is homozygous for a dominant gene involved in the depth of endodormancy, but the hypothesis was rejected based on a chi-square test. Therefore, it was suggested that pear plant had quantitative trait loci (QTL) to decide CR as the genetic factor. It has recently been reported that the fifth chromosome of apricots or ninth chromosome of apples possesses QTL that influence the CR for breaking endodormancy (van Dyk *et al*., 2010; Campoy *et al*., 2011).

In addition to these researches, it has been investigated about biochemical or molecular biological changes during induction or breaking periods of endodormancy in the buds of deciduous fruit trees. Previously researches about cold-hardening during winter in many higher plants shown the change of the lipid composition within cellular membrane (Uemura and Steponkus, 1994; Uemura *et al*., 1995) or the accumulation of compatible solute within the cell (Koster and Lynch, 1992; Wanner and Junttila, 1999; Kamata and Uemura, 2004). In the peach buds, the accumulating period of total soluble sugar contents and water contents during endodormancy seasons were different between two cultivars having difference of CRs, even though plants were of the same specie (Yooyongwech *et al*., 2009). Moreover, Yooyongwech *et al*. (2009) have shown that expression levels of Pp-PIP1 and Pp-γTIP1 genes encoding aquaporin regulating water transport in tonoplast and plasma membrane increased in the peach buds of high-chilling cultivars than that of low-chilling cultivars before endodormancy breaking. In addi-

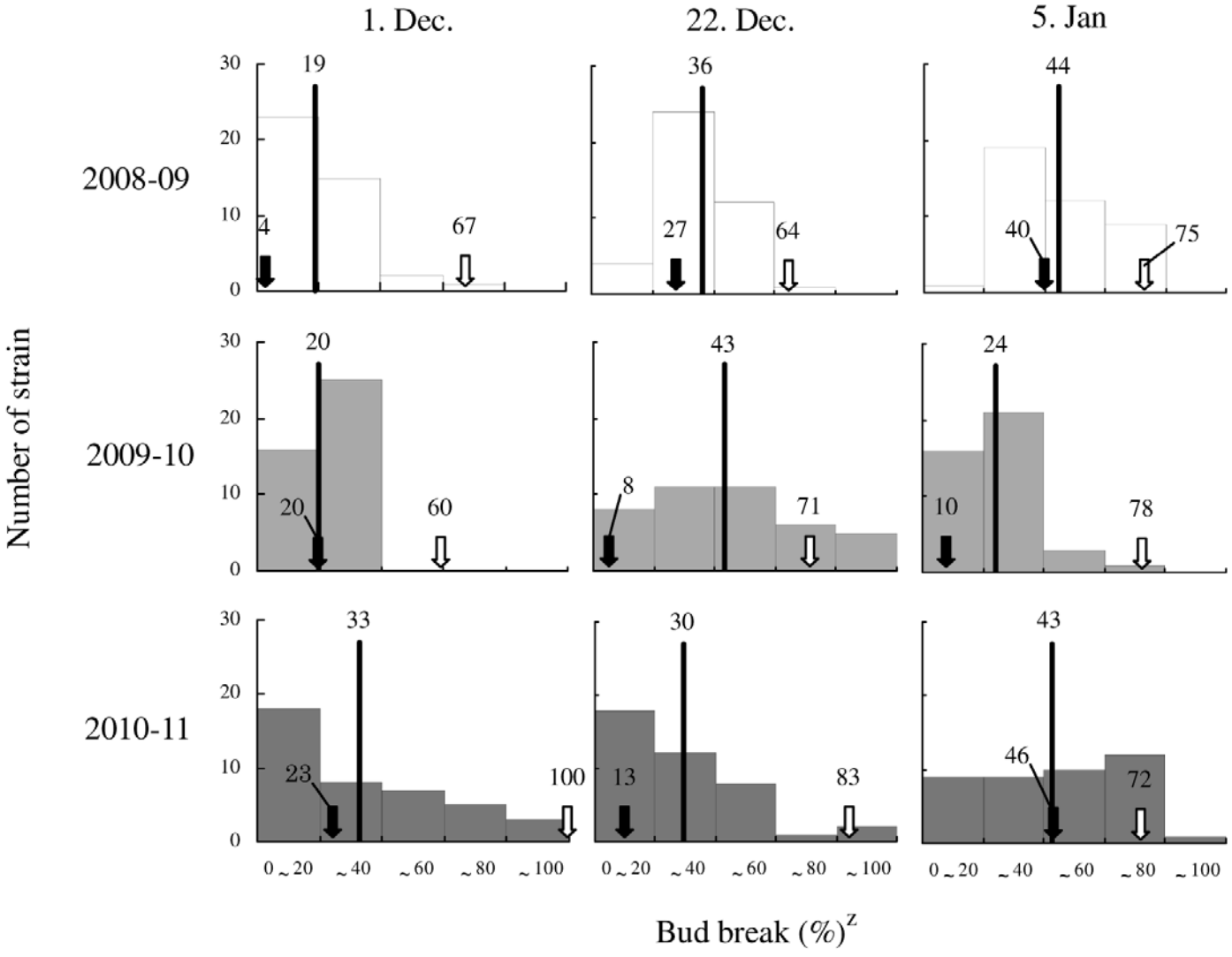


Fig. 3 - Distribution of percentage budbreak on F1 seedlings of 'TH3' × 'Yokoyama' (Takemura *et al*., 2012).
(z) 28 days after forcing at 23°C.
Vertical bars indicate mean of percentage budbreak on F1 seedlings.
: Budbreak of 'TH3' and 'Yokoyama, respectively.

tion, it has been reported that the change of dehydrin levels has been associated with cold hardiness, endodormancy and the content/state of water in the tissue of tree (Arora *et al*., 1997, 2003; Erez *et al*., 1998; Karlson *et al*., 2003). Yamane *et al*. (2006) reported that the role of dehydrin in the bark tissue during the dormant season is common to all *Prunus* species from research compared dehydrin expression level between evergreen and deciduous peach genotypes. However, Yakovlev *et al*. (2008) found that the expression of some dehydrin genes in Norway spruce (*Betula pubescens* Ehrh.) gradually decreased when approaching bud burst, and suggested that the observed changes cannot be related to winter dormancy. From results in our research using bud in Japanese pear, it was not clear that dehydrin genes is involved in development of endodormancy because the results cannot be find the common pattern in two cultivars having difference CRs (Fig.4; A13F). On the other hands, expression levels of gene encoding GAST-like gibberellin (GA) regulated protein increased with development of endodormancy in both two Japanese pear cultivars (Fig.4; B9C).

In previous research, there is a lot of reports focused on association between endodormancy breaking and phytohormone, including GA (Erez *et al*., 1979), Ethylene (Wang *et al*., 1985), Auxin (Nakano *et al*., 1980), Cytokinin (Broome and Zimmerman, 1976; Sterrett and Hipkins, 1980) and Abscisic acid (ABA) (Corgan and Peyton, 1970; Seeley and Powell, 1981; Tamura *et al*., 1992, 1993).

Among them, ABA is termed 'dormin' or 'dormancy inductor' (Addicott, 1983), and considered the most important growth inhibitor. On endodormancy induction period, it has been thought that the accumulation of ABA in shoots of grape controlled the induction or development of endodormancy (Düring and Alleweldt, 1973). In addition, the contents of ABA within leaf bud in Japanese pear on mid-November increased in only the open field condition which confirmed inducing endodormancy (Fig. 5) (Takemura *et al*., 2011). Moreover, Tamura *et al*. (1992) reported the decrease of ABA contents and the increase of GAs contents on endodormancy breaking period in leaf bud of Japanese pear.

The vernalisation has been controlled by GAs interacting closely with ABA and assessed similarities with dor-

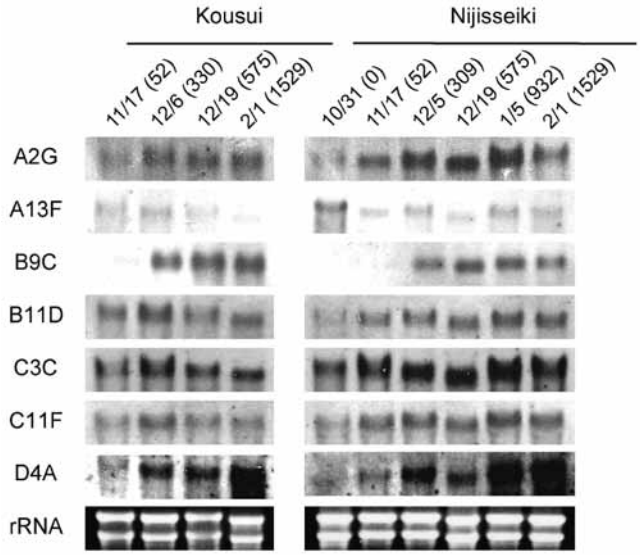


Fig. 4 - Northern blot analysis of the candidate genes is shown in Table 4 in Japanese pear buds of 'Kousui' and 'Nijisseiki'. CU is shown as number in parentheses on the right side of calendar day.

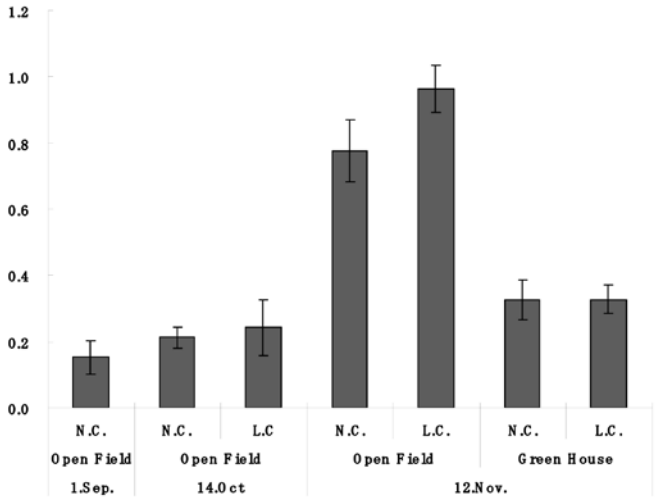


Fig. 5 - The change of ABA contents on induction period of endodormancy in the leaf bud of Japanese pear. N.C. and L.C. are shown as natural day-length condition and long-day condition, respectively (Takemura *et al*., 2011).

Table 4 - Results of sequence homology search for the candidate genes related to endodormancy

Reference clone	cDNA size (bp)	Best database match	E-value
A2G	596	Proline-rich protein 1 (*Vitis vinifera*; AAL02329.1)	7.00E-41
A13F	826	Dehydrin 1 (*Eriobotrya japonica* ; ACL01288.2)	2.00E-16
B9C	468	Putative GAST-like gibberellin regulated protein (*Prunus dulcis*; ABR13302.1)	1.00E-23
B11D	794	PREDICTED: early light-induced protein, chloroplastic (*Vitis vinifera*; XP_002283398.1)	2.00E-59
C3C	927	Cysteine protease inhibitor cystain (*Malus x domestica*; AAO19652.1)	2.00E-137
C11F	503	PREDICTED: uncharacterized RNA-binding protein C23E6.01c-like isoform 1 (*Vitis vinifera*; XP_002285479.1)	3.00E-25
D4A	1049	Unnamed protein product (*Vitis vinifera*; CBI31586.3)	3.00E-18

mancy release (Chouard, 1960). In Arabidopsis, the vernalisation after prolonged exposure to low temperatures was regulated by FLOWERING LOCUS C (FLC) which is MADS-box gene (Sung and Amasino, 2005). In evergrowing peach, Bielenberg *et al*. (2008) reported a cluster of six MADS-box transcription factors (DORMANCY-ASSOCIATED MADS-BOX: DAM) as candidate genes for the regulation of terminal bud formation. The expression of two of these genes, DAM5 and DAM6, is suppressed by chilling temperatures and inversely correlated with bud break rate in peach (Jimenez *et al*., 2010 b), whereas DAM4 and DAM6 expression is promoted by short photoperiods (Li *et al*., 2009). Recent studies have also shown that DAM genes are differentially expressed in response to seasonal dormancy transitions in other plant, inclucing raspberry (*Rubus idaeus* L.) (Mazzitelli *et al*., 2007), Japan apricot (*Prunus mume*) (Yamane *et al*., 2008), peach (Jimenez *et al*., 2010 a; Leida *et al*., 2010) and pear (Ubi *et al*., 2010).

4. Conclusions

Endodormancy in temperate-zone deciduous fruit trees is an essential mechanism to defend buds from unfavourable conditions during winter. To predict the day of breaking bud endodormancy is very important for cultivating. Especially on the forcing culture, a lack of CR for breaking endodormancy caused growth inhibitor as non-germination during spring. Therefore, molecular markers which can estimate whether plants are broken to the endodormancy or not should be developed. Until now, detail of mechanism regulating the induction and the breaking of endodormancy is still unknown. In the future, protein analysis related endodormancy in addition to these genetic approaches will lead us the final conclusion of the mechanisms.

On the other hands, it is suggested that pear and other plants had QTL to decide CR as the genetic factor. To provide for global warming in the future, deciduous fruit tree having lower CR should be bread in the future.

The data of the present paper showed that endodormancy in Japanese pear was induced by low temperature, without effect of day length. Japanese pear had quantitative trait loci (QTL) as genetic factor to decide chilling requirement (CR) for breaking endodormancy. The increase of abscisic acid (ABA) and gibberellin (GA) concentrations in the bud of Japanese pear is related with induction and breaking of endodormancy.

References

ADDICOTT F.T., 1983 - *Abscisic acid in abscission*, pp. 269-300. - In: ADDICOTT F.T. (Ed.), *Abscisic acid*. Praeger, New York.

ARORA R., ROWLAND L.J., PANTA G.R., 1997 - *Chill-responsive dehydrins in blueberry: are they associated with cold hardiness or dormancy transitions?* - Physiol. Plant., 101: 8-16.

ARORA R., ROWLAND L.J., TANINO K., 2003 - *Induction and release of bud dormancy in woody perennials: a science comes of age*. - HortScience., 38: 911-921.

ASANO S., OKUNO T., 1990 - *Period of breaking the rest and the quality of chilling requirement of 'Kosui' and 'Hosui' Japanese pears*. - Bul. Saitama Hort. Expt. Sta., 17: 41-47.

BIELENBERG D.G., WANG Y., LI Z.G., ZHEBENTYAYEVA T., FAN S.H., REIGHARD G.L., SCORZA R., ABBOTT A.G., 2008 - *Sequencing and annotation of the evergrowing locus in peach* Prunus persica *(L.) Batsch reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation*. - Tree Genet. Genomes., 4: 495-507.

BROOME O.C., ZIMMERMAN R.H., 1976 - *Breaking bud dormancy in tea crabapple (*Malus hupehenisis *(Pamp.) Rehd.) with cytokinins*. - J. Amer. Soc. Hort. Sci., 101: 28-30.

CAMPOY J.A., RIUZ D., EGEA J., DAVID J.G., CELTON J.M., MARTÍNEZ-GÓMEZ P., 2011 - *Inheritance of flowering time in apricot* (Prunus armeniaca L.) *and analysis of linked quantitative trait loci (QTSLs) using simple sequence repeat (SSR) markes*. - Plant Mol. Biol. Rep., 28: 560-568

CHEN C., TSAI A.A., LIN M.C., KANG Y.D., 1995 - *The influence of scion sources and warm water dipping on dormancy breaking and fruit of 'Kosui' and 'Hosui' pear grafted to 'Hungshan' pear in Taiwan*. - Acta Horticulturae, 395: 141-147.

CHOUARD P., 1960 - *Vernalization and its relations to dormancy*. - Annu. Rev. Plant Physiol., 11: 191-238.

CORGAN J.N., PEYTON C., 1970 - *Abscisic acid levels in dormant peach flower buds*. - J. Amer. Soc. Hort. Sci., 95: 770-774.

DÜRING H., ALLEWELDT G., 1973 - *The annual cycle of abscisic acid in vegetative organs of grapevines*. - Vitis., 12: 26-32.

EREZ A., COUVILLON G.A., HENDERSHOTT C.H., 1979 - *Quantitative chilling enhancement and negation in peach buds by high temperatures in daily cycle.* - J. Amer. Soc. Hort. Sci., 104: 536-540.

EREZ A., FAUST M., LINE M.J., 1998 - *The change in water status in peach buds on induction, development and release from dormancy*. - Sci. Hortic., 73: 111-123.

EREZ A., FISHMAN S., LINSLEY-NOAKES G.C., ALLAN P., 1990 - *The dynamic model for rest completion in peach buds*. - Acta Horticulturae, 276: 165-174.

FAUST M., EREZ A., ROWLAND L.J., WANG S.Y., NORMAN H.A., 1997 - *Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release*. - HortScience., 32: 623-629.

HALALY T., PANG X., BATIKOFF T., CRANE O., KEREN A., VENKATESWARI J., OGRODOVITCH S.A., LAVEE S., OR E., 2008 - *Similar mechanisms might be triggered by alternative external stimuli that induce dormancy release in grape buds*. - Planta, 228: 79-88.

HORIUCHI S., NAKAGAWA S., KATO A., 1981 - *General characteristics of bud dormancy in the vine*. - J. Japan. Soc. Hort. Sci., 50: 176-184.

JIMENEZ S., LI Z.G., REIGHARD G.L., BIELENBERG D.G., 2010 a - *Identification of genes associated with growth cessation and bud dormancy entrance using a dormancy-incapable tree mutant*. - BMC Plant Biol., 10: 25.

JIMENEZ S., REIGHARD G.L., BIELENBERG D.G., 2010 b - *Gene expression of DAM5 and DAM6 is suppressed by chill-*

ing temperatures and inversely correlated with bud break rate. - Plant Mol. Biol., 73: 157-167.

KAMATA T., UEMURA M., 2004 - *Solute accumulation in wheat seedlings during cold acclimation: contribution to increased freezing tolerance*. - CryoLetters., 25: 311-322.

KARLSON D.T., ZENG Y., STIRM V.E., JOLY R.J., ASHWORTH E.N., 2003 - *Photoperiodic regulation of a 24-kDa dehydrin-like protein in red-osier dogwood (*Cornus sericea *L.) in relation to freeze-tolerance.* - Plant Cell Physiol., 44: 25-34.

KAWASE M., 1961 - *Growth substances related to dormancy in Betula*. - Proc. Amer. Soc. Hort. Sci., 78: 532-544.

KEILIN T., PANG X., VENKATESWARI J., HALALY T., CRANE O., KEREN A., OGRODOVITCH A., OPHIR R., VOLPIN H., GALBRAITH D., OR E., 2007 - *Digital expression profiling of grape EST collection leads to new insight into molecular events during grape-bud dormancy release*. - Plant Sci., 173: 446-457.

KINGSTON C.M., KLINAC D.J., EPENHUIJSEN C.W., 1990 - *Floral disorders of nashi (*Pyrus serotina*) grown in New Zealand*. - N.Z. J. Crop. Hortic. Sci., 18: 157-159.

KLINAC D.J., GEDDES B., 1995 - *Incidence and severity of the floral bud disorder "budjump" on nashi (*Pyrus serotina*) grown in the Waikato region of New Zealand*. - N.Z. J. Crop. Hortic. Sci., 23: 185-190.

KOSTER K.L., LYNCH D.V., 1992 - *Solute accumulation and compartmentation during the cold acclimation of puma rye*. - Plant Physiol., 98: 108-113.

LANG G.A., 1987 - *Dormancy: a new universal terminology*. - HortScience., 22: 817-820.

LEIDA C., TEROL J., MARTI G., AGUSTI M., LLACER G., BADENES M.L., RIOS G., 2010 - *Identification of genes associated with bud dormancy release in* Prunus persica *by suppression subtractive hybridization*. - Tree Physiol., 30: 655-666.

LI Z., REIGHARD G.L., ABBOTT A.G., BIELENBERG D.G., 2009 - *Dormancy-associated MADS genes from the EVG locus of peach [*Prunus persica *(L.) Batsch] have distinct seasonal and photoperiodic expression patterns*. - J. Exp. Bot., 60: 3521-3530.

MAZZITELLI L., HANCOCK R.D., HAUPT S., WALKER P.G., PONT S.D.A., MCNICOL J., CARDLE L., MORRIS J., VIOLA R., BRENNAN R., HEDLEY P.E., TAYLOR M.A., 2007 - *Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus *L.) buds*. - J. Exp. Bot., 58: 1035-1045.

NAKANO M., YUDA E., NAKAGAWA S., 1980 - *Studies on rooting of the hardwood cuttings of grapevine, cv. 'Delaware'*. - J. Japan. Soc. Hort. Sci., 48: 385-394.

NEILL S.J., DESIKAN R., CLARKE A., HURST R.D., HANCOCK J.T., 2002 - *Hydrogen peroxide and nitric oxide as signalling molecules in plants*. - J. Exp. Bot., 53: 1237-1247.

OR E., VILOZNY I., EYAL Y., OGRODOVITCH A., 2000 - *The transduction of the signal for grape bud dormancy breaking induced by hydrogen cyanamide may involve the SNF-like protein kinase GDBRPK*. - Plant Mol. Biol., 43: 483-494.

OR E., VILOZNY I., FENNELL A., EYAL Y., OGRODOVITCH A., 2002 - *Dormancy in grape buds: Isolation and characterisation of catalase cDNA and analysis of its expression following chemical induction of bud dormancy release*. - Plant Sci., 162: 121-130.

PANG X., HALALY T., CRANE O., KEILIN T., KEREN K.A., OGRODOVITCH A., GALBRAITH D., OR E., 2007 - *Involvement of calcium signalling in dormancy release of grape buds*. - J. Exp. Bot., 58: 3249-3262.

PÉREZ F.J., LIRA W., 2005 - *Possible role of catalase in post-dormancy bud-break in grapevines*. - J. Plant Phisiol., 162: 301-308.

PÉREZ F.J., VERGARA R., RUBIO S., 2008 - *H_2O_2 is involved in the dormancy-breaking effect of hydrogen cyanamide in grapevine buds*. - Plant Growth Regul., 55: 149-155.

PETRI J.L., HERTER F., 2002 - *Nashi pear (*Pyrus pyrifolia*) dormancy under mild temperate climate conditions*. - Acta Horticulturae, 587: 353-361.

PETRI J.L., LEITE G.B., YASUNOBU Y., 2002 - *Studies on the causes of floral bud abortion of Japanese pear (*Pyrus pyrifolia*) in Southern Brazil*. - Acta Horticulturae, 578: 375-380.

SAURE M.C., 1985 - *Dormancy release in delicious fruit trees*. - Hort. Rev., 7: 239-300.

SEELEY S.D., POWELL L.E., 1981 - *Seasonal changes of free and hydrolyzable abscisic acid in vegetative apple buds*. - J. Amer. Soc. Hort. Sci., 106: 405-409.

STERRETT J.P., HIPKINS P.H., 1980 - *Response of apple buds to pressure injection of abscisic acid and cytokinin*. - J. Amer. Soc. Hort. Sci., 105: 917-920.

SUGIURA T., HONJO H., 1997 - *A dynamic model for predicting the flowering date developed using 15 an endodormancy break model and a flower bud development model in Japanese pear*. - J. Agr. Meteorol., 52: 897-900.

SUGIURA T., KURODA H., SUGIURA H., 2007 - *Influence of the current state of global warming on fruit tree growth in Japan*. - Hort. Res. (Japan)., 6: 257-263.

SUNG S., AMASINO R.M., 2005 - *Remembering winter: towards a molecular understanding of vernalization*. - Annu. Rev. Plant Biol., 56: 491-508.

TAKEMURA Y., KUROKI K., MATSUMOTO K., NAKATA N., TAMURA F., 2012 - *Characteristics of endodormancy of F1 hybrids between japanese pear TH3 and taiwanese pear Yokoyama*. - Hort. Res., 11: 181-187.

TAKEMURA Y., KUROKI K., MATSUMOTO K., TAMURA F., 2013 - *Cultivar and areal differences in the braking period of bud endodormancy in pear plants*. - Sci. Hortic., 154: 20-24.

TAKEMURA Y., SUDO S., IKEDA T., MATSUMOTO K., TAMURA F., 2011 - *Chilling induced bud endodormancy in japanese pear "Gold Nijisseiki"*. - Hort. Res., 10: 87-92.

TAMURA F., 2006 - *II-4 Pear 1. Japanese Pear*, pp. 50-57. - In: JSHS. The Japanese society for horticultural science (eds.) *Horticulture in Japan*. Shoukadoh Publication, Dept. of Publishing of Nakanishi Printing Co., Ltd., Kyoto, Japan, pp. 334.

TAMURA F., TANABE K., BANNO K., 1992 - *Effect of chilling treatment on intensity of bud dormancy, respiration and endogenous growth regulators in Japanese Pear 'Nijisseiki'*. - J. Japan. Soc. Hort. Sci., 60: 763-769.

TAMURA F., TANABE K., IKEDA T., 1993 - *Relationship between intensity of bud dormancy and level of ABA in Japanese pear 'Nijisseiki'*. - J. Japan. Soc. Hort. Sci., 62: 75-81.

TAMURA F., TANABE K., ITAI A., 1997 - *A model for estimating rest completion for 'Nijisseiki' pear*. - Environment Control in Biol., 35: 185-189.

TAMURA F., TANABE K., ITAI A., MORIMOTO M., 2001 - *Variation in the chilling requirement for breaking leaf bud endodormancy in wild pear species and pear cultivars*. - J. Japan. Soc. Hort. Sci., 70: 596-598.

TOHBE M., MOCHIOKA R., HORIUCHI S., OGATA T., SHIOZAKI S., KUROOKA H., 1998 - *The role of glutathione on the onset of endodormancy of Grape buds*. - J. Japan. Soc. Hort. Sci., 67: 912-916.

UBI B.E., SAKAMOTO D., BAN Y., SHIMADA T., ITO A., NAKAJIMA I., TAKEMURA Y., TAMURA F., SAITO T., MORIGUCHI T., 2010 - *Molecular cloning of dormancy associated MADS-box gene homologs and their characterization during seasonal endodormancy transitional phases of Japanese pear*. - J. Am. Soc. Hortic. Sci., 135: 174-182.

UEMURA M., JOSEPH R.A., STEPONKUS P.L., 1995 - *Cold acclimation of* Arabidopsis thaliana*: effect on plasma membrane lipid composition and freeze-induced lesions*. - Plant Physiol., 109: 15-30.

UEMURA M., STEPONKUS P.L., 1994 - *A Contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance*. - Plant Physiol., 104: 479-496.

VAN DYK M.M., SOEKER M.K., LABUSCHAGNE I.F., REES D.J.G., 2010 - *Identification of a major QTL for time of initial vegetative budbreak in apple (*Malus x domestica *Borkh.)*. - Tree Genet. Genom., 6: 489-502.

WANG S.Y., FAUST M., STEFFENS G.L., 1985 - *Metabolic changes in cherry flower bud associated with breaking of dormancy in early and late blooming cultivars*. - Physiol. Plant, 65: 89-94.

WANNER L.A., JUNTTILA O., 1999 - *Cold-induced freezing tolerance in* Arabidopsis. - Plant Physiol., 120: 391-400.

WESTWOOD M.N., 1978 - *Temperate-zone pomology,* pp. 199-303. - W.H. Freeman and Co., San Francisco, CA, USA, pp. 428.

YAKOVLEV I.A., ASANTE D.K.A., FOSSDAL C.G., PARTANEN J., JUNTTILA O., JOHNSEN O., 2008 - *Dehydrins expression related to timing of bud burst in Norway spruce*. - Planta., 228: 459-472.

YAMANE H., KASHIWA Y., KAKEHI E., YONEMORI K., MORI H., HAYASHI K., IWAMOTO K., TAO R., KATAOKA I., 2006 - *Differential expression of dehydrin in flower buds of two Japanese apricot cultivars requiring different chilling requirements for bud break*. - Tree Physiol., 26: 1559-1563.

YAMANE H., KASHIWA Y., OOKA T., TAO R., YONEMORI K., 2008 - *Suppression subtractive hybridization and differential screening reveals endodormancy-associated expression of an SVP/AGL24-type MADS-box gene in lateral vegetative buds of Japanese apricot*. - J. Am. Soc. Hortic. Sci., 133: 708-716.

YOOYONGWECH S., SUGAYA S., SEKOZAWA Y., GEMMA H., 2009 - *Differential adaptation of high- and low-chill dormant peaches in winter through aquaporin gene expression and soluble sugar content*. - Plant Cell Rep., 28: 1709-1715.

Adv. Hort. Sci., 2013 27(1-2): 33-43

Evidence for non-occurrence of node-to-node or stem-to-bud transfer of chilling temperature signal for dormancy release

M. Bonhomme *, **, André Lacointe *, **, Rémy Rageau *, **

* *INRA, UMR547 PIAF, 5 chemin de Beaulieu, F-63100 Clermont-Ferrand, France.*

** *Clermont Université, Université Blaise Pascal, UMR547 PIAF, F-63000 Clermont-Ferrand, France.*

Key words: chilling perception, endodormancy release, *Prunus persica,* temperature sensing.

Abbreviations: CU: Chilling unit, GDH: Growing degree hour, MTB: Mean time until bud break, PCU: Positive chilling unit.

Abstract: In the current context of global changes, phenology is expected to be one of the major processes affected by temperature increase, notably through the dynamics of endodormancy release. However, the actual impact on bud break pattern is difficult to predict due to poor knowledge about the spatial extent of chilling sensing, which likely affects bud break heterogeneity. Indeed, contrary to a widely held opinion, the strictly local perception of the chilling air signal has never been demonstrated. The present experiment addresses this issue through local chilling or heating of selected nodal groups of buds on shoots of peach tree under endodormancy. A temperature-conditioned localizable air-jet device was designed to provide the sharpest possible temperature contrast between selected treated buds and the 'not-treated' rest of the tree structure, including adjacent axis tissue. Different chilling doses were tested over two experimental seasons, and a heat treatment was applied on single nodes in a cold environment. Chilled vegetative buds did not break when the local chilling dose received was less than 50% of the requirements even though neighboring axis tissue received the standard chilling dose. The maximum rate of bud break reached 80% at chilling completion and most of the broken buds produced long shoots. We conclude that temperature sensing occurs on a very local scale with the signal not reaching neighboring untreated buds, meaning that endodormancy release should be considered a very local process. The local response permits analysis of the intra-canopy heterogeneity of bud break and the possible relationship between bud status and intra-canopy heterogeneity of bud temperature.

1. Introduction

Endodormancy is defined as a phase of suspended growth in the meristematic part of a plant structure that is controlled from within the same plant structure (Lang *et al.*, 1987). Endodormancy thus characterizes not a whole plant but individual plant structures (buds, cambial zones, seeds), and the endodormancy release enabling this meristematic part to recover full growth ability needs to be understood at this individual structure level. This allows for heterogeneity between buds in the tree structure regarding endodormancy status, which is not taken into account in standard phenological models. However, studies on temperate fruit tree species cultivated in tropical or subtropical conditions, i.e. experiencing limiting chilling conditions, have widely reported strong heterogeneity in bud break timing and spacing attributed to heterogeneity in endodormancy release (Crossa-Raynaud, 1955; Guerriero and Scalabrelli, 1982; Dennis, 1987; Bernardi, 1988; Mauget and Rageau, 1988; Lam Yam, 1990; Zguigal, 1995). This heterogeneity in endodormancy release has been shown to result in major impairment of bud break/blooming patterns, including bud-breaking rate with extended and heterogeneous bud breaking delay which in turn results in low leafing rates, the production of 'rosettes', and branching deficiency (Zguigal, 1995). In light of current global warming, such disturbances could potentially extend to new areas, starting in the south of the current temperate zone. This hypothesis is supported by recent observations: since 1988 (the start of continuous increase of yearly mean temperature in France) bloom date has advanced, compared to the 1976-1988 period, whereas endodormancy release date has tended to retreat, most visibly in southern Europe (Chmielewski *et al.,* 2004; Legave *et al.,* 2008) and Japan (Honjo, 2007; Primack *et al.,* 2009). In order to model blooming/bud-breaking date in a context of global change, an accurate endodormancy release module is needed, requiring greater knowledge of chilling signal

(1) Corresponding author: marc.bonhomme@clermont.inra.fr

Received for publication 14 January 2013
Accepted for publication 21 May 2013

sensing, transduction and response. As a step towards understanding and integrating bud break heterogeneity into relevant models, it is important to identify how locally the chilling air temperature signal is perceived and whether it is transduced between structures (buds).

Dormancy release has been investigated at more macroscopic scales, ranging from a twig up to a whole branch. Coville (1920) chilled or warmed a whole branch of blueberry plants, Timmis and Worrall (1974) chilled a whole branch of Douglas fir, Krassnosselskaya and Richter (1942) applied a warm bath on sections of poplar and ash branches, and Witkowska-Zuk (1970) applied a warm bath on a section of *Populus x berolinensis* branch. In reviews covering older works, Nooden and Weber (1978) and Saure (1985) concluded that the influence of endodormancy release factors appears confined to the buds of the treated parts; however treatments were not applied at bud scale and, consequently, did not permit identification of exactly which structure (buds, inter-node, or branch axis) hosted the actual perception zones sensing the endodormancy releasing factors (mainly temperature signal). In his review, Perry (1971) concluded that 'although there is much evidence for the concept of a control center of dormancy processes in buds and leaves, the possibility that other plant parts may be involved is not excluded'.

This issue could be partially resolved by investigations at a smaller spatial scale. Witkowska-Zuk (1970) applied warm baths to terminal buds of long shoots of *Populus x berolinensis*. Other studies used endodormancy-releasing chemicals on single buds on the shoot: Denny and Stanton (1928) applied ethylene chlorohydrin on lilac; Wang and Faust (1987) applied thidiazuron on apple. When plants or branches were subsequently exposed to mild temperatures, only the treated buds broke, prompting the conclusion that buds were effective perception zones of these endodormancy breaking factors and that only the treated bud was able to receive the signaling process borne in it.

However, with warm bath treatments it is impossible to distinguish the effects of temperature, tissue moistening and their interaction, such as the oxygen limitation that Saure (1985) highlighted as an important factor. In addition, Perry (1971) pointed out that 'all that the treatments used for breaking dormancy are severe. Often the temperature or chemical concentrations used border on being lethal. Many of the chemicals used are poisonous at relative low concentrations'. So the interpretation of such results remains questionable, even in the most convincing case of lilac bud treatment by Denny and Stanton (1928). Surprisingly, to date no experimental study at bud level has used chilling temperature, although it is undoubtedly the main natural factor driving bud endodormancy release at bud level.

The temperature signal was also investigated by authors working on vernalization signal, but the treatment was not applied strictly to the bud; for example Metzger (1988) chilled the upper part of the twig. Furthermore, the process investigated is not exactly the same because, in the vernalization process, leaves are involved (Crosthwaite and Jenkins, 1993).

Thus, contrary to a widely held opinion, a strictly local perception of the chilling air signal has never been demonstrated.

The present experiment addresses this issue through local application of different temperatures to either buds or neighboring non-bud areas in an attempt to answer the following questions: 1) which structures are able to perceive the chilling signal inducing dormancy release - bud only, non-bud area only, or both; 2) is the chilling signal applied to one bud able to break dormancy in other buds on the twig; 3) is the signal applied to non-bud area able to break dormancy in buds; 4) is non-bud chilling required in addition to bud chilling for dormancy release?

2. Materials and Methods

The experiment was conducted on 'Redhaven' peach trees (three years old in 2003) grown outside in 200-l containers filled with a peat-local soil mixture. Thermal conditioning was applied locally to parts of one-year-old shoots at bud scale, with two contrasting levels: either (i) target buds were submitted to chilling temperature while the rest of the tree structure, including shoot tissues adjacent to these buds, remained at non-chilling temperature (T>15°C in a greenhouse, experiments 1 and 2); or (ii) a symmetrical treatment, with target buds kept at non-chilling temperature while the rest of the tree structure remained at chilling temperature in a cold chamber (experiment 3). As the focus of the study was on endodormancy release in relation to outgrowth and branching pattern, the term 'target buds' refers to vegetative buds only; the one or two floral buds that could be on either side of the single vegetative bud on each node, sharing the same thermal conditions, were not taken into account.

Conditioning device

Local thermal conditioning was achieved by a thermostated air-jet (Fig. 1). Low-pressurized air was circulated in plastic pipes from a thermostated water bath and delivered through nozzles (modified pipette tip cones) directly blowing onto node buds. The neighboring shoot axis structures were kept isolated from the air jet by a plastic deflector set up at the base of each air-chilled or air-warmed bud group.

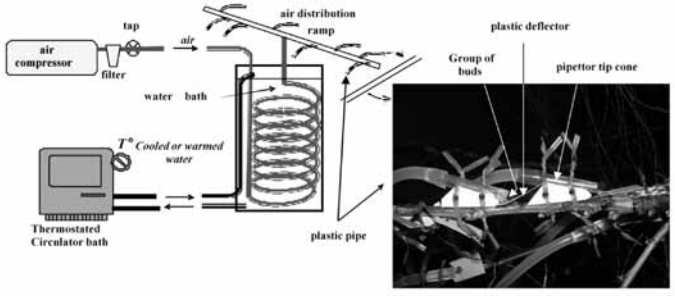


Fig. 1 - Schematic diagram and picture of the device developed for selectively chilling or heating buds (for the warming treatment, warm water replaced cooled water in the water bath).

Bud temperature was monitored by thermocouples inserted between the basal scales of one floral bud (Fig. 2), and internode temperature corresponded to ambient temperature. Thermocouples inserted into the bark of the shoot axis 3-5 mm away from the bud point connection made it possible to verify that adjacent axis tissues (AAT), very close to the buds but protected by the deflector (Fig. 1), were not significantly affected by the cooled air jet. This device allowed efficient local, differential thermal control of buds and the non-bud area (Fig. 3). Thermocouple data were recorded on a Delta T logger (Delta-T-Device-SIIS, Cambridge, UK) every 10 min. Ambient temperature, corresponding to the inter-node temperature, was recorded (time lapse: 10 min) through a thermistor (Hobo Temp Pro 64K, Prosensor).

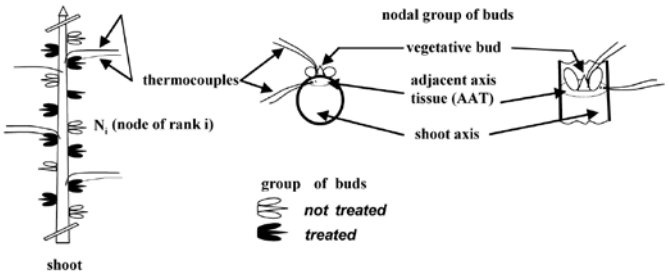


Fig. 2 - Schematic diagram of the experimental design for the treatment of bud groups and for collecting the temperature data for treated bud groups and the corresponding adjacent axis tissues.

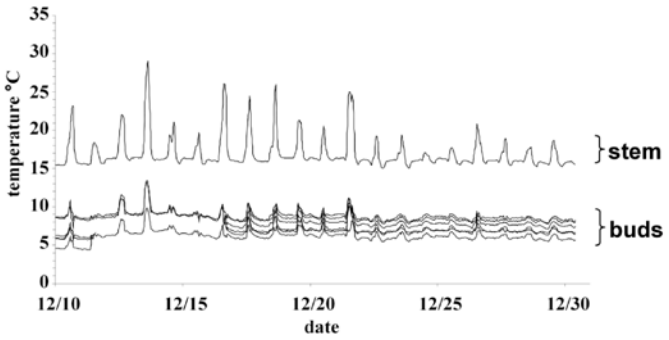


Fig. 3 - Temperature applied at stem and bud level in experiment 1 (partial recording as example).

Selective chilling of buds on cold-deprived trees under endodormancy (experiments 1 and 2)

Trees were cold-deprived from the time of deepest endodormancy onwards by transferring them to a greenhouse kept at a temperature that was high enough to prevent any chilling effect (heating threshold: 15°C) and moderate enough to prevent heat shocks (cooling threshold: 25°C).

Experiment 1. In 2003, six trees were transferred from outside into the greenhouse on October 14. Due to technical constraints, two 80-cm-long shoots were selected on one of the six trees for the cooling treatment. On each shoot (*Sx*), eight bud groups at *Nx* node position from the terminal (one vegetative bud with one or in most cases two floral buds) were chilled under the cooled air-jet from November 18 onwards. On December 23, a thermocouple was inserted under the bark of the shoot axis at mid-internode between nodes N14 (not chilled) and N15 (chilled) of shoot S2. The data (not shown) revealed that during the cooling period, mid-internode temperature was very close to the ambient temperature as recorded with the thermistor. This enabled us to map the temperature dynamics of each shoot section (buds, internodes) of the treated zone.

The buds of nodes N6 and N9 on shoot S1 fell at the beginning of December due to mechanical injury while setting up the air device. They were replaced by two new groups, i.e. nodes N5 and N7 on S1. On December 30, the cooling systems on all buds were stopped. We noticed damage on the buds at node N5, which were consequently discarded from the analyses. Consequently, this treatment actually had two levels: a 'short chilling' treatment (buds of node N7 on S1) and a 'long chilling' treatment (the other buds).

Experiment 2. In order to investigate the impact of insufficient chilling on dormancy release and bud breaking patterns, a second experiment, similar to experiment 1, was conducted with low and medium chilling doses. In 2004, six trees were transferred from outside into the greenhouse on October 10. Four one-year-old shoots on one of the six trees and six bud groups were selected on each shoot. Air jet cooling started on 11 February 2005 (Fig. 2). It was terminated on March 15 for shoots S1 and S4 ('short chilling' treatment) and on April 6 for shoots S2 and S3 ('long chilling' treatment'). On March 15, when switching off the nozzles, we noticed damage on the buds at node N12 on S1; the corresponding data were discarded.

Calculating chilling and heating doses. The chilling doses received by different parts of the treated shoots were calculated from the thermocouple (cooled buds) or thermistor data (other parts of the shoot) by applying the classical Utah model (CU) from Richardson *et al.* (1974), the Positive Utah Chill Units (PCU) model (Linsley-Noakes *et al.,* 1994) and the Dynamic model (Fishman *et al*., 1987 a, b). The PCU model was chosen because it appears more relevant in warm country (with October 1 as start date and hourly temperature data) and the Dynamic model because it is, described as the most accurate model for walnut by Luedelling *et al*. (2009 a, b; 2011).

For each experiment, we calculated the heat unit doses between the end of the bud cooling phase and the mean date of bud break. We used the classical Growing Degree Hours (GDH) model, with 4.5°C as base temperature (Richardson *et al.,* 1975).

Selective warming of buds on chilled trees under endodormancy (experiment 3)

Our objective was to condition targeted buds at temperatures unable to release endodormancy (>15°C) while the rest of the tree structure, including shoot tissues adjacent to these buds, remained at chilling temperatures. Two trees were cold-deprived by transferring them on 15 September 2008 to a greenhouse maintained at a temperature that was high enough to prevent any chilling effect on the trees (heating threshold: 15°C). On 17 October 2008, they were transferred into a cold chamber set around 8°C with

a short photoperiod (8 h) for 52 days, which corresponded to the target accumulation of ca. 1000 CU calculated with the PCU or Utah models as chilling requirements were estimated as 870 CU by Richardson *et al*. (1974, 1975) and 1180 CU by Werner *et al.* (1988) for Redhaven peach trees. The corresponding value for the Dynamic model was initially set as 75 'portions' for this variety (Erez *et al.,* 1988) but later revised to 45 'portions' (Erez, personal communication) and optimized by Bonhomme *et al.* (2010) at 48 'portions'. During this period, the same bud conditioning device was used to provide warm air (>15°C) to selected buds on six twigs of two trees. Finally, the trees were transferred on December 8 into a greenhouse where the temperature was kept above 15°C until bud break.

Biological observations

Dormancy assessment. To ascertain if vegetative buds were endodormant at the start of the chilling or warming treatments, we characterized dormancy status using the "single node cuttings" forcing test at 25°C (Rageau, 1978; Dennis, 2003) on buds from non-treated shoots. Mean time to bud break (MTB) was then calculated. At the end of both treatments, MTB was calculated to verify that endodormancy had not been released in experiments 1 and 2 for non-chilled buds but had been released in experiment 3 for non-warmed buds.

Bud phenology and shoot growth. Each of the buds of treated shoots was checked twice a week after the end of the cooling or warming treatment (i.e. non-chilling); the buds, like the other parts of the trees, were kept under the forcing temperatures (>15°C) of the greenhouse. We watched for bud break in vegetative buds, at "green tip" stage, #09 in BBCH scale (Meier, 2001).

After the cooling treatment, shoot length was measured several months after bud break, in late May and June in experiments 1 and 2, respectively, when growth had almost completely stopped, in order to check for alterations in shoot growth. The same control checks were done in June for experiment 3.

Statistical analysis

Bud breaking rates obtained for the different levels of chilling doses were compared with Fisher's exact test.

The relationship between bud break delay (DB) and chilling doses received, and between the length of shoots (L) and chilling doses were analyzed by linear correlation.

The effect of year (experiment) on DB and L was tested using the non-parametric Kruskal-Wallis test.

All the analyses were performed using the R software package (R Development Core Team 2011; R Foundation, http://www.r-project.org/).

3. Results

Chilling doses received by the different parts of the shoots

Accumulated chilling doses (in CU, PCU and portions) received by the different parts of the shoots were computed according to experiment and chilling treatment duration (Table 1).

Table 1 - Chilling amounts (computed with PCU and CU and Dynanic models) received by different organs and tissues: all internodes and at cooled and non-cooled node buds according to experiments 1 and 2 on shoots (Si) and nodes (Nj)

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 1					
"Short chilling" treatment					
S1	N5	**medium**	**622**	**537**	**24**
S1	N7	*medium*	*620*	*540*	*20*
"Long chilling" treatment					
S1	N11	*high*	*1060*	*970*	*37*
S1	N12	**high**	**1078**	**993**	**38**
S1	N13	*high*	*1060*	*970*	*37*
S1	N14	*high*	*1060*	*970*	*37*
S1	N15	*high*	*1060*	*970*	*37*
S1	N17	**high**	**991**	**906**	**37**
S2	N6	**high**	**1061**	**976**	**38**
S2	N8	*high*	*1060*	*970*	*37*
S2	N9	*high*	*1060*	*970*	*37*
S2	N11	**high**	**1083**	**998**	**37**
S2	N13	*high*	*1060*	*970*	*37*
S2	N15	*high*	*1060*	*970*	*37*
S2	N16	*high*	*1060*	*970*	*37*
S2	N19	**high**	**1079**	**993**	**37**
Chilling amounts at all internodes and non-cooled-node buds		**very low**	**97**	**0**	**3**
Experiment 2					
"Short chilling" treatment					
S1	N8	**low**	**469**	**334**	**30**
S1	N10	*low*	*500*	*370*	*30*
S1	N15	**medium**	**629**	**499**	**32**
S1	N17	**low**	**531**	**400**	**31**
S1	N20	**medium**	**652**	**523**	**32**
S4	N9	**low**	**451**	**313**	**30**
S4	N12	*low*	*440*	*300*	*30*
S4	N17	**low**	**378**	**231**	**28**
S4	N21	**low**	**528**	**386**	**30**
S4	N24	*low*	*440*	*300*	*30*
S4	N29	**low**	**399**	**249**	**29**
"Long chilling" treatment					
S2	N10	**low**	**558**	**410**	**29**
S2	N11	*medium*	*750*	*610*	*29*
S2	N13	**medium**	**674**	**534**	**29**
S2	N17	**medium**	**711**	**571**	**29**
S2	N20	**medium**	**862**	**726**	**30**
S2	N23	*medium*	*750*	*610*	*29*
S3	N9	**medium**	**761**	**626**	**30**
S3	N11	**medium**	**773**	**639**	**30**
S3	N16	**medium**	**887**	**982**	**30**
S3	N19	**high**	**1025**	**756**	**31**
S3	N24	**medium**	**806**	**667**	**30**
S3	N29	*medium*	*810*	*730*	*30*
Chilling amounts at all internodes and non-cooled node buds		**very low**	**129**	**0**	**11**

For non-thermocoupled buds, chilling amounts were estimated as means (rounded to the nearest 10 PCU or CU) of all experimental values from the same class of chilling on the same shoot in the same experiment and indicated in italics

Based on the recorded thermistor data, some chilling units were accumulated during experiments 1 and 2 (97 and 129 units, respectively, which is very low compared to the chilling requirements as mentioned above) at the inter-node tissues as well as at buds and AAT at non-cooled nodes. About half of this chilling occurred before transfer to the greenhouse, the rest occurred before the cooling treatment.

At the cooled nodes, chilling doses were computed, either from actual data for the thermocoupled buds or non thermocoupled buds, as means of all experimental values of the same chilling class from the same experiment (Table 1). Four classes of chilling dose were defined: 'very low' (<150 PCU), 'low' (350-550 PCU), 'medium' (600-900 PCU) and 'high' (>950 PCU). Internodes and non-cooled buds received very low doses (Table 1), mainly before the transfer into the greenhouse.

In experiment 3, the accumulated chilling dose of non-warmed buds reached 973 PCU (Table 2). As the temperature was around 8°C, almost the same value was obtained with the classical Utah model, thus meeting the chilling requirements of the Redhaven peach bud (≈900 CU). For warmed buds, the PCU dose was very low (32 PCU maximum, i.e. only 3% of chilling requirements, 1 portion i.e. around 2% of chilling requirements for Dynamic model) for the coldest bud, and zero for the warmest bud. The classical Utah model yielded zero CU for all warmed buds.

Endodormancy status

After trees were transferred to the greenhouse, the MTB values of non-chilled vegetative buds decreased over the first two to three months and leveled down to ca. 22.5±0.5 days in experiment 1, and 21.5±0.5 days in experiment 2 (data not shown). At periods marking the start of bud cooling, MTB was 31.5±0.5 d in experiment 1 and 21.5±0.5 d in experiment 2, thus indicating that vegetative buds were actually endodormant [MTB threshold between endodormancy and ecodormancy for vegetative 'Redhaven' peach tree buds is cited as 12 days by Bonhomme *et al.* (2000)].

In experiment 3, at the beginning of the treatment, the MTB value of the non-chilled vegetative buds was 32±10 d (data not shown), thus indicating that the vegetative buds were actually endodormant and close to the maximum of endodormancy. When trees were transferred from the cold chamber to the greenhouse for bud break forcing, the MTB value was 12±1.5 d, indicating endodormancy release for these chilled buds. It was impossible to determine MTB of the warmed buds due to insufficient replications. Nevertheless, bud break did not occur, which clearly indicated that endodormancy was not released.

Vegetative bud response

Vegetative bud behavior resulting from chilling dose in experiments 1 and 2. Table 3 presents the vegetative bud responses to the chilling doses received: break, abscission, or no detected event. Neither very low (<115 PCU) nor low (mean: 469 PCU) doses resulted in bud break. Consequently the bud break rate obtained with medium chilling dose is significantly higher ($p<0.0001$) than very low and

Table 2 - Chilling amounts (computed with PCU and CU and Dynamic models) received by different organs and tissues: all internodes and at non-cooled node buds according to experiment 3; at warmed nodes, shoots (Si) and nodes (Nj)

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 3					
"Warming" treatment					
S1	N16	**very low**	**0**	**0**	**0**
S1	N17	**very low**	**18**	**0**	**0**
S1	N21	**very low**	**0**	**0**	**0**
S1	N24	*very low*	*6*	*0*	*0*
S2	N16	**very low**	**30**	**0**	**1**
S2	N21	*very low*	*27*	*0*	*1*
S2	N27	**very low**	**24**	**0**	**1**
S3	N16	**very low**	**0**	**0**	**0**
S3	N21	*very low*	*0*	*0*	*0*
S3	N24	**very low**	**0**	**0**	**0**
S3	N28	*very low*	*0*	*0*	*0*
S3	N29	**very low**	**0**	**0**	**0**
S4	N16	**very low**	**0**	**0**	**0**
S4	N18	*very low*	*0*	*0*	*0*
S4	N20	**very low**	**0**	**0**	**0**
S5	N13	**very low**	**0**	**0**	**0**
S5	N20	**very low**	**0**	**0**	**0**
S5	N24	*very low*	*0*	*0*	*0*
S6	N14	**very low**	**3**	**0**	**0**
S6	N18	*very low*	*14*	*0*	*0*
S6	N23	**very low**	**32**	**0**	**1**
S6	N24	*very low*	*14*	*0*	*0*
S6	N28	**very low**	**6**	**0**	**0**
Chilling amounts at all internodes and non-warmed node buds		**high**	**973**	**941**	**41**

For non-thermocoupled buds, chilling amounts were estimated as means (rounded to the nearest 10 PCU or CU) of all experimental values from the same class of chilling on the same shoot in the same experiment and indicated in italics.

low rates. Almost no buds fell after very low doses, but 20% of buds fell after low doses. Medium and high doses (mean: 745 and 1057 PCU, respectively) both resulted in about 10% bud fall and in 69 and 80% bud break, respectively, values which were not statistically different.

On the shoots with medium and high doses, none of the non-cooled vegetative buds broke (Fig. 4a, 4b), either on the equipped tree or on the other five trees kept under the greenhouse.

Figure 5a shows that within each experiment, the individual delay to bud break (DB), i.e. the time between the end of cool air application and bud break, was not dependent on the PCU received by the bud (for experiment 1, $R^2 = 0.22$, $p = 0.12$; for experiment 2, $R^2 = 0.10$, $p =$

0.41; and if both experiments 1 and 2 were analyzed as one single dataset, $R^2 = 0.08$, $p = 0.21$). There was a clear experiment effect on the mean DB ($p < 0.001$): 35.5 d in experiment 1 and 28.0 d in experiment 2. This difference could be mainly explained based on mean temperature under the greenhouse after the end of bud cooling, which was higher in experiment 2 (19.1°C) than experiment 1 (16.2°C). In addition, Figure 5b shows that the length of a growth unit sprouted from a broken bud was not dependent on the cumulated PCU that had been received by that bud (for experiment 1, $R^2 = 0.05$, $p = 0.51$; for experiment 2, $R^2 = 0.08$, $p = 0.47$; and if both experiments 1 and 2 were analyzed as one single dataset, $R^2 = 0.008$, $p = 0.79$). The

Table 3 - Vegetative bud responses to the received chilling doses

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 3					
"Warming" treatment					
S1	N16	**very low**	**0**	**0**	**0**
S1	N17	**very low**	**18**	**0**	**0**
S1	N21	**very low**	**0**	**0**	**0**
S1	N24	*very low*	*6*	*0*	*0*
S2	N16	**very low**	**30**	**0**	**1**
S2	N21	*very low*	*27*	*0*	*1*
S2	N27	**very low**	**24**	**0**	**1**
S3	N16	**very low**	**0**	**0**	**0**
S3	N21	*very low*	*0*	*0*	*0*
S3	N24	**very low**	**0**	**0**	**0**
S3	N28	*very low*	*0*	*0*	*0*
S3	N29	**very low**	**0**	**0**	**0**
S4	N16	**very low**	**0**	**0**	**0**
S4	N18	*very low*	*0*	*0*	*0*
S4	N20	**very low**	**0**	**0**	**0**
S5	N13	**very low**	**0**	**0**	**0**
S5	N20	**very low**	**0**	**0**	**0**
S5	N24	*very low*	*0*	*0*	*0*
S6	N14	**very low**	**3**	**0**	**0**
S6	N18	*very low*	*14*	*0*	*0*
S6	N23	**very low**	**32**	**0**	**1**
S6	N24	*very low*	*14*	*0*	*0*
S6	N28	**very low**	**6**	**0**	**0**
Chilling amounts at all internodes and non-warmed node buds		**high**	**973**	**941**	**41**

For very low chilling level of the non-treated shoots in experiments 1 and 2, the number (Nb) of non-broken buds was not exactly determined (>1000) but corresponded to all the buds (100%) of the six trees placed under the greenhouse.
Significantly different rates, as assessed by Fisher's exact test, are indicated by different letters.

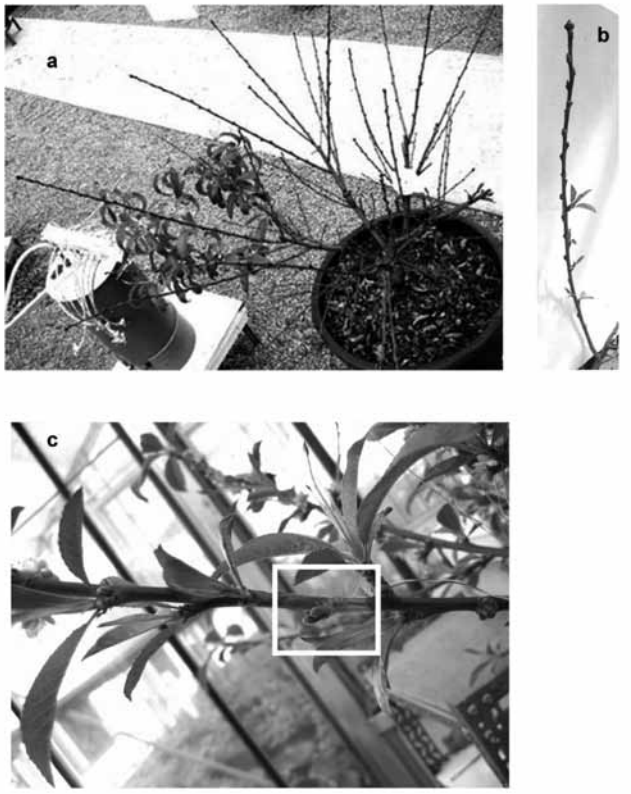


Fig. 4 - Bud break in the localized-chilled experiment (a and b) and localized-warmed experiment (c).

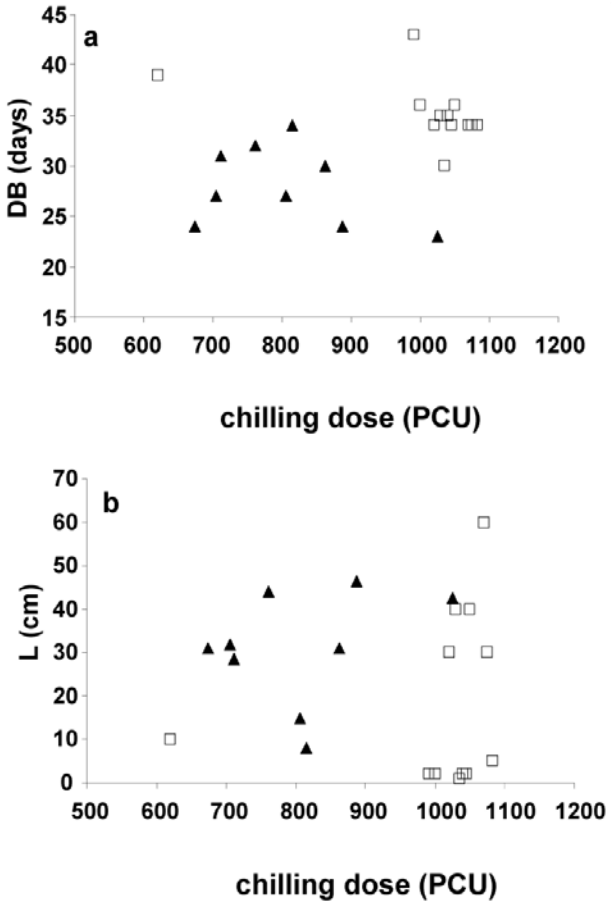


Fig. 5 - Responses of vegetative buds according to chilling dose. a: individual delay to bud break (DB); b: individual final length (L) of shoots in experiments 1 (□) and 2 (▲).

experiment effect on sprouted shoot length was not significant ($p = 0.06$).

It can be concluded that selective chilling of a nodal group of buds was effective in releasing the endodormancy of its vegetative bud. No endodormancy release of buds of any other node was observed, which shows that the signal is not transmitted.

Vegetative bud behavior resulting from the warming treatment in experiment 3. With a few exceptions at the stem bases, almost all buds chilled in the cold chamber broke, corresponding to the classical bud break pattern observed in temperate climates (82% of bud break on the control twigs). On the treated tree, not a single warmed vegetative bud broke, indicating that the chilling signal received at the stem was not transmitted to non-chilled buds (Fig. 4c).

For non-warmed trees, bud break was observed on 9 January 2009 for terminal buds and 16 January 2009 for axillary buds, corresponding to 8419 and 10479 GDH (with the 4.5°C base) after chilling, respectively, i.e. around 10% higher than was calculated for axillary buds in experiments 1 and 2.

Mean temperature in the greenhouse over the 39-day period between the end of chilling and axillary bud break was 15.8°C.

4. Discussion and Conclusions

Vegetative bud behavior resulting from chilling dose in experiments 1 and 2

Contrasting with medium and high chilling doses, the low chilling dose treatment allowed some events to take place in the buds during further forcing, that ultimately led to abscission of a significant percentage of these buds.

The selective chilling of a nodal group of buds was effective in releasing endodormancy of its vegetative bud, and did not result in endodormancy release of buds of any other node.

Nevertheless, some other aspects of the results were unexpected. Medium chilling doses (620-887 PCU) were almost as effective as high doses (991-1083 PCU): the percentage of broken buds was only slightly lower (Table 3) and the shoots borne from the broken buds showed normal growth. The chilling requirement for Redhaven peach trees is cited at 870 CU (Utah model) by Richardson *et al.* (1975), 1180 CU by Werner *et al.* (1988) and 45 portions in the dynamic model (Erez, personal communication), optimized to 48 portions (Bonhomme *et al*., 2010) from one-node cutting test results. This points out the three critical elements in the models: (1) the determination of the starting date for chilling accumulation, (2) taking into account (or not) the negative temperatures and (3) the reality and intensity of the reverse effect of mild temperatures and the duration during which the reversion is possible (a short period in Dynamic model, without limits in Utah model, 24 h in PCU).

The computed heat requirement for bud break (around 9000 GDH) was much higher than the value given by Richardson *et al.* (1975) for 'Redhaven', i.e. 4922 GDH (for the same 4.5°C baseline) for floral buds (full bloom) with 870 CU of prior chilling, and closer to that found by Scalabrelli and Couvillon (1986), i.e. 8000 GDH (same base) for vegetative bud break with 900 CU of prior chilling. The heat requirements would, surprisingly, have been quite similar for buds under medium chilling doses (8900 GDH) and buds under high chilling doses (9200 GDH). This suggests that chilling requirements may have been overestimated and/or that these buds were in deeper endodormancy in experiment 1 than in experiment 2 when chilling started.

Although some shoots had not totally stopped growing when the shoot lengths were recorded, Figure 5b shows that the length of a growth unit borne from a broken bud was not dependent on the cumulated chilling dose received by this bud.

Vegetative bud behavior resulting from warming treatment in experiment 3

Excluding some buds at the base of the twigs, almost all the buds chilled in the cold chamber broke, thus reproducing the classical bud break pattern observed in temperate climates. Not a single warmed vegetative bud broke, indicating that the chilling signal received at the stem was not transmitted to the non-chilled buds.

Normal bud break occurred at a chilling mean temperature of around 9.3°C, showing once again that the Weinberger model is not accurate, even under temperate climates. In this range of chilling temperatures, the Utah and PCU models gave the same cumulated chilling (990 CU), close to the chilling requirement cited by Richardson *et al.* (1974).

The heat requirement for bud break estimated in this experiment was a little higher than that found by Scalabrelli and Couvillon (1986), i.e. 8000 GDH (same base) for vegetative bud break with 900 CU of prior chilling, and also than the amounts deduced from experiments 1 and 2. The small difference (2-3 days) could probably be attributed to the uncertainty in determining bud break date or starting date (i.e. endodormancy release date).

All these results indicate that the temperature for endodormancy release has to be applied at the level of the structure, where the cause of growth blockage is located, i.e. the bud itself, according to the definition of endodormancy given by Lang *et al.* (1987).

Accuracy of the chilling and heat requirements

These requirements are very approximate determinations that are not fully suitable for modeling the impacts of global changes. Figures on chilling requirements for endodormancy release are often confounded by the chilling received during the period between growth arrests or leaf fall and bud break. Heat requirements given in the literature are also approximations, as the thermal threshold permitting growth is generally unknown. Moreover, heat and chilling action could combine at mild temperature

(around 10°C), and determination of the start date for heat action remains problematic.

Further, the delay to bud break (or bloom) and the heat requirements calculated may also be dependent on factors other than the sole bud response to temperature, such as bud water and nutrient uptake rate and, consequently, the temperature of the roots as revealed in Young *et al.* (1987).

Thus, the questions about chilling and heat requirements will probably have to be revisited before chilling and heat requirement figures can be used to model climate change impacts.

Temperature signal transfer

From the literature, the most clear response is that the chilling (temperature) signal is not transferred from one terminal bud of a branch to the terminal bud of another branch.

Regarding vernalization process studies, it is clear that a signal is transferred from the leaves of sugar beet to the terminal meristem (Crosthwaite and Jenkins, 1993) but this signal could be a thermic signal or a secondary biochemical signal. This signal could also be originated in roots (Metzger *et al.,* 1992). Some grafting approaches (Metzger, 1988) seem to show that the vernalized condition is not transferred to other buds but it is not exactly the same process (transfer of a status and not simultaneous transfer of the signal received by one bud to another bud). Moreover, vernalization seems to be correlated with the presence of dividing cells and the dormancy release process occurs on buds with their cell cycle blocked in G1 phase (Cottignies, 1987).

Therefore, even if vernalization and dormancy release seem to be very similar (Metzger, 1996), the literature does not make it possible to conclude about the absence of transfer for the chilling signal from the close part of the twig to the bud or from one bud to another bud located on the next internode or the complementarity of both tissues.

Synthesis: interpretation in terms of chilling signal transfer

Candidates for the chilling receptive zone are the different nodal bud groups and the axis; candidates for the meristematic targets are the vegetative buds of different nodal groups (only two groups represented). With regard to possible paths, we considered 1) 'univocal' paths, i.e. endodormancy release response can only result from the signal coming from one sensing zone; 2) 'parallel' paths, i.e. each of the signals borne in either a bud group or an axis results in effective endodormancy release in a given bud; 3) 'cooperative' paths, i.e. effective endodormancy release in a given bud needs signals from both bud and axis; 4) 'short' paths, i.e. the signal reaching a bud comes from its bud group, and 5) 'long' paths, i.e. the signal reaching a bud comes from another group.

This can be translated into different hypotheses:

Hypothesis 1: a local application of chilling or warming induces a local response for dormancy release. As chilled buds broke and warmed buds did not, the present results are consistent with this hypothesis.

Hypothesis 1': the chilling (or warming) signal can be transmitted to another bud. As on a twig in warm conditions only chilled buds broke, this hypothesis has to be rejected.

Hypothesis 2: a chilling signal applied to a stem could reach the bud and permit bud break. As warmed buds on stems placed in a cold environment did not break, this hypothesis has to be rejected.

Hypothesis 3: chilling applied to buds does permit bud break even if parallel warming on the stem is applied. As chilled buds broke on a twig in warm conditions, the present results are consistent with this hypothesis. The reciprocal hypothesis - chilling applied to a twig does permit bud break even if parallel warming on the bud is applied - is rejected consequent to the rejection of hypothesis 2.

Hypothesis 3': the chilling signal applied to a bud could be transmitted to other buds, permitting bud break, even if a parallel warm signal is applied to the stem. As only chilled buds broke on a twig in warm conditions, this hypothesis has to be rejected.

Hypothesis 4: chilling applied to buds needs additional chilling on the stem to permit bud break. As chilled buds broke on a twig in warm conditions, this hypothesis has to be rejected.

Hypothesis 4': chilling applied to buds could be transmitted to other buds if additional chilling is applied to the stem. Since in a cold environment (i.e. not-warmed buds and twig are chilled), warmed buds did not break, this hypothesis has to be rejected.

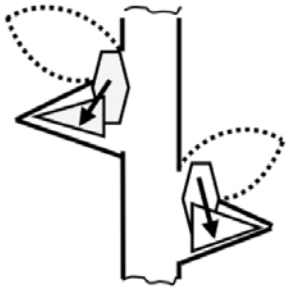
Figure 6 summarizes the different hypotheses tested and the possible "chilling receptive zone → vegetative meristematic target" pathways for endodormancy release signal.

In conclusion, our results validated hypotheses 1 and 3; the other cases are to be rejected. Thus, chilling clearly has to be applied to the given bud to be effective for its subsequent endodormancy release. Chilling the stem without chilling the bud is not effective for endodormancy release. Chilling on the bud does not need additional chilling on the stem.

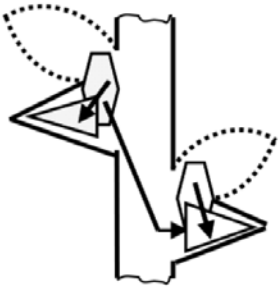
Consequently, the physiological processes involved in chilling-temperature response for endodormancy release have to be investigated at bud level. This is not necessarily (and probably not actually) the case for primordial growth in buds during the ecodormancy phase, as water and nutrient uptake fluxes from roots are needed.

Acknowledgements

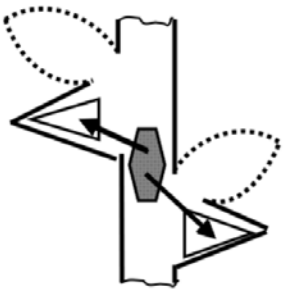
This work was supported by the INRA (Institut National de la Recherche Agronomique), Environment and Agronomy Department. We thank C. Bodet, M. Crocombette and J.P. Richard for technical assistance and ATT for language editing of the manuscript.



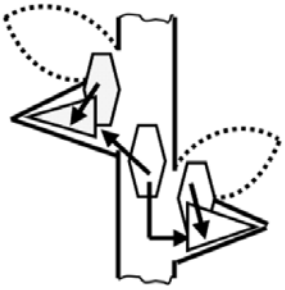
Hyp 1: chilling or warming application on group of buds, local response.
Result : Hyp 1 valid



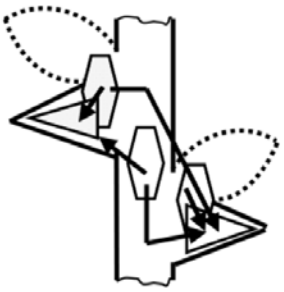
Hyp 1': chilling application on buds, possible transmission of signal to others buds
Result : Hyp 1' not valid



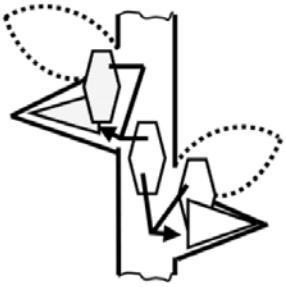
Hyp 2: chilling application on stem, possible transmission of signal to buds.
Result : Hyp 2 not valid



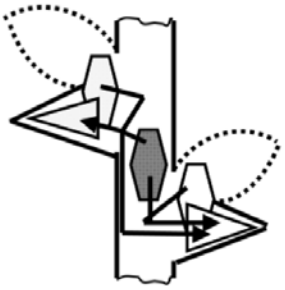
Hyp 3: response of chilled buds did not need parallel chilling signal from stem.
Result : Hyp 3 valid



Hyp 3': the chilling signal could be transmitted to other buds even if parallel warm temperature signal is applied on stem.
Result : Hyp 3' not valid



Hyp 4: response of chilled buds need additional chilling signal from stem.
Result : Hyp 4 not valid



Hyp 4': the chilling signal could be transmitted to other buds if additional chilling signal is applied on stem.
Result : Hyp 4' not valid

'perception zone' with effective chilling dose in experiments

'perception zone' of warm temperature in experiments

Bud break observed i.e. endodormancy released

No bud break observed i.e. endodormancy not released

Fig. 6 - Schema of the different hypotheses tested for "chilling perception" → "meristematic response" spatial paths in the endodormancy release response to chilling; possible and rejected cases for the vegetative buds of peach trees, based on our experimental findings.

References

BERNARDI J., 1988 - *Behaviour of some apple cultivars in the subtropical region of Santa Catarina, Brazil*. - Acta Horticulturae, 232: 46-50.

BONHOMME M., RAGEAU R., GEDRAUD M., 2000 - *ATP, ADP and NTP contents in vegetative and floral peach buds during winter: are they useful for characterizing the type of dormancy?,* pp. 245-257. - In: VIÉMONT J.D., and J. CRABBÉ (eds.) *Dormancy in plants*. CAB International, Wallingford, UK, pp. 386.

BONHOMME M., RAGEAU R., LACOINTE A., 2010 - *Optimization of endodormancy release models, using series of endodormancy release data collected in France*. - Acta Horticulturae, 872: 51-59.

CHMIELEWSKI F.M., MÜLLER A., BRUNS E., 2004 - *Climate changes and trends in phenology of fruit trees and field crops in Germany, 1961-2000*. - Agric. For. Met., 121: 69-78.

COTTIGNIES A., 1987 - *Dormance*. - Annales des Sciences naturelles, botanique, Paris 13e Serie, 8: 93-142.

COVILLE F.V., 1920 - *The influence of cold in stimulating the growth of plants*. - J. Agric. Res., XX(2): 151-160.

CROSSA-RAYNAUD P., 1955 - *Effets des hivers doux sur le comportement des arbres fruitiers à feuilles caduques. Observations faites en Tunisie à la suite de l'hiver 1954-1955*. - An. Ser. Bot. et Agr. Tunisie, 28: 1-22.

CROSTHWAITE S.K., JENKINS G.I., 1993 - *The role of leaves in the perception of vernalization temperatures in sugar beet*. - J. Exp. Bot., 44(286): 801-806.

DENNIS F.G., 1987 - *Producing temperature-zone fruits at low latitudes: An overview*. - HortScience, 22: 1226-1227.

DENNIS F.G., 2003 - *Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of wood plants*. - HortScience, 38: 347-350.

DENNY F.E., STANTON E.N., 1928 - *Localization of response of woody tissues to chemical treatments that break the rest period.* - Amer. J. Bot., 15: 337-344.

EREZ A., FISHMAN S., GAT Z., COUVILLON G.A, 1988 - *Evaluation of winter climate for breaking bud rest using the dynamic model*. - Acta Horticulturae, 232: 76-89.

FISHMAN S., EREZ A., COUVILLON G.A., 1987 a - *The temperature dependence of dormancy breaking in plants: mathematical analysis of a two-step model involving a cooperative transition*. - J. Theor. Biol., 124(4): 473-483.

FISHMAN S., EREZ A., COUVILLON G.A., 1987 b - *The temperature dependence of dormancy breaking in plants: computer simulation of processes studied under controlled temperatures.* - J. Theor. Biol., 126(3): 309-321.

GUERRIERO R., SCALABRELLI G., 1982 - *Relationships between bud dormancy and growing and fruiting behaviour on different apricot varieties along the tuscan cost line. a) Changes of one year shoot morphogenetic gradient during rest period*. - Acta Horticulturae, 121: 85-92.

HONJO H., 2007 - *Effects of global warming on dormancy and flowering behavior of temperate fruit crops in Japan.* - Hortic. Res., 6: 1-5.

KRASNOSSELSKAYA T.A., RICHTER A.A., 1942 - *Transport of break of winter dormancy of buds along branches of woody plants*. - Dokl. A. N. SSSR., 35: 184-186.

LAM YAM L., 1990 - *Contribution to the study on the growth and fruiting of peaches (*Prunus persica *L. Batsch) under tropical climatic conditions of Reunion. Part two: the concept of bud dormancy in the tropics*. - Fruits (Paris), 45: 37-42.

LANG G.A., EARLY J.D., MARTIN G.C., DARNELL R.L., 1987 - *Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research*. - HortScience, 22: 371-377.

LEGAVE J.M., FARRERA I., ALMERAS T., CALLEJA M., 2008 - *Selecting models of apple flowering time and understanding how global warming has had an impact on this trait*. - J. Hortic. Sci. Biotech., 83: 76-84.

LINSLEY-NOAKES G.C., ALLAN P., MATTHEE G., 1994 - *Modification of rest completion models for improved accuracy in South African stone fruit orchards*. - J. S. Afr. Soc. Hort. Sci., 4: 13-15.

LUEDELING E., GIRVETZ E.H., SEMENOV M.A., BROWN P.H., 2011 - *Climate change affects winter chill for temperate fruit and nut trees*. - Plos One, 6.

LUEDELING E., ZHANG M.H., GIRVETZ E.H., 2009 a - *Climatic changes lead to declining winter chill for fruit and nut trees in california during 1950-2099*. - Plos One, 4.

LUEDELING E., ZHANG M.H., MCGRANAHAN G., LESLIE C., 2009 b - *Validation of winter chill models using historic records of walnut phenology*. - Agric. For. Met., 149: 1854-1864.

MAUGET J.C., RAGEAU R., 1988 - *Bud dormancy and adaptation of apple tree to mild winter climates.* - Acta Horticulturae, 232: 101-108.

MEIER U., 2001 - *Stades phénologiques des mono-et dicotylédones cultivées. BBCH Monographie*. - Centre Fédéral de Recherche Biologiques pour l'Agriculture et les Forêts. http://www.jki.bund.de/fileadmin/dam_uploads/_veroeff/bbch/BBCH-Skala_franz%C3%B6sisch.pdf, p. 166.

METZGER J.D., 1988 - *Localization of the site of perception of thermoinductive temperature in* Thlaspi arvense *L.* - Plant Physiol., 88: 424-428;

METZGER J.D., 1996 - *A physiological comparison of vernalization and dormancy chilling requirement*, pp. 147-155. - In: LANG G.A. (ed.) *Plant dormancy*. CAB International, Wallingford, UK, pp. 386.

METZGER J.D., DENNIS E.S., PEACOCK W.J., 1992 - *Tissue specifity of thermoinductive processes:* Arabidopsis *roots respond to vernalization*. - Plant Physiol., S99: 52.

NOODEN L.D., WEBER J.A., 1978 - *Environmental and hormonal control of dormancy in terminal buds of plants*, pp. 221-268. - In: CLUTTER M.E (ed.) *Dormancy and developmental arrest*. Academic Press, New York, USA, pp. 316.

PERRY T.O., 1971 - *Dormancy of tree in winter*. - Science, 171: 29-36.

PRIMACK R.B., HIGUCHI H., MILLER-RUSHING A.J., 2009 - *The impact of climate change on cherry trees and other species in Japan*. - Biol Conserv., 142: 1943-1949.

RAGEAU R., 1978 - *Croissance et débourrement des bourgeons végétatifs de pêcher (*Prunus persica *L. Batsch) au cours d'un test classique de dormance*. - C.R. Acad. Sci. Paris, Série D, 287: 1119-1122.

RICHARDSON E.A., SEELEY S.D., WALKER D.R., 1974 - *A model for estimating the completion of rest for "Redhaven" and "Alberta" peach trees*. - Hortscience, 9: 331-332.

RICHARDSON E.A., SEELEY S.D., WALKER R., ANDERSON J.L., ASHCROFT G.L., 1975 - *Pheno-climatography of spring peach bud development*. - HortScience, 10: 236-237.

SAURE M.C., 1985 - *Dormancy release in deciduous fruit trees*. - Hortic. Rev., 7: 239-300.

SCALABRELLI G., COUVILLON G.A., 1986 - *The effect of temperature and bud type on rest completion and the GDH°C requirement for bud break in 'Redhaven' peach*. - J. Amer. Soc. Hort. Sci., 111: 537-540.

TIMMIS R., WORRALL J., 1974 - *Translocation of dehardening and bud break promoters in climatically 'split' Douglas fir*. - Can. J. For. Res., 4: 229-237.

WANG S.Y., JI Z.L., FAUST M., 1987 - *Metabolic changes associated with bud break induced by thidiazuron*. - J. Plant Growth Regul., 6: 85-95.

WERNER D.J., MOWREY B.D., YOUNG E., 1988 - *Chilling requirement and post-rest heat accumulation as related to difference in time of bloom between peach and western sand cherry*. - J. Amer. Soc. Hort. Sci., 113: 775-778.

WITKOWSKA-ZUK I., 1970 - *Investigations on the bud dormancy of* Populus x berolinensis *Dipp. V. Relation between the growth apexes on neighbouring shoots*. - Acta Soc. Bot. Pol., 39: 285-296.

YOUNG E., MOTOMURA Y., UNRATH R., 1987 - *Influence of root temperature during dormancy on respiration, carbohydrates, and growth resumption in apple and peach*. - J. Amer. Soc. Hort. Sci., 112: 514-519.

ZGUIGAL Y., 1995 - *Evolution et caracteristiques de la dormance des bourgeons de pommier (*Malus domestica *Borkh., cv Golden delicious) dans un climat à hiver doux (Région de Meknes, Maroc).* - PhD Thesis, Institut agronomique et veterinaire Hassan II (MA).

Adv. Hort. Sci., 2013 27(1-2): 44-54

Ground cover management strategies in an Apulian oil-producing olive grove: agronomic and ecological assessment proposals

M. Fracchiolla, D. Caramia, C. Lasorella, P. Montemurro
Dipartimento di Scienze Agroambientali e Territoriali, Università degli Studi di Bari, Via G. Amendola, 165/a, 70126 Bari, Italy.

Key words: chopping, cover crop, herbicides, *Olea europaea* L., soil management, weeds.

Abstract: Several studies have pointed out that ground flora in olive groves, such as in any orchard, should ideally combine adequate positive effects on the agro-environment with only marginal negative competitive effects on the olive plants. This paper reports the results of an experiment carried out in an irrigated olive orchard (cv. Leccino), located in the area of Savelletri, Puglia (southern Italy), regarding the effects of ground flora as a consequence of different management techniques. An aggregate index is proposed, able to provide a comprehensive evaluation of flora from both an ecological and agronomic point of view. Four different weed control strategies were compared: A) seeding, every other year, of a cover crop (*Vicia sativa* L.) chopped in springtime; B) weed control using a mixture of a systemic herbicide and a residual herbicide; C) weed control using a systemic herbicide only; D) chopping. The results revealed that the different management practices largely influenced the ground cover values in each study year, but not the yield. Ground cover features, assessed both from an agronomic and ecological point of view varied in particular, as was well reflected by the applied index, which proved to easily and effectively describe the flora features in different plots.

1. Introduction

Although olives are grown in other world regions, such as California, Australia and Argentina, the most important production areas are found in the Mediterranean basin where olive finds its best growing conditions, in particular in Spain, Portugal, Italy, Greece, Albania, North Africa and the Middle East. Olive tree has had traditional importance in the Mediterranean region since ancient times (Loumou and Giourga, 2003) and its cultivation retains importance in this area for its social, environmental and economic value. Currently a large part of the total world olive plantations are found in the Mediterranean basin. The olive tree defines the Mediterranean region within the Holarctic Kingdom (Ubaldi, 2003) and it is considered one of the most typical species of this area, where it represents a very important element in defining the "identity" of the rural landscape.

The ecological function of rural landscapes and the promotion of multifunctional agriculture is an important topic in agricultural and agri-environmental policy within the European Union (Gerowitt *et al*., 2003). Vegetation related to olive orchards plays an important ecological function that can be efficiently used to improve the multifunctional role of olive growing in the Mediterranean region (Margaris, 1980). For instance, Saavedra (1998) reports over 500 species in the olive area of Córdoba province. In a selection of plantations in western Andalucía, 75 plant species were recorded prior to the spring cultivation (Rodenas *et al.*, 1977). In Greece, traditionally managed olive groves have been identified as important habitats that often support a rich ground flora, which may include species with habitats threatened by land-use changes (Allen *et al*., 2006).

Surveys in several olive orchards of south-western Albania report more than 80 species belonging to 14 botanical families (Huqi *et al*., 2009). In Italy, Viggiani (2009) reports more than 50 species as typical of olive groves, most of them having also ethno-botanical importance. The number could be even higher considering the species that can be found along field margins, i.e. roads, stone walls and other traditional human infrastructures typical of olive orchard landscapes.

The presence of a significant number of plant species in olive groves offers favourable conditions for a multitude of animals such as arthropod fauna, reptiles, mammals and birds (Beaufoy, 2000; Loumou and Giourga, 2003). This is due not only to primary production in the food chain, but also to the provision of cover and reproduction sites (Marshall *et al.*, 2003). Potts *et al.* (2006) assessed the biodiversity value of six common habitats on the Greek island of Lesvosmic. They found that man-

Received for publication 27 February 2013
Accepted for publication 21 March 2013

aged olive groves had the highest diversity of bees, comparable with natural habitats such as oak woodlands and pine forests.

Natural flora in olive orchards and the related fauna are also important sources of food for many species of birds, with consequent internationally important effects related to the migration of these animals (Guzman Alvarez, 1999). For example, in southern Italy about 6% of olive groves are included in "*Natura 2000 habitats*" (Birds Directive 92/43/EEC). From an agronomic point of view, natural flora is often able to enhance pest control because it can be an alternative host or direct food source for beneficial organisms (Marshall *et al.*, 2003; Norris, 2005).

In addition, ground cover can positively affect the diversity of soil biota, improving the soil ecosystem function. This effect was shown in a trial conducted in a rain-fed olive orchard, located in south-eastern Spain by Moreno *at al.* (2009) in which covered soils exhibited greater bacterial biomass and diversity, as well as higher microbial functional diversity than non-covered soils.

Conservative flora management can also increase CO_2 fixation and enhance the capacity of olive orchards to accumulate significant amounts of biomass and humus (Sofo *et al.*, 2004; Palese *et al.*, 2005).

Vegetation cover also has an important function in significantly reducing soil erosion (Hernandez *et al.*, 2005), one of the most serious and widespread environmental problems in many areas of the Mediterranean region (Pastor Muñoz-Cobo and Castro, 1995) where olive groves are often located in marginal soils and on steep slopes (Gomez *et al.,* 2003; Francia Martínez *et al.*, 2006).

However, olive tree vegetation and yield can be significantly damaged if weed flora is not correctly managed, especially under rain-fed conditions. In addition to competing with olive for water, nutrients and - at early crop stages - even for light, weeds may also hamper olive picking. Moreover, during summer, dead weed residues can catch fire and seriously damage olive plants in cases where the residues are abundant.

Traditional soil management is based on tillage, keeping the soil bare of vegetation all year round. This practice, in addition to undoing the potential benefits of natural flora, is also labour-intensive and expensive, hence it must be considered not sustainable. Several experiments show that it is possible to obtain the same or better productive results by adopting practices, such as chemical weeding or mowing, reducing or eliminating soil tillage and maintaining weed flora density at a level that is not dangerous for olive plants. Some significant results are reported by Huqi *et al.* (2009) in Albania, Montemurro and Mastropirro (1995), Montemurro *et al.* (2002) and Toscano *et al.* (2004) in southern Italy, Hernandez *et al.* (2005) in central Spain, Pastor Muñoz-Cobo (1990; 1991) in Spain, and Kabourakis (1999) in Greece.

These alternative strategies could represent a remarkable sustainable approach for the maintenance of the environment both in intensive systems, mitigating the environmental impact of olive growing, and in low-intensity farming systems located in marginal areas. In these latter areas, reduced tillage could reduce management costs and contribute to preventing abandonment of these groves and preserve natural and cultural resources (Duarte *et al.*, 2008). The effects could be beneficial on a large portion of the European territory; olive groves occupy approximately 5.4 million hectares, or about 4% of the utilisable agricultural area (Source: European Community).

Each soil management system provides different conditions of the growth for weeds. Tillage destroys the annual flora, but can also create favourable conditions for new germinations and, moreover, it benefits perennial weeds by fragmenting and scattering vegetative reproductive organs such as rhizomes, tubers, bulbs and stolons. Foliar herbicides, such as glyphosate, are able to control both annual and perennial plants, but they could exert a selection pressure on tolerant or resistant species. Residual herbicides, such as oxyfluorfen, keep the soil weed-free for a longer period of time. Generally, chemical weed control can cause a simplification of the flora spectrum with fewer species that are often more problematic to manage. Mowing can encourage those species that are able to re-sprout after cutting. Cover crop (living mulches) may contribute significantly to weed suppression providing early soil coverage and reducing the number of established weed seedlings.

Research proposal

Weed flora, also as a consequence of different management practices, can have both positive and negative effects on olive orchards as well as on the agro-ecosystem. Several studies have focused specifically on the ecological importance of natural flora in olive groves; other experiments have been performed in order to suggest the best control practices and to reduce the negative effects of weeds. It is reasonable to suppose that ground flora in olive groves, such as in any orchard, should ideally combine adequate positive effects on the agro-environment with only marginal negative competitive effects on the olive plants.

The objective of the current work is to report data on flora communities established as a consequence of different management techniques and to suggest an aggregate index able to give a comprehensive evaluation of flora, both from the ecological and agronomic point of view. The effects on olive production and oil yield are also considered.

2. Materials and Methods

The experimentation was carried out between November 2005 and December 2010 in an irrigated olive orchard located in the area of Savelletri (Puglia -southern Italy) made up of 11-year-old cv. Leccino plants, vase shape trained and spaced 7 x 7 m. The soil that hosted the orchard was loamy-textured (16.9% clay - 35.8%

silt - 47.3% sand), with a moderate presence of shallow pebbles (7.5 to 25 cm in size).

The trial involved the comparison of the following four different weed control strategies: i) Seeding, every other year, of a cover crop (*Vicia sativa* L.) chopped in springtime. The subsequent infestation was controlled by chopping (VE); ii) Weed control using a mixture of a glyphosate-based systemic herbicide and a residual herbicide containing oxyfluorfen, at a rate of 1.08 and 0.12 l ha^{-1} respectively (GLY + OX); iii) Weed control using glyphosate only at a rate of 1.08 l ha^{-1} (GLY); iv) Chopping (TRI).

Herbicides were diluted in a water volume of 400 l ha^{-1} and applied with a hand-pump spray bottle, equipped with flat fan nozzles. Weeds were chopped using a shredder. The vetch was sown broadcast at the rate of 80.0 kg ha^{-1}, burying the seed by a shallow harrowing.

The different management strategies of ground flora were applied following the general principle of applications so as to keep the orchard fully free of natural flora in the peak vegetative growth period, i.e. in spring-summer, when the flora reached a mean height of about 10-15 cm. The dates of weeding operations for each treatment are detailed in Table 1.

The other agronomic and plant protection practices were applied using the techniques commonly used in the research area.

The experimental plots, covering an area of 441.00 m^2 (21 x 21 m), were arranged in the field following a randomised block design, with four replicates; for flora surveys a central test area of 196.0 m^2 was used including the four plants on which vegetation production surveys were conducted.

Flora surveys and data processing

Plot flora surveys were run in April and October of each year and in the two peak growth periods of weeds. In these surveys, made prior to the execution of scheduled weed control operations, species were divided into the following two groups: a) species distributed uniformly in the test area; b) species represented either by solitary plants or distributed in restricted patches. Afterwards, for each species of the first group, a percent ground cover value related to the reference test area was estimated. These data have been used to calculate the Specific Contribution (CS), dividing the ground cover of each single species by the total cover (sum of the covers attributed to each single species) and multiplying it by 100. Moreover, the presence of each botanical family was obtained by summing the percent ground covers of each species belonging to it. Nomenclature refers to Pignatti (1982).

To provide an estimate of the ground cover features of each treatment, an index defined as Ground Cover Quality Index (GCQI) was proposed. This index is calculated by the following formula: $GCQI = [\ \Sigma_i(CSi \times V_i)\ ]/6$, where V_i is a total score assigned to each species having a uniform distribution in the test area, calculated by summing the values assigned to the following parameters:

- ability to cover the soil and protect it from erosion processes (0 = negligible; 1 = average; 2 = good);
- general ability to improve/preserve the chemical and physical soil properties through biomass production, nitrogen fixation or development of bunched roots (e.g. grass plants) that increase its porosity (0 = negligible; 1 = good);
- competitive ability against the orchard (0 = very competitive; 1 = normal; 2 = negligible);
- flammability in summer periods (0 = plant that leaves much dry biomass easily flammable; 1 = thin plant that produces little biomass potentially flammable or whose biomass is easily degraded prior to the warm season or that remains green in summer periods).

It follows that the value of $<V_i>$ could range between 0 and 6 and that, by the indicated formula, the Specific Contribution (CS) of each species, based on their morphologic and eco-physiological features, can take a weight varying between 0 and 6 times its value.

The specified parameters are proposed as a general indication, based on the specific needs of the test area. This does not exclude the possibility of using other ones based on other needs related to different conditions (e.g. aesthetic contribution, ability to be intermediate hosts of predators or of hyper pests, etc.).

This work presents only the flora data and the relevant analyses for 2006, 2008 and 2010, i.e. only in the presence of vetch (VE treatment) and in heavy years, considering olive alternate bearing.

Surveys on olive plants

For each of the four plants included in the test area, plants were tested for mean shoot growth recorded between April and October of each year, by selecting four shoots per plant arranged along the four cardinal direc-

Table 1 - Calendar of applied practices in different ground cover management strategies

Strategies	Practices	2005	2006		2007			2008		2009			2010	
		Nov	April	Oct	April	July	Oct	April	Oct	April	Oct	Nov	April	Oct
1) VE	Sowing of *Vicia sativa*	x					x					x		
	Chopping		x	x	x	x		x		x			x	x
2) GLY + OX	Chemical weeding	x	x	x	x		x	x	x	x	x		x	
3) GYI	Chemical weeding	x		x	x			x	x	x	x		x	
4) TRI	Chopping	x	x	x	x	x	x	x	x	x	x		x	x

tions. The data concerning each plot was thus obtained as an average of 16 values (four shoots per plant x four plants). At the beginning and end of the trial, the trunk diameter of each plant included in the plot area was also measured, and the mean growth occurred in that period was obtained by difference.

Olive harvesting was carried out in alternate years, i.e. in December 2006, 2008 and 2010. The fruits produced by the four plants of the test area were weighed. The oil yield was measured on a randomly chosen 2 kg sample from all olives harvested in each plot. The applied procedure complied with the guidelines of Annex XV of the EC Reg. No 2568/1991.

All data were submitted to variance analysis and the means were compared using Duncan's test.

Climate pattern

Figures 1 and 2 show the climate pattern observed during the experiment. Each year, in accordance with the climate pattern of the test area, the hottest months were June, July and August, whereas the coldest ones were December, January and February. The highest positive deviations (from +2.0 to +3.6°C) were recorded for the mean temperatures of January, June, July and August of 2007, January 2008 and November 2010; the highest negative difference was observed in February 2009 (-2.0°C compared to the plurennial mean). As to rainfall, the rainiest years were 2009 and 2010 with values exceeding the plurennial mean of the area (577.2 mm) by 243.5 mm and 162.1 mm, respectively. In those years, the months that deviated most from the average were January (+110.7 mm), March (+57.5 mm) and October (+64.6 mm) in 2009, and May (+72.9 mm) and October (+147.3 mm) in 2010.

3. Results

Table 2 lists the species found during the experiment. A total of 60 were identified; only 34 had a uniform distribution in the plots. The results obtained with regard to the uniformly distributed species are addressed in this section.

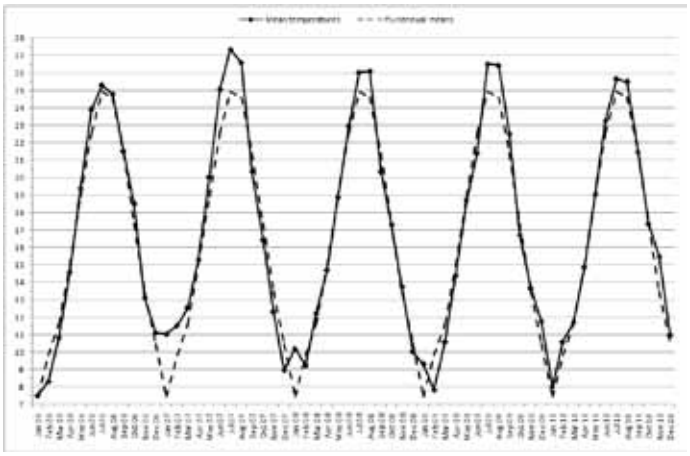


Fig. 1 - Mean monthly temperatures recorded during the trial and pluriannual means (1951-2001).

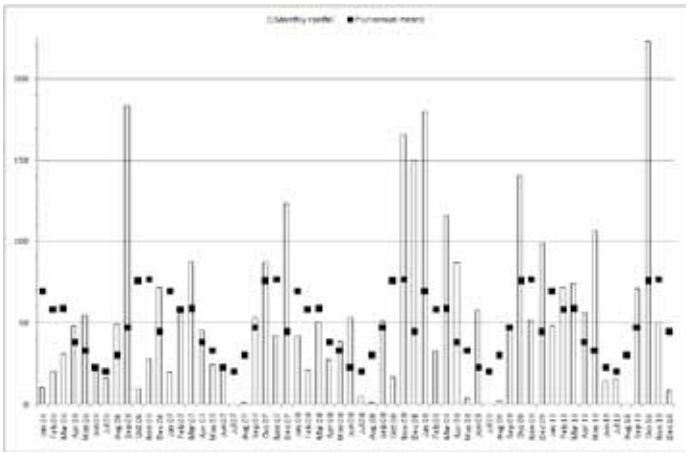


Fig. 2 - Monthly rainfall recorded during the trial and pluriannual means (1951-2001).

Spring flora surveys

In 2006 (Table 3), the statistically lowest total ground cover values were observed in treatments GLY and GLY+OX with 35.6 and 33.9%, respectively, followed by vetch-sown plots with 91.5%. The highest mean number of species, equal to 23.0, was recorded in vetch-sown areas, whereas the highest number of families (10.0) was observed in the plots subjected to chopping. Table 4 reveals that in the TRI treatment the statistically highest mean ground cover values were found for *Gramineae*, *Compositae and Leguminsae,* equal to 113.9-12.8 and 12.5% respectively, whereas the lowest values were recorded in treatment GLY (10.2% *Graminae* and 0.4% *Leguminosae*), GLY+OX (13.3% *Graminae*, 5.7% *Compositae* and 0.1% *Leguminosae*) and VE (6.1% *Compositae*). As to single species (Table 5), the highest specific contributions were calculated in chemically weeded plots for *Malva sylvestris* L. with 16.7% in treatment GLY+OX and 12.8% in GLY; these values were significantly higher than those observed in the plots of treatments VE and TRI. For *Avena sterilis* L.*, Bromus sterilis* L. and *Lolium rigidum* Gaudin, in chopped or vetch-sown plots higher specific contributions were observed than in chemically weeded plots. On the contrary, the specific contribution of *Hordeum murinum* L. and *Setaria verticillata* (L.) Beauv. was significantly higher in the GLY+OX treatment. With regard to the Ground Cover Quality Index in treatments TRI and VE, the observed values (52.4 and 55.1 respectively) were shown to be statistically higher than those calculated for chemically weeded treatments (Table 3).

In 2008, the highest values of total infestation and mean number of species (Table 3) were found in chopped (83.7% - 18.0) and vetch-sown plots (85.3% - 18.0), whereas the number of families was lower in treatment VE. The family *Gramineae* had a ground cover equal to 63.8% in treatment TRI, which is statistically higher than

Table 2 - Species found in experimental plots [z]

Treatments	VE			GLY+OX			GLY			TRI		
	2006	2008	2010	2006	2008	2010	2006	2008	2010	2006	2008	2010
Adonis aestivalis L.									+			
Anagallis arvensis L.			X			X	X		X	X		X
Anthemis arvensis L.			X			X			X	X		X
Arum italicum Miller.	+						+			+		
Asparagus acutifolius L.	+	+	+	+	+	+	+	+	+	+	+	+
Asphodelus fistulosus L.						+						
Aster squamatum (Sprengel) Hieron.	+		+	+	+	+	+	+	+	+	+	
Avena sterilis L.	X	X	X	X	X	X	X	X	X	X	X	X
Bellardia trixago (L.) Ali.	X		X	X		X			X	X	X	X
Briza maxima L.												+
Bromus sterilis L.	X	X	X	X	X	X	X	X	X	X	X	X
Calendula arvensis L.		X	X		X	X		X	X		X	X
Capsella bursa-pastoris (L.) Medicus	X	X			X			X			X	
Catapodium rigidum (L.) Hubbard			+			+			+			+
Cerinthe major L.		+										
Chrysanthemum segetum L.	X	X	X	X	X	X	X	X	X	X	X	X
Convolvulus arvensis L.	X			X	X		X	X		X		
Conyza canadensis (L.) Cronq.	X		X	X	X	X	X	X	X	X	X	X
Cynodon dactylon (L.) Pers.	X	X		X	X		X	X	X	X	X	X
Digitaria sanguinalis (L.) Scop.	+			+			+			+		
Diplotaxis erucoides (L.) DC.	X	X	X	X	X	X	X	X	X	X	X	X
Diplotaxis muralis (L.) DC.		X						X			X	
Dittrichia viscosa Greuter.								+	+			
Erodium malacoides (L.) L'Hér.	X	X	X	X	X	X	X	X	X	X	X	X
Euphorbia chamaesyce L.	+		+	+		+	+			+		+
Galactites tomentosa Moench.		+	+								+	+
Geranium molle L.			+					+		+	+	+
Heliotropium erupaeum L.	X	X		X	X		X	X		X	X	
Hippocrepis unisiliquosa L.												+
Hordeum murinum L.	X	X	X	X	X	X	X	X	X	X	X	X
Lactuca serriola L.			+									
Lamium purpureum L.				X								
Lolium rigidum Gaudin	X	X	X	X	X	X	X	X	X	X	X	X
Lotus ornithopodioides L.									+			+
Malva sylvestris L.	X	X	X	X	X	X	X	X	X	X	X	X
Medicago hispida Gaertner	X	X	X	X	X	X	X	X	X	X	X	X
Melilotus indica (L.) All.						+						
Mercurialis annua L.								+				+
Muscari neglectum Guss.					+		+	+		+		
Ononis natrix L.			+			+			+			+
Oxalis pes-caprae L.	X	X	X	X	X	X	X	X	X		X	X
Papaver rhoeas L.	X	X		X		X	X	X	X	X	X	X
Phalaris paradoxa L.	X	X	X			X		X	X		X	X
Portulaca oleracea L.	X			X			X			X		
Raphanus raphanistrum Strobl.		+	+		+			+			+	
Scorpiurus muricatus L.	X								X	X		X
Serapias sp.									+			+
Setaria verticillata (L.) Beauv.	X	X		X			X			X	X	
Sherardia arvensis L.			+			+			+			
Solanum nigrum L.	X			X			X			X		
Sonchus oleraceus L.	X	X	X	X	X	X	X	X	X	X	X	X
Sonchus tenerrimus L.			X			X			X			X
Tetragonolopus purpureum Moench.											+	+
Trifolium campestre Shreber	X		X	X			X		X	X		X
Trifolium fragiferum L.		+	+			+			+		+	+
Trifolium repens L.	X	X	X	X	X	X	X	X	X	X	X	X
Trifolium scabrum L.	X	X	X	X		X	X		X	X	X	X
Trifolium tomentosum L.	X	X	X	X		X	X		X	X	X	X
Valerianella eriocarpa Desv.		+	+	+	+	+		+	+	+	+	+
Verbascum sinuatum L.	+						+			+	+	+
Total species with uniform distribution (n.)	27	22	22	25	18	22	24	21	25	26	24	25
Total others (n.)	6	6	12	5	5	10	7	8	11	9	9	15

[z] X = Uniformly distributed species; + = Others: solitary plants or restricted to patchy areas.

the values recorded for the other strategies under consideration (Table 4). The *Leguminosae*, instead, showed the highest cover value (16.2%) in chopped plots. The data presented in Table 5 point out that the statistically highest CS value of *A. sterilis* was found in vetch plots VE (18.1%), whereas that of *B. sterilis* was higher with only chopping, where the lowest specific contribution of *L. rigidum* was also calculated. Within *Leguminosae*, the most represented species were *Medicago hispida* Gaertner, *Trifolium repens* L. and *Trifolium tomentosum* L., whose specific contributions were higher in chopped plots. *Conyza canadensis* (L.) Cronq. and *M. sylvestris*, instead, showed the significantly highest CS in the experimental plots weeded only by Glyphosate. As for the Ground Cover Quality Index (Table 3), the highest statistical value was calculated in chopped plots (61.0), followed by treatment VE (54.6).

In 2010, the statistically highest total cover percentage (Table 3) was observed in the chopped plot (107.5%), followed by vetch plots (74.5%). The lowest mean number of families and species (4.5 and 12.5, respectively) was recorded in OX treatment. The data included in Table 4 point out that the most significant cover value of *Graminae* and *Leguminosae* was found, respectively, in the plots with vetch (51.9%) and in TRI treatment (63.5%). As for single species (Table 5), *A. sterilis* and *B. sterilis* showed the statistically highest mean values of CS in the VE treatment (21.3 and 22.0% respectively); the CS of *L. rigidum* and *Trifolium campestre* Shreber were found to be, instead, the lowest in statistical terms in treatments TRI (4.2%) and GLY+OX (0.0%). With regard to the GCQI (Table 3), the statistically highest mean value (80.9) was observed in chopped plots among all compared treatments.

Autumn flora surveys

In 2006, the statistically highest infestation value (Table 6) was observed in chopped plots (34.6%). The statistically lowest mean number of species and families, 13.0 and 9.0 respectively, was recorded in the TRI treatment. In TRI and VE treatments the mean cover values of *Gramineae* species were 22.4 and 21.5% respectively, statistically higher values compared to treatments GLY and GLY+OX (Table 7). As to specific contributions, Table 8 shows that *L. rigidum* is the species with the highest mean data of all the monitored species; more specifically, it accounted for 63.5% of cover in the VE treatment and 61.1% in TRI, both values being significantly higher than those observed in chemically weeded plots. The highest GCQI (Table 6),

Table 3 - Total ground cover, number of families and species, agro-ecological indices in spring surveys

Ground cover management strategies (2)	Crop year 2006				Crop year 2008				Crop year 2010			
	GLY	GLY+OX	TRI	VE	GLY	GLY+OX	TRI	VE	GLY	GLIY+OX	TRI	VE
Total ground cover (%)	35.6 c	33.9 c	155.7 a	91.5 b	45.3 c	61.5 b	83.7 a	85.3 a	74.2 b	58.2 c	107.5 a	74.5 b
Botanical families (n.)	8.5 b	8.0 b	10.0 a	7.7 b	9.0 a	9.0 a	9.0 a	8.0 b	7.0 a	4.5 c	5.5 b	6.0 b
Species	16.0 c	15.5 c	20.7 b	23.0 a	17.0 b	17.0 b	18.0 a	18.0 a	16.2 a	12.5 b	15.7 a	15.7 a
GCQI	53.2 B	49.6 C	52.4 A	55.1 A	53.1 C	53.9 C	61.0 A	54.6 B	57.5 B	58.4 B	80.9 A	57.3 B

(z) Values that do not have a letter in common are significantly different at 0.01 P (capital letter) or at 0.05 P (small letter) (Duncan's test).

Table 4 - Ground cover (%) of the botanical families found in spring surveys

Weeds	Crop year 2006				Crop year 2008				Crop year 2010			
	GLI	GL+OX	TRI	VE	GLY	GLIY+OX	TRI	VE	GLY	GLY+OX	TRI	VE
Gramineae	10.2 c	13.3 c	113.9 a	68.5 b	22.7 d	40.0 c	52.4 b	63.8 a	23.2 b	22.4 b	30.0 b	51.9 a
Compositae	9.1 b	5.7 c	12.8 a	6.1 c	8.2 a	7.4 a	7.3 a	5.9 b	21.5 a	20.0 a	6.7 b	6.6 b
Leguminosae	0.4 c	0.1 c	12.5 a	7.3 b	1.4 d	2.9 c	16.2 a	6.7 b	12.0 b	11.1 b	63.5 a	10.5 b
Cruciferae	1.6 a	1.6 a	1.0 b	0.6 b	1.6 b	1.7 ab	0.5 c	1.9 a	--	--	--	--
Primulaceae	2.6 a	0.0 c	1.9 b	0.0 c	--	--	--	--	4.5 b	0.0 d	5.7 a	1.9 c
Scrofuliaraceae	0.0 b	0.0 b	2.0 a	0.1 b	0.0	0.1	0.6 a	0.0	1.9 a	0.5 b	0.5 b	0.5 b
Convolvulaceae	5.1	4.9	4.9	4.7	2.3 a	2.2 a	0.0 b	0.0 b	--	--	--	--
Geraniaceae	--	--	--	--	2.0 b	1.9 b	2.9 a	2.6 ab	--	--	--	--
Boraginaceae	--	--	--	--	0.9 b	1.7 a	2.4 a	1.9 a	--	--	--	--
Labiatae	0.0	0.5	0.0	0.0	--	--	--	--	--	--	--	--
Malvaceae	4.5 b	5.6 a	3.2 c	0.0 d	5.7 a	2.6 b	1.0 c	0.6 c	7.0 a	3.6 b	0.5 c	2.5 b
Papaveraceae	0.1 c	0.3 c	1.3 b	3.0 a	0.4 b	0.0 b	0.4 b	2.0 a	4.0 a	0.5 b	0.5 b	0.0 b
Portulacaceae	1.9	1.8	2.0	1.5	--	--	--	--	--	--	--	--

(z) Values that do not have a letter in common are significantly different at 0.05 P (Duncan's test).

equal to 54.9, was calculated in the plot submitted to chopping; this data is statistically different from that observed in the other treatments.

In 2008, the statistically highest values of total cover percentage and number of species (Table 6) were observed in chopped plots (49.6% and 13.7%, respectively). For the number of families (Table 6), which varied between 6.0 and 6.5, no sharp differences were found. The statistically highest mean cover values of *Gramineae* (21.8%), *Leguminosae* (5.0%) and *Compositae* (14.5%) were recorded in the TRI treatment (Table 7). The data shown in Table 8 point out that the highest specific contributions were found for: a) *A. sterilis* in the treatment weeded by the mixture of Glyphosate and Oxyfluorfen (22.9%); b) *B. sterilis* (12.6%) and *Oxalis. pes-caprae* L. (14.6%) in VE; c) *C. canadensis* (47.0%) in the plots weeded by Glyphosate only; d) *Heliotropium erupaeum* L. in both chemically weeded plots; and e) *M. hispida* in the plots submitted to chopping

Table 5 - Specific contributions (%) calculated for the species found in spring surveys

Weeds	Crop year 2006				Crop year 2008				Crop year 2010			
	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE
Anagallis arvensis L.	7.3 a [z]	0.0 c	1.2 b	0.0 c	--	--	--	--	6.1 a	0.0 c	5.3 ab	2.6 b
Anthemis arvensis L.	0.0 b	0.0 b	1.2 a	0.0 b	--	--	--	--	4.8 a	0.9 b	0.4 b	3.3 a
Avena sterilis L.	3.3 c	3.9 c	20.2 a	17.1 b	0.5 c	2.1 c	13.0 b	18.1 a	0.6 c	5.1 b	3.8 b	21.3 a
Bellardia trixago (L.) Ali.	0.0 c	0.0 c	1.3 a	0.1 b	0.0 c	0.2 b	0.7 a	0.0 c	2.7	0.9	0.5	0.7
Bromus sterilis L.	3.0 c	3.9 c	19.9 a	16.5 b	23.5 c	25.6 b	29.2 a	17.9 d	9.4 c	12.7 b	14.3 b	22.0 a
alendula arvensis L.	--	--	--	--	0.3 b	0.2 b	0.2 b	1.1 a	--	--	--	--
Capsella bursa-pastoris (L.) Medicus	0.0 b	0.0 b	0.0 b	0.2 a	0.2	0.2	0.1	0.2	--	--	--	--
Chrysanthemum segetum L.	3.2 b	4.8 a	1.3 c	1.0 c	5.9 a	4.4 b	3.2 c	3.2 c	6.2 a	8.5 a	1.1 b	4.0 a
Conyza canadensis (L.) Cronq.	9.8 a	11.0 a	2.9 b	0.5 c	3.2 a	0.4 b	0.0 b	0.0 b	12.7 b	16.2 a	0.9 c	0.9 c
Convolvulus arvensis L.	14.3 a	14.7 a	3.2 b	5.1 b	5.1 a	3.7 b	0.0 c	0.0 c	--	--	--	--
Cynodon dactylon (L.) Pers.	14.8 a	14.4 a	3.1 b	0.2 c	3.2 a	2.5 b	0.4 d	2.1 c	--	--	--	--
Diplotaxis erucoides (L.) DC.	4.4 a	4.8 a	0.6 b	0.5 b	4.4 a	3.1 b	3.5 b	3.0 b	--	--	--	--
Hordeum murinum L.	0.3 d	6.3 a	2.1 c	3.5 b	2.1	2.8	2.8	2.2	2.6 bc	0.8 c	4.6 b	5.4 a
Lamium purpureum L.	0.0 b	1.3 a	0.0 b	0.0 b	1.3 c	14.0 a	13.6 ab	12.9 b	--	--	--	--
Lolium rigidum Gaudin	5.6 c	6.4 c	27.1 b	34.7 a	24.9 a	24.8 a	6.9 b	25.8 a	16.7 a	17.8 a	4.2 b	20.0 a
Malva sylvestris L.	12.8 b	16.7 a	2.1 c	0.0 c	12.7 a	4.3 b	1.2 c	0.7 c	9.5 a	6.3 a	0.5 b	3.4 a
Medicago hispida Gaertner	0.7 b	0.1 b	1.9 a	0.0 b	1.9 d	3.9 b	6.6 a	2.9 c	4.1	7.9	5.2	2.0
Papaver rhoeas L.	0.3 b	0.9 b	0.8 b	3.3 a	1.0 b	0.0 c	0.5 bc	2.3 a	5.5 a	0.8 b	0.4 b	0.0 b
Phalaris paradoxa L.	0.0 b	0.0 b	0.0 b	0.1 a	0.0 b	0.0 b	0.0 b	0.1 a	1.6 a	1.7 a	0.9 b	1.6 a
Portulaca oleracea L.	5.5 a	5.3 a	1.3 b	1.7 b	--	--	--	--	--	--	--	--
Setaria verticillata (L.) Beauv.	1.7 b	4.3 a	0.7 b	2.4 b	--	--	--	--	--	--	--	--
Scorpiurus muricatus L.	0.0 c	0.0 c	0.2 b	1.1 a	--	--	--	--	0.7	0.0	0.4	0.0
Sonchus oleraceus L.	12.5 a	1.1 d	2.9 c	5.1 b	8.8 a	6.9 b	5.4 c	2.5 d	5.5 b	8.5 a	3.8 b	1.0 c
Trifolium campestre Shreber	0.3 b	0.1 b	1.5 a	1.8 a	--	--	--	--	3.4 a	0.0 b	5.2 a	3.4 a
Trifolium repens L.	0.1 b	0.1 b	1.3 a	1.4 a	1.1 c	0.8 c	5.2 a	1.8 b	0.6 c	1.2 c	11.8 a	3.4 b
Trifolium scabrum L.	0.1 b	0.1 b	1.6 a	1.9 a	0.0 c	0.0 c	1.4 a	0.9 b	1.9 b	2.7 b	21.9 a	2.0 b
Trifolium tomentosum L.	0.1 b	0.1 b	1.5 a	1.8 a	0.0 c	0.0 c	6.3 a	2.2 b	5.5 b	7.8 b	14.9 a	3.4 b

[z] Values that do not have a letter in common are significantly different at 0.05 P (Duncan's test).

Table 6 - Total ground cover, number of families, number of species and agro-ecological indices in autumn surveys

	Crop year 2006				Crop year 2008				Crop year 2010			
	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE
Total ground cover	23.4 c [z]	23.6 c	34.6 a	31.9 b	19.1 c	20.7 c	49.6 a	39.8 b	8.2 b	9.1 b	24.0 a	22.0 a
N° of families	10.0 a	10.0 a	9.0 b	9.7 a	6.5	6.5	6.0	6.2	6.7 ab	5.7 bc	5.5 c	7.5 a
N° of species	14.0 a	14.0 a	13.0 b	13.7 a	8.7 c	11.5 b	13.7 a	11.7 b	9.7	7.7	8.2	10.7
GCQI	53.3 b	52.7 b	54.9 a	53.6 b	42.2 c	55.7 a	54.2 a	52.6 b	58.8	62.0	58.4	56.8

[z] Values that do not have a letter in common are significantly different at 0.05 P (Duncan's test).

(10.1%). The GCQI with the lowest statistical value was in the treatment weeded by Glyphosate only (42.0), whereas the values calculated in the other treatments were not statistically different from each other (Table 6).

In 2010, the highest total cover values were observed in TRI and VE treatments, with 24.0 and 22.0%, respectively (Table 6). The highest mean number of families (7.5) was recorded for treatment VE. As to the number of species, statistical analysis did not point out any reliable difference between the values of different strategies that ranged between 7.7 for treatment GLY+OX and 10.7 for VE. The data in Table 7 show that *Graminae* and *Compositae* had a statistically higher mean cover value in TRI and VE plots, whereas the cover values of *Leguminosae* species did not show any remarkable difference between each other. As to single species (Table 8), the highest CS values in statisti-

Table 7 - Ground cover (%) of the botanical families found in autumn surveys

Weeds	Crop year 2006				Crop year 2008				Crop year 2010			
	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE
Graminaceae	11.1 b (z)	11.6 b	22.4 a	21.5 a	1.1 d	6.3 c	21.8 a	9.3 b	1.3 b	1.7 b	4.3 a	5.9 a
Compositae	2.5 a	2.7 a	2.9 a	1.5 b	10.8 b	6.1 c	14.5 a	11.5 b	2.2 b	3.5 b	6.6 a	6.6 a
Leguminosae	1.2 c	1.1 c	3.3 a	1.7 b	0.1 c	0.3 bc	5.0 a	0.6 b	0.9	1.0	1.5	1.2
Cruciferae	2.1 a	2.2 a	1.3 c	1.9 b	0.3 b	0.5 b	0.2 b	6.0 a	0.1	0.1	0.2	0.7
Convolvulaceae	2.6	2.4	2.4	2.3	--	--	--	--	--	--	--	--
Geraniaceae	0.5 b	0.5 b	0.5 b	1.0 a	0.0	0.0	0.0	0.1	1.0	0.2	0.4	1.0
Boraginaceae	0.6	0.6	0.8	1.0	5.2 b	5.1 b	5.1 b	6.5 a	--	--	--	--
Malvaceae	1.3 a	1.0 ab	0.9 b	0.7 b	0.1	0.1	0.0	0.0	1.6 a	0.4 b	0.2 b	0.7 b
Oxalidacee	1.0 a	1.0 a	0.1 b	0.0 b	1.5 d	2.2 c	2.9 b	5.8 a	0.6 d	2.1 c	10.2 a	5.5 b
Primulaceae	--	--	--	--	--	--	--	--	0.3	0.3	0.5	0.4
Solanacee	0.4 a	0.5 a	0.2 b	0.2 b	--	--	--	--	--	--	--	--

(z) Values that do not have a letter in common are significantly different at 0.05 P (Duncan's test).

Table 8 - Specific contributions (%) calculated for the species found in autumn surveys

Weeds	Crop year 2006				Crop year 2008				Crop year 2010			
	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE	GLI	GLI+OX	TRI	VE
Anagallis arvensis L.	--	--	--	--	--	--	--	--	4.1	0.5	1.9	2.0
Avena sterilis L.	--	--	--	--	0.0 c (z)	22.9 a	5.0 b	3.7 b	--	--	--	--
Bromus sterilis L.	--	--	--	--	0.0 c	1.3 b	11.6 a	12.6 a	2.4 c	1.5 c	13.3 b	25.0 a
Calendula arvensis L.	--	--	--	--	8.9 b	1.7 c	10.6 b	16.3 a	4.3 b	1.1 b	20.1 a	16.0 a
Chrysanthemum segetum	--	--	--	--	--	--	--	--	3.8	0.0	0.9	2.5
Conyza canadensis (L.) Cronq.	6.5 b	7.6 a	4.4 c	1.4 d	47.0 a	3.6 c	8.6 b	0.0 c	11.0 a	12.9 a	0.1 b	1.6 b
Convolvulus arvensis L.	10.9 a	10.3 a	6.9 b	7.2 b	--	--	--	--	--	--	--	--
Cynodon dactylon (L.) Pers.	5.4 a	5.3 a	3.6 b	3.9 b	5.9 a	5.3 a	2.2 b	0.4 c	3.5	0.0	1.1	0.0
Diplotaxis erucoides (L.) DC.	4.9 a	5.0 a	3.3 b	5.5 a	1.4	2.4	0.3	13.8	1.5	1.2	0.9	3.2
Diplotaxis muralis (L.) DC.	4.2 a	4.1 a	0.4 b	0.5 b	0.1 bc	0.0 c	0.2 b	1.3 a	--	--	--	--
Erodium malacoides (L.) L'Hér.	2.1 b	2.0 b	1.4 c	3.1 a	0.1	0.0	0.0	0.1	12.4 a	2.5 b	1.7 b	4.6 b
Heliotropium europaeum L.	2.7	2.7	2.4	3.1	27.5 a	24.5 a	10.3 c	16.4 b	--	--	--	--
Hordeum murinum L.	--	--	--	--	0.0 b	0.7 b	10.1 a	0.1 b	--	--	--	--
Lolium rigidum Gaudin	42.3 b	43.9 b	61.1 a	63.5 a	0.0 b	0.2 b	9.6 a	0.2 b	10.0 ab	16.5 a	3.7 bc	1.6 c
Malva sylvestris L.	5.4 a	4.1 b	2.5 c	2.4 c	0.5	0.7	0.0	0.0	19.9 a	4.1 b	1.1 b	3.2 b
Medicago hispida Gaertner	0.9 c	0.9 c	7.0 a	2.0 b	0.3 c	1.4 b	10.1 a	1.6 b	12.2	12.0	6.1	4.2
Oxalis pes caprae L.	4.3 a	4.1 a	0.0 b	0.3 b	7.7 c	10.9 b	5.9 c	14.6 a	8.5 c	25.0 b	42.6 a	25.8 b
Setaria verticillata (L.) Beauv.	--	--	--	--	0.0 b	0.0 b	5.6 a	6.3 a	--	--	--	--
Sonchus oleraceus L.	--	--	--	--	0.5 d	24.2 a	10.1 c	12.6 b	2.9	5.4	0.9	4.0
Sonchus tenerrimus L.	4.2	4.1	3.8	3.1	--	--	--	--	3.3 b	16.9 a	5.2 b	5.2 b
Solanum nigrum L.	1.9 a	2.2 a	0.6 b	0.4 b	--	--	--	--	--	--	--	--
Trifolium campestre Shreber	4.3	3.7	2.5	3.5	--	--	--	--	--	--	--	--

(z) Values that do not have a letter in common are significantly different at 0.05 P (Duncan's test).

cal terms were observed: a) for *B. sterilis* (25.0%) in the plots of treatment VE; b) for *Calendula arvensis* L. both in VE (16.0%) and TRI (20.1%); c) for *C. canadensis* in both chemically weeded plots; and d) for *O. pes-caprae* in the chopped treatment (42.6%).The GCQI (Table 6) ranged between 62.0 in treatments GLY+OX and 56.8 in treatment VE and did not show any significant differences between the values of the strategies being compared. Finally, Table 2 lists the other species found during the experiment, with low cover percent values and a non-uniform distribution. The occurrence of these 26 species increased during the years for all treatments, although none of them attained a uniform distribution over time.

Vegetation surveys

Shoot growth and trunk diameter did not show any significant differences between the mean values measured for the compared treatments (Table 9) during the study three years.

Olive production and oil yield

As shown by the data in Table 10, no statistical differences were observed between the mean recorded values during the study period for the different strategies being compared with regard to olive production and oil yield per plant.

4. Discussion and Conclusions

In this work diachronic analysis was not carried out given the limited number of years under study, however synchronic comparisons have supplied data that can lead to some interesting conclusions.

The different management practices employed in the study largely influenced the ground cover values in the various years, both quantitatively and qualitatively, but not the yield. In all surveys, the most represented families, both in terms of ground cover and number of species, were *Graminae*, *Leguminosae* and *Compositae*.

With regard to single species, significant specific contributions were recorded for *A. sterilis*, *B. sterilis*, *C. canadensis*, *L. rigidum*, *M. sylvestris*, *O. pes-caprae* and *Trifolium* spp. The largest differences, in terms of total ground cover, were observed between the chemically weeded plots and plots submitted to chopping only or sown with vetch. Moreover, at the time of surveys, no important differences were found between the treatment with the systemic herbicide only and the plot supplied also with the residual herbicide; this is presumably due to the low application rate of oxyfluorfen. In particular, in spring and autumn surveys, the highest total infestation was found in chopped and vetch-sown plots. The latter, although covering the whole plot area at spring surveys, has only partially limited the growth of weeds, especially grasses (*Graminae*).

The number of species having a uniform distribution in springtime in the plots controlled by chopping or through the sowing of the cover crop rarely exceeded the value observed under different management practices. The number calculated in autumn, instead, was virtually equal for all strategies.

The species that showed the highest specific contributions in chemically weeded plots include *C. canadensis* and *M. sylvestris*, which might be related to the fact that those species are tolerant to the applied rates of herbicides or maybe, for *C. canadensis*, resistant to Glyphosate (Montemurro, 2008; Herbicide Resistance Action Committee, 2012). In the two other conditions, in general, no single species was found to be markedly present.

The most influenced families in spring surveys were *Leguminosae* and *Graminae*; in particular, the latter seemed to be facilitated by vetch sowing, whereas the former was aided by chopping, conditions that were more evident in spring than in autumn surveys when all differences in general seemed to be less marked.

Table 9 - Shoot and trunk growth measured during the trial

Treatments	Shoots (cm)			Trunk diameter (cm)
	April-October 2006	April-October 2008	April-October 2010	April 2006-October 2010
VE	15.9	14.2	15.0	3.0
GLI + OX	13.5	16.6	14.0	3.5
GLI	16.4	17.3	13.4	3.9
TRI	13.1	16.6	14.8	3.1

Table 10 - Olive production per plant and oil yield

	Production per plant (Kg)			Oil yield (%)		
	2006	2008	2010	2006	2008	2010
GLI	13.6	11.7	14.8	18.0	19.1	21.6
GLI+OX	13.9	10.8	15.0	19.0	20.0	21.7
TRI	15.8	11.1	15.6	19.0	19.0	22.3
VE	12.8	10.9	15.1	17.9	20.1	20.9

The Ground Cover Quality Index calculated in spring 2006 and 2008 was on average higher in the chopped or vetch-sown plots. In 2010 the value calculated in chopped plots was markedly higher than in the other treatments, which, instead, did not show any differences for this parameter. This would suggest a shifting, over time, of vegetation towards a higher quality composition which was more accentuated in the case of the strategy involving chopping only. These effects are well summarized by the applied index, which indicates that for the same weed species assortment influenced the ground cover quality.

Differences in the flora composition, both quantitatively and qualitatively, did not affect olive yield or vegetation, since weeds were however controlled during the plants' critical periods. What varied was above all the ground cover features, assessed both from an agronomic and ecological point of view. From our perspective, this feature is well reflected by the applied index, which easily and effectively described the flora features in different plots. In this regard it should be said that this index is obviously influenced by the value attributed to each species that may vary in relation to the objectives of weed management, as previously mentioned. In our case emphasis was placed on competition, protection from erosion and on the capacity to preserve or even increase fertility: these features coincide with the objectives that olive growers normally try to achieve, especially in our areas. In other situations different parameters could be applied, namely by varying the index numerically while still keeping its functional meaning. Moreover, in the case under study, the GCQI value was largely influenced by the total ground cover because none of the weeds found in the trial was assigned a zero score (V_i). In the event that undesired species were found in among the cover composition, the index would certainly have been less dependent on total ground cover.

Finally, since different cover crop and ground flora management practices seemed to give results, in terms of yield, that were not different from each other, a long-term approach could be applied for their selection. Currently it seems possible to prefer a ground cover management strategy that enables a sustainable use of olive agro-ecosystems and emphasizes the different roles of wild flora, including landscaping. This keeps in mind the fact that in 2006 the Puglia Regional Government enacted a law regarding the protection and enhancement of monumental olive trees and of the olive agro-ecosystems of its region (L.R. N. 39 del 03/10/2006) and that the location where the trial was conducted falls within the areas of highest density of ancient and traditional olive tree landscapes. The value of this area was also further declared by its inclusion among the "High Nature Value Farmland" areas (European Environment Agency, 2004).

References

ALLEN H.D., RANDALL R.E., AMABLE G.S., DEVEREUX B.J., 2006 - *The impact of changing olive cultivation practices on the ground flora of olive groves in the messara and Psiloritis regions, Crete, Greece.* - Land Degrad. Develop., 17: 249-273.

BEAUFOY G., 2000 - *The environmental impact of olive oil production in the European Union: practical options for improving the environmental impact*. - Report produced by the European Forum on Nature Conservation and Pastoralism and the Asociación para el Análisis y Reforma de la Política Agro-rural. Published by European Commission, Environment Directorate-General. Available online at: http://ec.europa.eu/environment/agriculture/pdf/oliveoil.pdf

DUARTE F., JONES N., FLESKENS L., 2008 - *Traditional olive orchards on sloping land: sustainability or abandonment?* - Journal of Environmental Management, 89: 86-98.

EUROPEAN ENVIRONMENT AGENCY, 2004 - *High nature value farmland characteristics, trends and policy challenges.* - Office for Official Publications of the European Communities, Luxembourg, pp. 32. Available online at: http://www.eea.europa.eu/publications/report_2004_1.

FRANCIA MARTÍNEZ J.R., DURÁN ZUAZO V.H., MARTÍNEZ RAYA A., 2006 - *Environmental impact from mountainous olive orchards under different soil-management systems (SE Spain).* - Science of the Total Environment, 358: 46-60.

GEROWITT B., BERTKE E., HESPELT S.K., TUTE C., 2003 - *Towards multifunctional agriculture - weeds as ecological goods?* - Weed Research, 43(4): 227-235.

GOMEZ J.A., BATTANY M., RENSCHLER C.S., FERERES E., 2003 - *Evaluating the impact of soil management on soil loss in olive orchards*. - Soil use and management, 19: 127-134.

GUZMAN ALVAREZ J.R., 1999 - *Olivicolture ed ecologia: la situazione in Spagna*. - Olivae, 78: 41-49.

HERBICIDE RESISTANCE ACTION COMMITTEE, 2012 - *The international survey of herbicide resistant weeds.* - http://www.weedscience.com.

HERNANDEZ A.J., LACASTA C., PASTOR J., 2005 - *Effects of different management practices on soil conservation and soil water in a rainfed olive orchard*. - Agricultural Water Management, 77: 232-248.

HUQI B., DHIMA K., VASILAKOGLOU I., KECO R., SALAKU F., 2009 - *Weed flora and weed management in established olive groves in Albania.* - Weed Biology and Management, 9: 276-285.

KABOURAKIS E., 1999 - *Codice di pratiche per i sistemi ecologici di produzione oleicola in Creta*. - Olivae, 77: 35-45.

LOUMOU A., GIOURGA C., 2003 - *Olive groves: "The life and identity of the Mediterranean"*. - Agriculture and Human Values, 20: 87-95.

MARGARIS N.S., 1980 - *Structure and dynamics of Mediterranean type vegetation.* - Portugaliae Acta Biologica, 16: 45-58.

MARSHALL E.J.P., BROWN V.K., BOATMAN N.D., LUTMAN P.J.W., SQUIRE G.R., WARD L.K., 2003 - *The role of weeds in supporting biological diversity within crop fields.* - Weed Research, 43: 77-89.

MONTEMURRO P., 2008 - Conyza canadensis *negli oliveti, un'espansione da contrastare*. - Terra e Vita, 27: 68-70.

MONTEMURRO P., FRACCHIOLLA M., GUARINI D., 2002 - *Results of a chemical weed control trial in an olive oil orchard.* - Acta Horticulturae, 586: 397-400.

MONTEMURRO P., MASTROPIRRO A., 1995 - *Un biennio di ricerche sulla gestione della vegetazione infestante nella coltura dell'olivo (*Olea europeaea *var.* sativa *L.) da olio in irriguo in Puglia*. - Atti Conv. Naz. "L'olivicoltura Mediterranea: Stato e prospettive della coltura e della ricerca" - Rende (CS), 26-28 January, pp. 425-433.

MORENO B., GARCIA-RODRIGUEZ S., CANIZARES R., CASTRO J., BENITEZ E., 2009 - *Rainfed olive farming in south-eastern Spain: Long term effect of soil management on biological indicators of soil quality.* - Agriculture, Ecosystems and Environment, 131: 333-339.

NORRIS R.F., 2005 - *Ecological bases of interactions beteween weeds and organisms in other pest categories*. - Weed Science, 53: 909-913.

PALESE A.M., CELANO G., PETRILLO G., GRAZIANO D., XILOYANNIS C., 2005 - *Gestione del suolo negli oliveti e conservazione delle risorse naturali.* - L'Informatore Agrario, 38: 41-45.

PASTOR MUÑOZ COBO M., 1990 - *La non lavorazione e altri sistemi di lavorazione ridotta nella coltivazione dell'olivo.* - Olivae, 34: 18-30.

PASTOR MUÑOZ COBO M., 1991 - *La non lavorazione e altri sistemi di lavorazione ridotta nella coltivazione dell'olivo (continuazione e fine).* - Olivae, 35: 35-49.

PASTOR MUÑOZ COBO M., CASTRO J., 1995 - *Sistemi di manutenzione del suolo ed erosione.* - Olivae, 59: 64-74.

PIGNATTI S., 1982 - *Flora d'Italia*. - Ed. Agricole, Bologna.

POTTS S.G., PETANIDOU T., ROBERTS S., O'TOOLE C., HULBERT A., WILLMER P., 2006 - *Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape*. - Biological Conservation, 129(4): 519-529.

RODENAS L.M., SANCHO R.F., RAMIREZ D.L., BERNALDEZ G.F., 1977 - *Ecosistemas del area de influencia de Sevilla*. - Monografia 18. Doñana: Prospeccìon e inventario de Ecosistemas, ICONA, Madrid, Spain (Cited by Beaufoy, 2000).

SAAVEDRA M., 1998 - *Flora del olivar y manejo de herbicidas*. - Paper presented at Universidad Internacional de Andalucía, Baeza, Spain (Cited by Beaufoy, 2000).

SOFO A., PALESE A.M., XILOYANNIS C., MONTANARO G. E MASSAI R., 2004 - *Il ruolo della frutticoltura nella mitigazione dell'effetto serra.* - L'informatore Agrario, 44: 27-31.

TOSCANO P., BRICCOLI-BATI C., GODINO G., DE SIMONE C., RAGLIONES M., LORENZONI P., ANGELINI R., ANTONUCCIO S., 2004 - *Effetti agronomici e pedologici di due diverse tecniche di gestione del suolo in un oliveto collinare del Meridione d'Italia*. - Olivae, 102: 21-26.

UBALDI D., 2003 - *Flora, fitocenosi e ambiente: elementi di geobotanica e Fitosociologia*. - Clueb Bologna, Italy, pp. 334.

VIGGIANI P., 2009 - *La Flora spontanea*. - In: PISANTE M., P. INGLESE, and G. LERCKER *L'ulivo e l'Olio*. - Coltura & Cultura. Collana ideata e diretta da Angelini Renzo. Bayer Crop Science. ART Servizi Editoriali S.p.A., Bologna, Italy, pp. 784.

Adv. Hort. Sci., 2013 27(1-2): 55-60

Trace and minor elements in bee honeys produced in Syria

A. Khuder* [(1)], M. Ahmad*, R. Hasan *, G. Saour**

* *Department of Chemistry, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria.*

** *Department of Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria.*

Key words: cluster analysis, honey, minerals, X-ray fluorescence.

Abstract: Eleven minerals (K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr) in 31 honey samples, including 24 Syrian, three imported and four honeys produced from bees fed with sugar were quantified using a dry ashing method for X-ray fluorescence analysis. The search for natural groups in the honey samples was carried out by cluster analysis, using complete linkage and Euclidean distance. The Syrian and imported samples clustered into three honey groups: 1) poor in element concentrations (citrus honey); 2) rich in minor and trace element concentrations (wild plants and jujube honeys); and 3) moderate mineral concentrations (multiflora, eucalyptus, crataegus, and sunflower honeys). Results are discussed in terms of the mineral concentrations in Syrian honeys and in comparison with international values.

1. Introduction

Honey is a natural substance formed when the nectar and sweet deposits from plants are gathered, modified and stored in honeycomb by honey bees (Azeredo *et al*., 2003; Wei *et al*., 2010). However, honey needs to satisfy numerous quality and certification criteria before commercialization (Devillers *et al*., 2004; Laube *et al*., 2010). Different methods based on parameters, such as nutritious, prophylactic properties, pollen and unique flavors analyses were applied to specify the quality of honeys. Although these methods have many advantages, they are not recommended for the fast routine procedure because their applications required for highly specialized personnel; furthermore, they are laborious and time-consuming (Chudzinska and Baralkiewicz, 2010; Wei *et al*., 2010).

Several researchers found the physicochemical analysis method as a most prevalent tool that could be used for detecting the origin of honey (Adebiyi *et al*., 2004; Felsner *et al*., 2004; Serrano *et al*., 2004; Corbella and Cozzolino, 2006; Cantarelli *et al*., 2008). For instance, Lachman *et al*. (2007) classified Czech Republic honey samples by combining between the mineral content and the electrolytic conuctivity analyses.

The elemental content of honeys is closely related to the soil and vegetation in the area where the raw material for honey was collected (Caroli *et al*., 1999; Bilandžić *et al*., 2012). For instance, Tuzen *et al*. (2007) determined the levels of several trace elements in honey from different botanical origins in Turkey and established a correlation between the content of trace elements and the botanical and geographical origin of honey. Pisani *et al*. (2008) showed the influence of botanical origin on the chemical composition of honey through analysis of various elements in 51 Italian honey samples. Likewise, Grembecka and Szefer (2012), using flame atomic absorption spectrometry, estimated honey quality from different locales in Poland and Europe in light of their mineral composition.

Syria has various flora-rich regions that have been considered suitable for apiculture. Unfortunately, data dealing with element concentrations in Syrian honeys has been ignored. Thus, the present work focuses on determining several elements in different types of honey from different natural and artificial sources using a dry ashing method for X-ray fluorescence (XRF) analysis. In addition, cluster analysis (CA) is also applied in the present study to group the analyzed samples with regard to their botanical origin. The ability of CA to discriminate between natural honeys and those produced from bees fed with sugar was also studied.

2. Materials and Methods

Honey samples

A set of 24 Syrian natural honey samples were analyzed. In addition to the local samples, three jujube honey samples (200 g each) imported from India (two samples)

[(1)] Corresponding author: scientific@aec.org.sy.

Received for publication 5 June 2012
Accepted for publication 28 March 2013

and Pakistan (one sample) were used for comparison purposes. The Syrian honey samples (500 g each) were collected directly from sedentary beehives in different parts of Syria during the late spring and early summer months. All samples were collected in clean, closed glass jars and immediately transferred to the laboratory. The samples were unpasteurized, stored in glass bottles and kept at 4-5°C in the dark until analysis. The Syrian honey samples under study belonged to six representative honey types: citrus (C, n=6), multiflora (M, n=6), *Eucalyptus* (Eu, n=5), crataegus (Crat, n=3), sunflower (Sun, n=2) and wild plants (W, n=2). The botanical origin of some honey samples was confirmed by pollen analysis, according to Louveaux *et al*. (1978). Additionally, two types of artificial honey samples based on honeys produced from bees fed with sugar were collected after feeding a sugar solution to bees in one apiary (one beehive). The two sugar solutions were prepared as follows: the first solution (HIS) was prepared by mixing sugar and water on a 1:1 basis. A total of 1.5 kg commercial sugar was completely dissolved in 1.5 l hot-ultrapure water (18.2 MΩcm specific resistivity) using an electrical heating plate at 60°C. The obtained solution was cooled at room temperature. Then, the following chemical salts were dissolved in the sugar solution: (0.7259 g) $FeCl_3.6H_2O$, (0.0372 g) $Ni(NO_3)_2.6H_2O$, (0.0424 g) $CuCl_2.2H_2O$, (0.1563 g) $ZnCl_2$; the solution was mixed with a glass rod. The final volume of the obtained sugar solution was 2480 ml. The second sugar solution (HSB) was prepared in the same way but no chemical salts were added. For statistical analysis, two indipendent HIS and HSB sugar solutions were prepared. Feeding to bees of the sugar solutions was carried out at seven-day intervals.

Reagents and solutions

All aqueous solutions and dilutions were prepared with ultrapure water obtained from a water purification system (New Human Power II, South Korea) with 18.3 MΩcm specific resistivity. The solutions of 14 N HNO_3 'Analar' (BDH) were used for the honey ash dissolutions. The stock standard solutions of K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr with concentrations of 1000 μg ml^{-1} each were used for the preparation of the multi-element reference targets for XRF calibration. A pure cellulose powder (AG) for analysis from Seelze (Hannover/Germany) was used as a binder for preparation of the XRF targets.

Analytical procedures

Eleven elements (K, Ca, Ti, Cr, Mn, Fe, Cu, Ni, Zn, Rb, and Sr,) were determined in the honey samples using a dry ashing method for XRF analysis (Khuder *et al*., 2010). Ten g of each honey sample were put in a 50-ml crucible and dried in an oven at 105°C for 72 h, covered, cooled in a desiccator and weighed. Each crucible-held dried sample was subjected to ashing in an electrical furnace. The temperature was increased in three steps: 200, 300, and 550°C; where the first and second steps lasted for 20 min each, while the third step lasted for 16 h. The ash of each honey sample was weighed and kept in the desiccator. Each obtained ash was dissolved in 1-ml volume of 6 N HNO_3 then removed to a small 5-ml volume vial. A volume of 100 μl of suspended cellulose solution (0.120 g ml^{-1}) was added to each dissolved ash. The obtained mixtures were thoroughly shaken using an electrical shaker (KS 125 basic, IKALABORTECHNIK Co., Japan) for 5 min, then removed to XRF spectro-cups with surface area of 4.91 cm^2 each, and dried under IR lamp. Finally, each obtained honey target was weighed and subjected to XRF analysis using Mo-secondary target for the determination of Fe, Ni, Cu, Zn, Rb, and Sr, and Cu-secondary target for the determination of K, Ca, Ti, Cr, and Mn.

Instrumental measurements

The XRF measurements were performed using an energy dispersive X-ray fluorescence instrument equipped with a 2 kW Mo tube and a Si (Li) semiconductor detector (PGT Co.) with an energy resolution of 140 eV at 5.9 keV. The operating conditions were differed, depending on the mode of the X-ray excitation: 7 mA and 17 kV, and 5 mA and 45 kV by using Cu- and Mo-secondary targets, respectively. The live time was 1000 s for both of the X-ray excitation modes.

The peak areas in the obtained spectra were evaluated using the AXIL-QXAS software package (IAEA, 2005). The XRF results were compared with those obtained by standardized AAS method using hollow cathode lamps (Rashed and Soltan, 2004). The accuracy, precision, and limits of detection (LOD) of the XRF were estimated using the method described by Khuder *et al*. (2010).

Statistical analysis

Basic statistics were carried out using the STATISTICA 6.0 statistical package for windows (Statsoft). Prior to chemometric processing, the root square of data was carried out in order to stabilize the variance. CA was used to group the analyzed honey samples with regard to their botanical origin. The Euclidean distance was used to measure the similarity as clustering method single linkage.

3. Results

XRF analysis

A typical XRF spectrum of a honey sample excited by means of Cu- and Mo-secondary targets is shown in figure 1. The spectra confirmed the presence of K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, and Sr in the analyzed samples. The detected elements were calibrated by constructing sensitivity curves (Fig. 2) and quantified using the AXIL-QXAS program. The validity of XRF was examined by estimating the precision, accuracy, and LOD parameters (Table 1). The LOD of K, Ca, Ti, Cr, and Mn were 0.40, 0.09, 0.06, 0.01, and 0.01 μg g^{-1}, respectively; while those of Fe, Ni, Cu, Zn, Rb, and Sr were 0.050, 0.032, 0.031, 0.030, 0.009, and 0.007 μg g^{-1}, respectively. A comparison

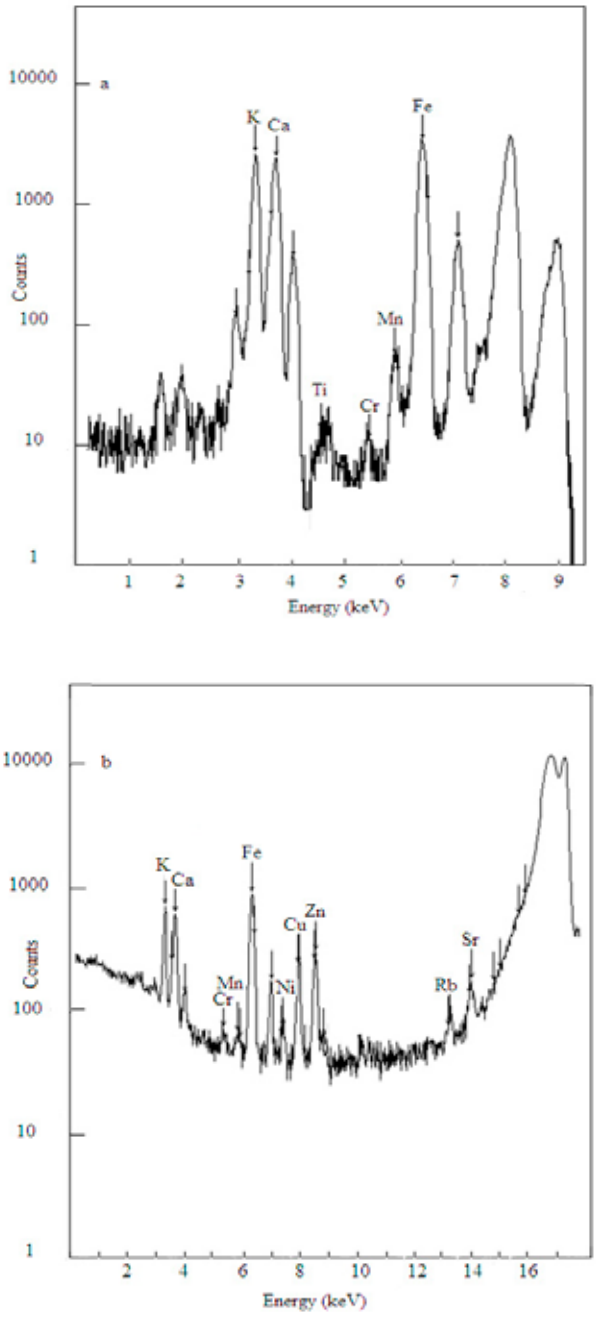


Fig. 1 - Typical spectra of a honey sample excited by X-ray Mo tube with (a) Cu-, and (b) Mo-secondary targets.

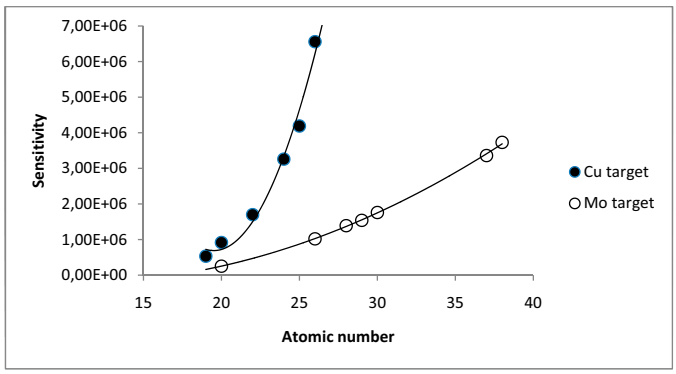


Fig. 2 - Calibration sensitivity curves obtained by X-ray excitation of elements using Cu- and Mo- secondary targets.

between XRF and AAS methods revealed a correlation coefficient above 0.990, indicating that the XRF was reliable and suitable to determine most of the elements in honey samples.

Table 1 - Determination of different elements in multi-element standard sample using Cu- and Mo-secondary targets for XRF analysis [z]

Secondary target	Elements	(Means±SD) [y]	A (%) [x]	RSD (%)[w]
Cu	K	9.68±0.35	-3.2	±3.62
	Ca	10.25±0.25	2.5	±2.44
	Ti	10.22±0.30	2.2	±2.94
	Cr	9.66±0.35	-3.4	±3.62
	Mn	9.72±0.33	-2.8	±3.40
Pooled (rms)[v]			2.85	
Mo	Fe	9.95±0.66	-0.5	±6.63
	Ni	9.50±0.55	-5	±5.79
	Cu	10.44±0.82	4.4	±7.85
	Zn	10.42±0.52	4.2	±4.99
	Rb	9.92±0.11	-0.8	±1.11
	Sr	9.95±0.08	-0.5	±0.80
Pooled (rms)[d]			3.24	

[z] The 'dark matrix' entered for running QXAS-AXIL program with Cu-secondary target mode was C (5%) and H by difference; while, that for Mo-secondary target was Si (1%) and O by difference.
[y] XRF results (µg) were obtained by measuring the multi-element standard three times; SD is the standard deviation.
[x] A is the accuracy calculated by the difference between the obtained and the used amounts (absolute amount is 10 µg).
[w] RSD is the relative standard deviation; RSD=(SD x Mean $^{-1}$) x 100.
[v] is the root mean square of elemental accuracy.

Honey analysis

Chemical analysis data (Table 2) concerning the studied honey samples differentiated two mineral groups: the most abundant and the trace elements. The first group was composed of K and Ca, having concentrations of more than 10 µg g^{-1}. The second mineral group comprised the trace elements: Fe, Cu, Zn, Rb, Ti, Cr, Mn, Ni, and Sr. Of these, two subgroups were noted: the trace elements Fe, Cu, Zn, and Rb with concentrations in the 1-10 µg g^{-1} range, and a second subgroup (Ti, Cr, Mn, Ni, and Sr) with concentrations < 1.0 µg g^{-1}.

The data were clustered in order to find the similarities between analyzed honey samples (Fig. 3) and also the elements (Fig. 4).

4. Discussion and Conclusions

The honey samples in the present work were subjected to an ashing process in order to increase the sensitivity of the XRF analysis, and the concentrations of the elements were much higher than the obtained LOD values.

Table 2 - Element concentrations in Syrian honeys from different botanical origins determined by using X-ray fluorescence analysis

Botanical origins		Element concentrations (μg.g^{-1})										
		K	Ca	Ti	Cr	Mn	Fe	Cu	Ni	Zn	Rb	Sr
M	Mean	138	76.1	0.212	0.029	1.04	5.87	2.34	0.23	1.21	1.03	0.63
	Min.	56.2	44.6	0.102	0.017	0.36	4.0	0.83	0.08	0.21	0.44	0.41
	Max.	183	118	0.285	0.036	1.69	9.42	4.53	0.39	3.82	2.2	1.03
Eu	Mean	121	92.6	0.292	0.016	1.74	8.35	3.82	0.18	2.44	1.05	0.64
	Min.	66.7	50.6	0.145	0.011	0.36	5.72	1.59	0.17	0.53	0.43	0.47
	Max.	228	127	0.401	0.024	3.16	12.4	6.09	0.20	7.16	1.78	0.79
C	Mean	40.3	45.5	0.085	0.026	0.50	1.74	1.14	0.13	2.42	0.32	0.31
	Min.	5.7	7.3	0.052	0.011	0.15	1.04	0.62	0.11	1.01	0.07	0.14
	Max.	84.6	78.5	0.099	0.054	1.06	2.43	1.59	0.14	4.30	0.94	0.70
Crat	Mean	125	43.3	0.103	0.028	0.90	7.57	2.95	0.20	3.50	1.57	0.48
	Min.	47.5	39.1	0.071	0.014	0.46	3.83	1.66	0.15	1.41	0.26	0.44
	Max.	179	46.1	0.125	0.055	1.34	13.1	4.39	0.25	7.33	2.53	0.50
Sun	Mean	107	34.4	0.142	0.024	0.32	4.83	3.10	0.162	3.83	0.81	0.65
	Min.	98.3	33.6	0.115	0.019	0.2	4.68	2.67	0.135	3.75	0.65	0.32
	Max.	115	35.1	0.168	0.029	0.42	4.98	3.53	0.188	3.91	0.98	0.99
W	Mean	198	50.3	0.30	0.029	2.54	17.0	3.4	0.265	2.88	4.52	0.38
	Min.	187	49.1	0.18	0.024	1.81	14.2	1.06	0.109	1.38	1.57	0.36
	Max.	208	51.4	0.42	0.034	3.26	19.7	5.73	0.420	4.37	7.47	0.41
J	Mean	250	58.5	0.17	<0.01	3.37	5.40	1.46	0.083	0.65	3.97	0.49
	Min.	219	38.7	0.12	-	0.60	4.98	0.83	0.081	0.27	2.69	0.13
	Max.	280	78.3	0.22	-	6.13	5.82	2.08	0.084	1.03	5.24	0.85
HSB	Mean	4.1	16.1	0.05	<0.01	0.11	1.35	1.34	0.069	3.19	0.08	0.09
	Min.	3.9	15.3	0.03	-	0.10	1.28	1.27	0.064	3.03	0.07	0.08
	Max.	4.3	16.9	0.07	-	0.20	14.2	1.41	0.074	3.35	0.08	0.10
HIS	Mean	5.1	17.0	0.05	<0.01	0.09	22.0	3.29	1.36	21.6	0.05	0.07
	Min.	4.8	16.1	0.03	-	0.08	20.9	3.12	1.28	20.5	0.04	0.06
	Max.	5.4	17.9	0.07	-	0.12	23.1	3.46	1.44	22.7	0.05	0.07

M, Eu, C, Crat, Sun, W, J, correspond to multiflora, *eucalyptus,* citrus, crataegus, sunflower, wild plants and jujube, honeys respectively. HSB and HIS were honeys produced from bees fed with sugar alone and sugar enriched with salts, respectively.

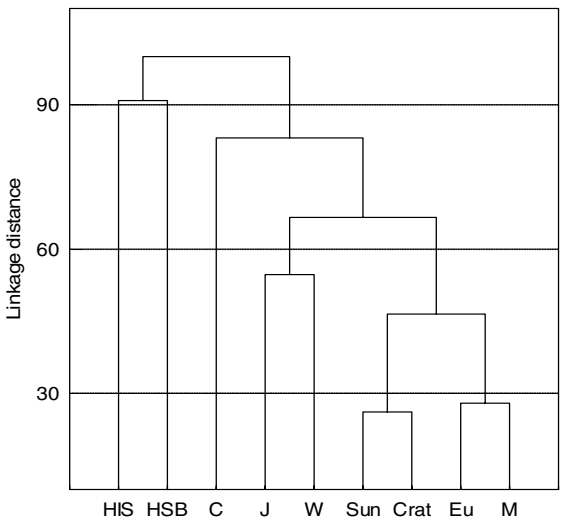


Fig. 3 - Dendrogram of cluster analysis of elements in different types of honeys (root square of data was treated by the linkage method with Euclidean distance as measure of similarity).
HIS and HSB= honeys produced from bees fed with sgar enriched with salts and sugar alone, C= citrus, J= jujube, W= wild plants, Sun= sunflower, Crat= crataegus, Eu= eucalyptus, M= multiflora.

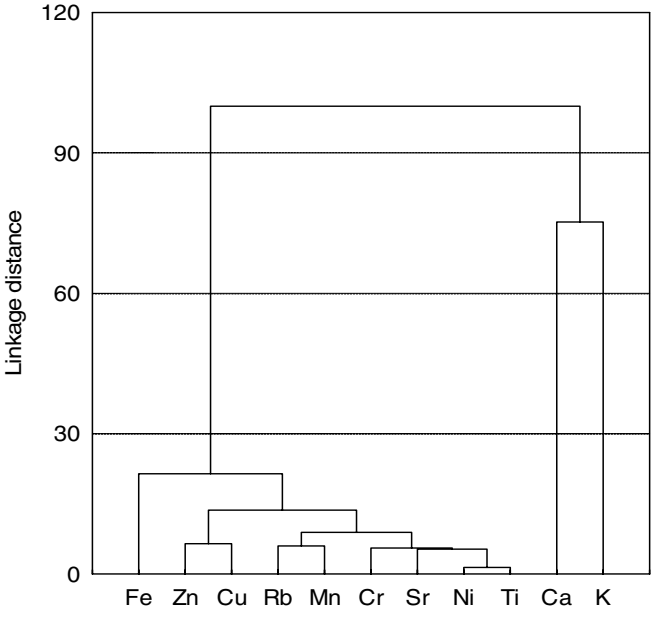


Fig. 4 - Dendrogram of 11 analyzed elements in different types of honey samples (root square of data was treated by the linkage method with Euclidean distance as measure of similarity).

Based on our data the minerals in Syrian natural, artificial, and imported honeys fell into two groups: the first group was composed of K and Ca, while the other included the trace elements Fe, Cu, Zn, Rb, Ti, Cr, Mn, Ni, and Sr.

Potassium represented the most abundant element in the Syrian honey samples with a mean concentration of 107 μg g^{-1}. This finding coincides with most other authors who consider this element to be the most quantitatively important in honey (Terrab *et al*., 2004; Fernandez-Torres *et al*., 2005; Nozal Nalda *et al*., 2005; Pisani *et al*., 2008). The highest and lowest K concentrations were found in Jujube (250 μg g^{-1}) and citrus (40.3 μg g^{-1}) honeys, respectively; the mean concentrations of K in Syrian honeys were very similar to those in honeys from Brazil (Sodré *et al*., 2007) and less than values obtained for Turkish honeys (Cantarelli *et al*., 2008). Calcium in Syrian honeys was the second most abundant element with a mean concentration of 61.9 μg g^{-1}; the values found for this element in Syrian honeys were similar to those of jujube honeys imported from Pakistan and India, as well as for honeys from other countries (Cantarelli *et al*., 2008; Chudzinska and Baralkiewicz, 2010).

The mean concentration of Fe and Cu in Syrian honeys was estimated to be 6.33 and 2.49 μg g^{-1}, respectively. Our value for Fe is very similar to that obtained by Cantarelli *et al*. (2008) for Turkish honeys, but higher than for honey from Argentina, Brazil and Switzerland (Bogdanov *et al*., 2007; Sodré *et al*., 2007; Cantarelli *et al*., 2008). It is worth mentioning that the concentrations of Cu in Syrian honeys were much higher than those for honeys from Poland, Switzerland, the Czech Republic, Brazil and Argentina (Bogdanov *et al*., 2007; Lachman *et al*., 2007; Sodré *et al*., 2007; Cantarelli *et al*., 2008; Chudzinska and Baralkiewicz, 2010). The mean concentration of Zn and Rb was 2.27 and 1.50 μg g^{-1}, respectively; the value for the former was comparable to those obtained for the imported honeys (1.89 μg g^{-1}), as well as for honeys from Poland, Argentina, Spain, Italy and Turkey (Cantarelli *et al*., 2008; Chudzinska and Baralkiewicz, 2010). The mean concentration of Rb in Brazilian honey was much lower than that obtained in the present work (Sodré *et al*., 2007). Latorre *et al*. (1999) reported a mean value of 1.5 μg.g^{-1} in Spain, which is similar to the mean Rb concentration found in Syrian honeys.

Titanium was found in the honey samples with a mean value of 0.184 μg g^{-1}, and Cr was identified in 20 samples (87% of the total) with a mean concentration of 0.025 μg g^{-1}. Sodré *et al*. (2007) found Ti in Brazilian honey samples (mean value 0.112 μg g^{-1}) while the mean concentrations of Cr in different honeys from Switzerland, Chile, and Brazil were 0.005, 0.070 and 0.038 μg g^{-1}, respectively (Fredes and Montenegro, 2006; Bogdanov *et al*., 2007; Sodré *et al*., 2007). Manganese and Ni were found in 21 and 14 honey samples, respectively, with mean concentrations of 1.29 and 0.204 μg g^{-1}. The mean concentrations of Mn in honeys from Argentina and the Czech Republic varied from 0.33 to 2.87 μg g^{-1} (Lachman *et al*., 2007; Cantarelli *et al*., 2008), and a mean value of 1.0 μg g^{-1} was found in Turkish honey (Tuzen *et al*., 2007). Concentrations of Ni in Syrian honeys were comparable to those obtained for honeys from Chile, the Czech Republic and Switzerland (Fredes and Montenegro, 2006; Bogdanov *et al*., 2007; Lachman *et al*., 2007). Strontium was verified in all samples with a mean concentration of 0.518 μg g^{-1}. Our data showed that the highest Sr concentration (1.03 μg g^{-1}) came from a multiflora honey obtained from a beehive placed near highways. Results similar to ours have been recorded previously: Fredes and Montenegro, (2006) found that the highest concentrations of Sr in Chilean honeys were noted in honeys harvested from beehives close to roads and highways.

Cluster analysis was applied to the data of the 11 elements in the nine types of studied honeys. At a similarity level of 60%, the natural honey samples were grouped into three clusters (Fig. 3). The first cluster contained only the citrus honey, the second Jujube and wild plant honeys, and the third the remaining honey types (sunflower, crataegus, eucalyptus, and multiflora). At the same similarity level, the two artificial honey samples (HIS and HSB) were well discriminated in two clusters. The hierarchical dendrogram (Fig. 4) discriminated between the elements according to their concentrations. The elements Zn, Fe, Rb, Cu, Mn, Ni, Sr, Cr, and Ti represented a group of elements with concentrations ≤10 μg g^{-1}; while K and Ca represented a group of elements with concentrations ≥10 μg g^{-1}. The hierarchical dendrogram shown in figure 4 could suggest a potential relationship between the samples' origin and the clusters of particular elements, which reflects the chemical composition of the botanical origin of honey.

In conclusion, cluster analysis grouped the natural honeys into different clusters with regard to their botanical origin. Citrus honeys, which were poor in element concentrations, formed an individual group while wild plants and jujube honeys formed the second group, which were rich in the determined minor and trace elements. Samples of mutiflora, eucalyptus, crataegus, and sunflower honeys formed the third natural group with moderate elemental concentrations. CA technique also revealed a very good discrimination between natural honeys and those produced from bees fed with sugar.

Acknowledgements

We would like to thank the general Director of AECS, Prof. I. Othman, and the Head of the Department of Chemistry, Dr. T. Yassine, for their help and support.

References

ADEBIYI F.M., AKPAN I., OBIAJUNWA E.I., OLANIYI H.B., 2004 - *Chemical/physical characterization of Nigerian honey*. - Pakistan J. Nutr., 3: 278-281.

AZEREDO L., DA C., AZEREDO M.A.A., DE SOUZA S.R., DUTRA V.M.L., 2003 - *Protein contents and physicochemi-*

cal properties in honey samples of Apis mellifera *of different floral origins*. - Food Chem., 80: 249-254.

BILANDŽIĆ N., ĐOKIĆ M., SEDAK M., VARENINA I., SOLOMUN-KOLANOVIĆ B., KONČURAT A., ŠIMIĆ B., RUDAN N., 2012 - *Content of five trace elements in different honey types from Koprivnica-Krievci county.* - Slov. Vet. Res., 49: 167-175.

BOGDANOV S., HALDIMANN M., LUGINBÜHL W., GALLMANN P., 2007 - *Minerals in honey: environmental, geographical and botanical aspects*. - J. Apic. Res., 46: 269-275.

CANTARELLI M.A., PELLERANO R.G., MARCHEVSKY E.J., CAMIÑA J.M., 2008 - *Quality of honey from Argentina: Study of chemical composition and trace elements*. - J. Argent. Chem. Soc., 96: 33-41.

CAROLI S., FORTE G., IAMICELI A.L., GALOPPI B., 1999 - *Determination of essential and potentially toxic trace elements in honey by inductively coupled plasma-based techniques*. - Talanta, 50: 327-336.

CHUDZINSKA M., BARALKIEWICZ D., 2010 - *Estimation of honey authenticity by multielements characteristics using inductively coupled plasma-mass spectrometry (ICP-MS) combined with chemometrics*. - Food Chem. Toxicol., 48: 284-290.

CORBELLA E., COZZOLINO D., 2006 - *Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics*. - LWT-Food Sci. Tech., 39: 534-539.

DEVILLERS J., MORLOT M., PHAM-DELEGUE M.H., DOR J.C., 2004 - *Classification of monofloral honeys based on their quality control data*. - Food Chem., 86: 305-312.

FELSNER M.L., CANO C.B., BRUNS R.E., WATANABE H.M., ALMEIDA-MURADIAN L.B., MATOS J.R., 2004 - *Characterization of monofloral honeys by ash contents through a hierarchical design*. - J. Food Compos. Anal., 17: 737-747.

FERNANDEZ-TORRES R., PEREZ-BERNAL J.L., BELLO-LOPEZ M.A., CALLEJON-MOCHON M., JIMENEZ-SANCHEZ J.C., GUIRAUM-PEREZ A., 2005 - *Mineral content and botanical origin of Spanish honeys*. - Talanta, 65: 686-691.

FREDES C., MONTENEGRO G., 2006 - *Heavy metals and other trace elements contents in Chilean honey*. - Cien. Inv. Agr., 33: 50-58.

GREMBECKA M., SZEFER P., 2012 - *Evaluation of honeys and bee products quality based on their mineral composition using multivariate techniques.* - Environ Monit. Assess., pp. 1-15.

IAEA, 2005 - *Quantitative X-ray Analysis System* - QXAS, Doc. Version 2.0 International Atomic Energy Agency, Vienna.

KHUDER A., AHMAD M., HASAN R., SAOUR G., 2010 - *Improvement of X-ray fluorescence sensitivity by dry ashing method for elemental analysis of bee honey*. - Microchem. J., 95: 152-157.

LACHMAN J., KOLIHOVÁ D., MIHOLOVA D., KOŠATA J., TITĚRA D., KULT K., 2007 - *Analysis of minority honey components: Possible use for the evaluation of honey quality*. - Food Chem., 101: 973-979.

LATORRE M.J., PENA R., PITA C., BOTANA A., GARCIA S., HERRERO C., 1999 - *Chemometric classification of honeys according to their type II. Metal content data*. - Food Chem., 66: 263-268.

LAUBE I., HIRD H., BRODMANN P., ULLMANN S., SCHNE-MICHLING M., CHISHOLM J., BROLL H., 2010 - *Development of primer and probe sets for the detection of plant species in honey*. - Food Chem., 118: 979-986.

LOUVEAUX J., MAURIZIO A., VORWOHL G., 1978 - *Methods of Melissopalynology by International Commission for Bee Botany of IUBS*. - Bee World, 59: 139-157.

NOZAL NALDA M.J., BERNAL YAGUE J.L., DIEGO CALVA J.C., MARTIN GOMEZ M.T., 2005 - *Classifying honeys from the Soria Province of Spain via multivariate analysis*. - Anal. Bioanal. Chem., 382: 311-319.

PISANI A., PROTANO G., RICCOBONO F., 2008 - *Minor and trace elements in different honey types produced in Siena Country (Italy).* - Food Chem., 107: 1553-1560.

RASHED M.N., SOLTAN M.E., 2004 - *Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys.* - J. Food Compos. Anal., 17: 725-735.

SERRANO S., VILLAREJO M., ESPEJO R., JODRAL M., 2004 - *Chemical and physical parameters of Andalusian honey: classification of citrus and eucalyptus honeys by discriminant analysis*. - Food Chem., 87: 619-625.

SODRÉ G.S., MARCHINI L.C., ZUCCHI O.L., FILHO V.F.N., OTSUK I.P., MORETI A.C., 2007 - *Determination of chemical elements in Africanized* Apis mellifera *(HYMENOPTERA: APIDAE) honey samples from the state of Piauí, Brazil*. - Quím. Nova., 30: 920-924.

TERRAB A., HERNANZ D., HEREDIA F.J., 2004 - *Inductively coupled plasma optical emission spectrometric determination minerals in thyme honeys and their contribution to geographical discrimination*. - J. Agric. Food Chem., 52: 3441-3445.

TUZEN M., SILICI S., MENDIL D., SOYLAK M., 2007 - *Trace element levels in honeys from different regions of Turkey*. - Food Chem., 103: 325-330.

WEI Z., WANG J., WANG Y., 2010 - *Classification of monofloral honeys from different floral origins and geographical origins based on rheometer*. - J. Food Eng., 96: 469-479.

Adv. Hort. Sci., 2013 27(1-2): 61-66

Protandrous-protogynous dimorphism in indigenous selections from North Western India and some exotic cultivars of Persian walnut (*Juglans regia* L.)

A. Kumar*, N. Sharma**

* *Division of Fruit Science, Sher-e-Kashmir University Agricultural Sciences and Technology, Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India.*

** *Division of Fruit Science, Sher-e-Kashmire University Agricoltural Sciences and Tecnology, Jammu, Chatta, Jammu, Jammy and Kashmir, India.*

Key words: dichogamy, low productivity, pollen shedding, stigma receptivity, walnut.

Abstract: Walnut (*Juglans regia* L.) is one of the most important temperate nuts grown worldwide. In India, however, most of the produce comes from age-old trees of unknown origin. Apart from non-adoption of standard farm practices, certain inherent problems like dichogamy are the major factors of low productivity. The problem of dichogamy is further aggravated by short period of pollen shedding and stigma receptivity. Dichogamy in walnut prevents self-pollination and necessitates cross-pollination to set fruits. For optimum fruit set and consequent yield, an attempt was made with 20 cultivars/selections grafted on seedling walnuts for three consecutive years to determine the nature and degree of dichogamy. Observations were recorded on the time of male and female flowering, duration of pollen shedding and stigma receptivity, nature and degree of dichogamy. Results indicate that out of 20 cultivars/selections fourteen have protandrous nature, 'Gobind' in the first and third year, 'KX Giant' in second and third year and 'Plant No. 45' in the second year have homogamous nature and only 'Gobind' have protogynous nature in the second year. Degree of dichogamy varies from zero to 100 per cent among various cultivars/selections. Findings of the present study emphasized on the interplanting of protandrous and protogynous cultivars or homogamous cultivars to ensure adequate pollination to obtain higher nut yields in walnut.

1. Introduction

Juglans regia plants are monoecious, anemophilous with unisexual flowers grouped in separate inflorescences (Manning, 1938; Bauckmann, 1974; Germain *et al*., 1981). Staminate flowers (catkins) develop from lateral buds in the axil of leaves on the previous season's growth. Pistillate flowers are borne terminally on the current season's growth, however in some cultivars, also develops in a lateral position on the last year's growth (Forde, 1977). Although *Juglans regia* cultivars are self fertile, but low yields in walnut is a cause of concern which is mainly attributed to inadequate pollination resulting from pistillate flower abscission (PFA) and adverse climatic conditions during pollen shedding and stigma receptivity. Apart from these factors, dichogamy is also a main cause for low yields in walnut which was steady and biological characteristics of walnut (Akca and Sen, 1997).

The phenomenon of dichogamy involves development on the same plant of male and female organs at different times. 'Protandry' refers to the shedding of pollen prior to stigma receptivity and 'protogyny' the reverse sequence (Gleeson, 1982). The mating system with both protandrous and protogynous plants within the species has been classified as heterodichogamy (Gleeson, 1982; Luza and Polito, 1988). The extent of heterodichogamy varies from almost complete overlap (homogamy) to complete separation of male and female bloom periods (McGranahan and Leslie, 1991). The degree and nature of dichogamy is a varietal character, but it is also greatly affected by the age of the tree, climate and geographic location (Wood, 1932). Walnut variety or locality is free from dichogamy. On the basis of these data it is possible to make combinations of cultivars, which ensure pollinating and enable regular harvest. The present work deals with protandrous-protogynous dimorphism in 20 walnut cultivars/selections. To overcome the problem of low yields and to identify suitable cultivars that can overlap in blooming time, the present study was conducted at the University of Horticulture and Forestry, Nauni-Solan for three consecutive years. On this basis we can define the most suitable combinations of cultivars for north-western region of India.

Received for publication 20 March 2013
Accepted for publication 24 April 2013

2. Materials and Methods

The present study was carried out over a three-year period (2006-2008) in the walnut germplasm collection block of the Department of Fruit Breeding and Genetic Resources, Dr. Y S Parmar University of Horticulture and Forestry, Nauni-Solan, Himachal Pradesh. The walnut block is located at an elevation of 1225 m a.m.s.l. and between 31°N latitude and 77°E longitude. The mean average rainfall of the study area during the flowering season (February to April) ranges from 125 to 200 mm. The minimum and maximum temperatures of the field area during the flowering season range between 10 and 30°C. The experiment was conducted with the following 20 cultivars/selections: 'ACO 38853', 'Blackmore', 'Gobind', 'Hartley', 'Plant No.10', 'Netar Akhrot', 'Roopa Akhrot', 'KX Giant', 'Rattan Akhrot', 'Lake English', 'Kandaghat Selection', 'Payne', 'Inder Akhrot', 'Plant No. 32', 'Xenia', 'Plant No. 45', 'Plant No. 46', 'Plant No. 47', 'Solding Selection' and 'Luxmi Akhrot'. These cultivars/selections were grafted on walnut seedlings and the age of the planting material during the study was 18-20 years old. The grafted plants were planted at a distance of 7x7 m and standard practices of orchard management were followed. Observations were taken with regard to time of male and female flowering, duration of pollen shedding and stigma receptivity, nature and degree of dichogamy. Nature of dichogamy was determined as

Protandry: Maturation of male catkins prior to stigma receptivity of female flower

Protogyny: Stigma receptivity prior to maturation of male catkins

Homogamy: Maturation of male catkins coinciding with stigma receptivity in female flowers.

Degree of dichogamy was calculated according to the formula suggested by Solar *et al*. (1997).

$$\text{Degree of dichogamy (\%)} = = 1 - \frac{\text{No. of days when male and female flowering coincides}}{\text{Number of days of female flowering}} \times 100$$

3. Results and Discussion

The results obtained from the three consecutive years of study showed variation with respect to time of male and female flowering, duration of pollen shedding and stigma receptivity, nature and degree of dichogamy. The earliest emergence of catkins in all three years was recorded in 'Plant No. 45' (i.e. 12 March, 17 March and 15 March in the first, second and third years, respectively) along with 'Luxmi Akhrot' (12 March) in the first year, and 'Netar Akhrot' and 'Inder Akhrot' (17 March) in the second year (Fig. 1). Late emergence of catkins was observed in 'Solding Selection' (25 March) in the first year; in the second and third years late emergence was observed in 'Gobind' (7 April and 28 March, respectively). 'Plant No. 45' showed early female flowering (15 March in the first year, 22 March in second and 17 March in the third), whereas late emergence of female flowering in the first year was observed in 'Rattan Akhrot' (31 March), in the second year in 'Plant No. 32' (8 April) and in the third in 'ACO 38853' and 'Luxmi Akhrot' (1 April).

Early pollen shedding in 2006 and 2008 was recorded in 'Plant No. 45' (17 March and 20 March, respectively) along with 'Hartley' and 'Luxmi Akhrot' in 2006, however, it was recorded in 'Netar Akhrot' in 2007 (24 March) (Fig. 2). As for late pollen shedding the following observations were made: 'Solding Selection' (30 March 2006) and 'Gobind' (10 April 2007 and 1 April 2008). In all three years 'Plant No. 45' had early stigma receptivity (19 March, 25 March and 21 March, respectively). However, there was late stigma receptivity in 'Rattan Akhrot' in the first year (5 April), 'Plant No. 32' and 'Xenia' in the second year (12 April) and 'ACO 38853', 'Blackmore' and 'Luxmi Akhrot' in the third year (5 April).

Regarding the nature of dichogamy, it is clear from Table 1 that 'Gobind' was found to be homogamous in the first and third years, 'KX Giant' in the second and third years and 'Plant No. 45' in the second year. However, during the study period only 'Gobind' showed a protogynous nature in the second year. While 'KX Giant', 'Plant No. 32' and 'Plant No. 45' in 2006, 'Netar Akhrot' and 'Xenia' in 2007 and again 'Plant No. 45' in 2008 revealed a slight protandrous nature. All the other cultivars were of protandrous nature in all years. 'ACO 38853', 'Blackmore', 'Hartley', 'Roopa Akhrot', 'Lake English', 'Inder Akhrot', 'Plant No. 47' and 'Luxmi Akhrot' were found to have a 100% degree of dichogamy during the course of study; 'KX Giant' and 'Plant No. 45' in the second year, and 'Gobind' in the third year showed a 0% degree of dichogamy. Other cultivars/selections had a wide range of degrees of dichogamy during the study.

From the present study it is clear that Persian walnut has a large variation with regard to nature and degree of dichogamy. The degree of dichogamy in various cultivars/selections varies from zero to 100%. It is generally assumed that protandrous cultivars are the most numerous, followed by protogynous types, while homogamous types are quite rare (McDaniel, 1957; Majackaja, 1969; Germain *et al*., 1981) as also revealed in the present study. The trend here is towards protandry with as many as fourteen protandrous cultivars/selections ('ACO 38853', 'Blackmore', 'Hartley', 'Plant No. 10', 'Roopa Akhrot', 'Rattan Akhrot', 'Lake English', 'Kandaghat Selection', 'Payne', 'Inder Akhrot', 'Plant No. 46', 'Plant No. 47', 'Solding Selection' and 'Luxmi Akhrot') as demonstrated from the extent of synchronization of male and female flowering, and especially the time of pollen shedding and stigma receptivity. 'Gobind' in the first and third years, 'KX Giant' in second and third and 'Plant No. 45' in the second year had a homogamous nature, whereas only 'Gobind' showed a protogynous nature in the second year. However, the trend towards protandry was also reported by earlier researchers. Germain *et al*. (1983 a) found among

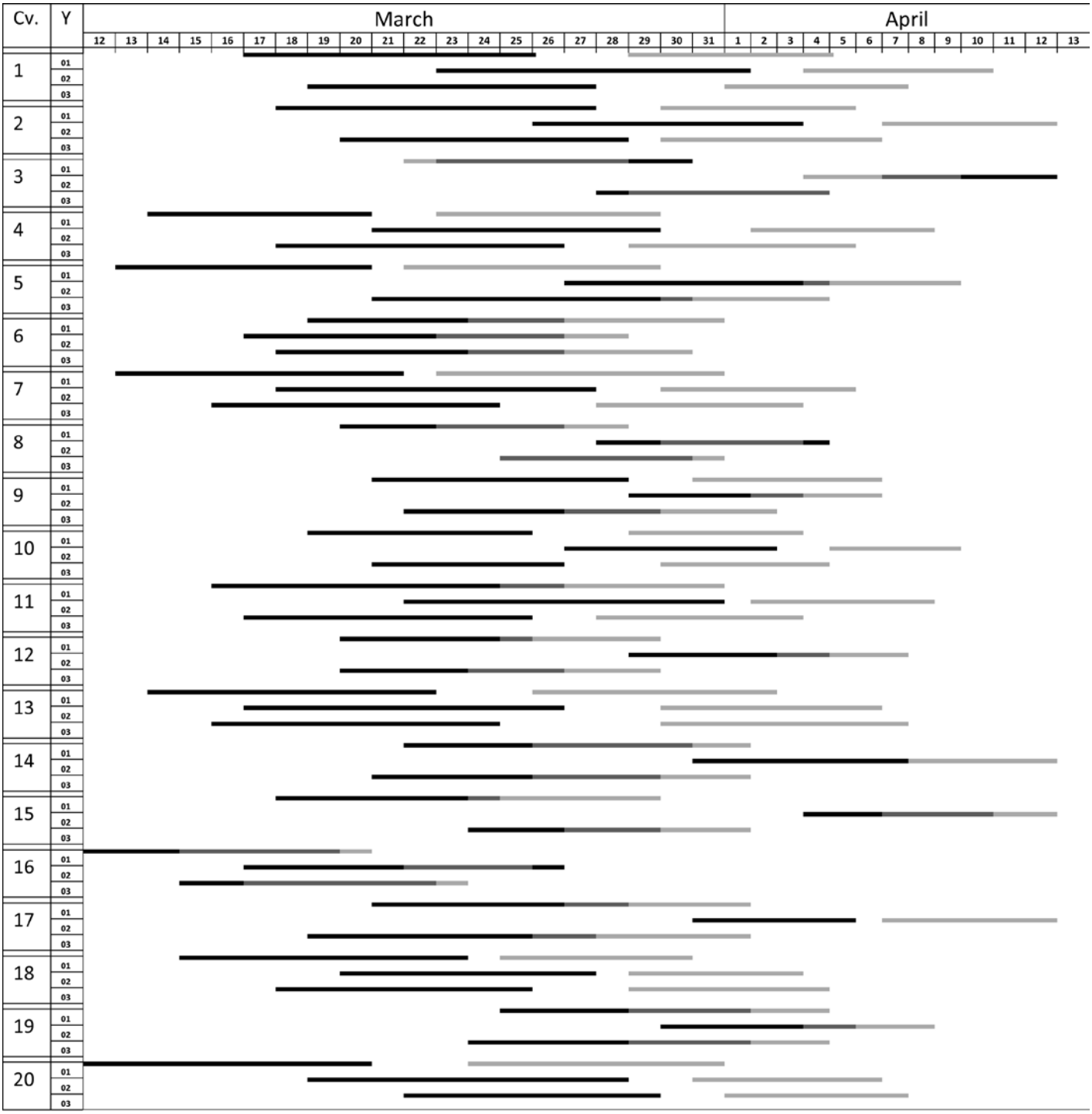


Fig. 1 - Time of male and female flowering in different walnut cultivars/selections. Cv= Cultivar; Y= Year; 01= 2006; 02= 2007; 03= 2008.

1	ACO 38853	5	Plant No. 10	9	Rattan Akhrot	13	Inder Akhrot	17	Plant No. 46
2	Blackmore	6	Netar Akhrot	10	Lake English	14	Plant No. 32	18	Plant No. 47
3	Gobind	7	Roopa Akhrot	11	Kandaghat Selection	15	Xenia	19	Solding Selection
4	Hartley	8	KX Giant	12	Payne	16	Plant No. 45	20	Luxmi Akhrot

Time of male flowering
Overlapping of male flowering and female flowering
Time of female flowering

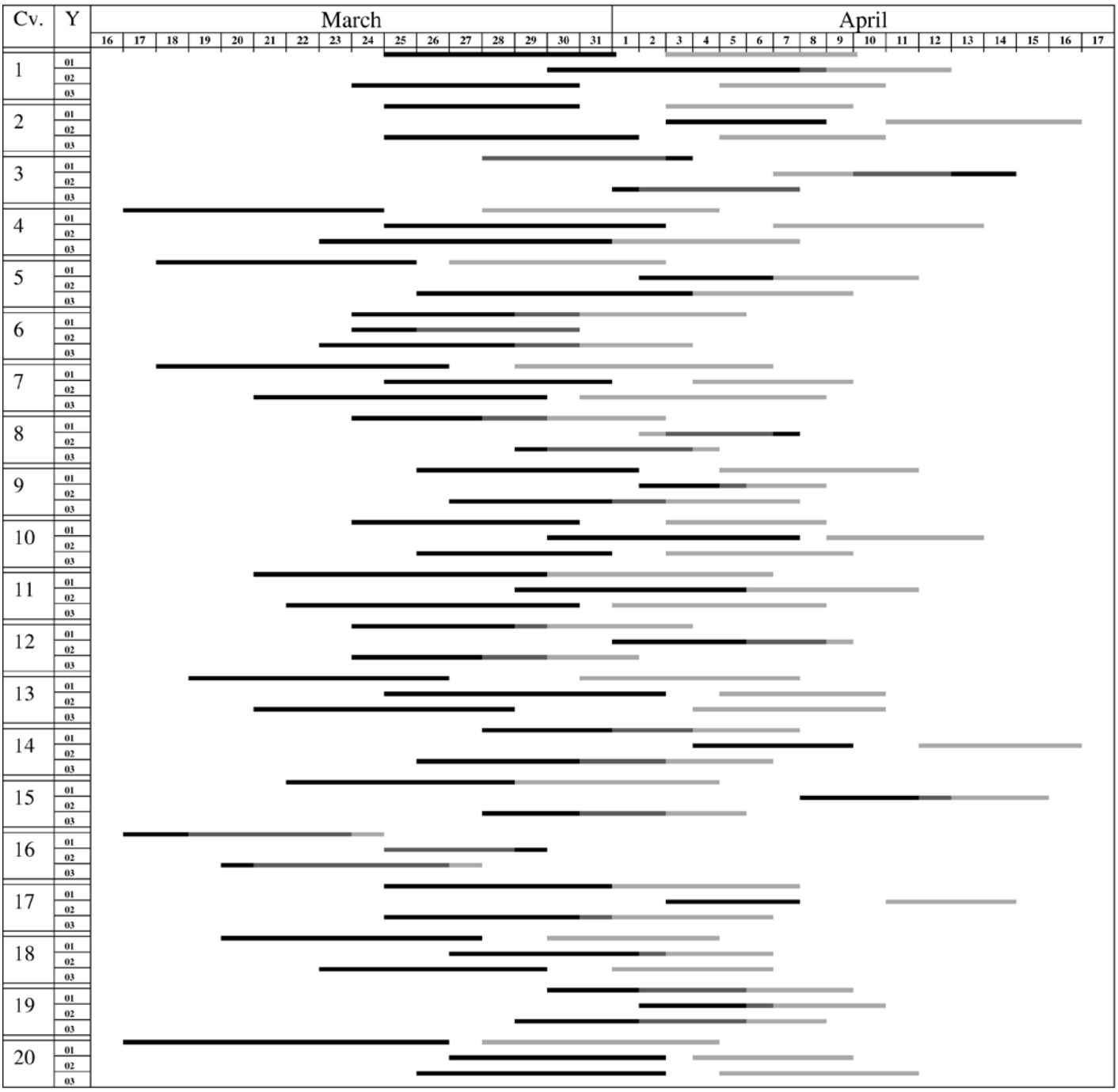


Fig. 2 - Duration of pollen shedding and stigma receptivity in different walnut cultivars/selections. Cv= Cultivar; Y= Year; 01= 2006; 02= 2007; 03= 2008.

1	ACO 38853	5	Plant No. 10	9	Rattan Akhrot	13	Inder Akhrot	17	Plant No. 46
2	Blackmore	6	Netar Akhrot	10	Lake English	14	Plant No. 32	18	Plant No. 47
3	Gobind	7	Roopa Akhrot	11	Kandaghat Selection	15	Xenia	19	Solding Selection
4	Hartley	8	KX Giant	12	Payne	16	Plant No. 45	20	Luxmi Akhrot

Duration of pollen shedding
Overlapping of pollen shedding and stigma receptivity
Duration of stigma receptivity

Table 1 - Nature and degree of dichogamy in different walnut cultivars/selections

Cultivar/selection	First year		Second year		Third year	
	Nature	Degree	Nature	Degree	Nature	Degree
ACO 38853	Protandrous	100	Protandrous	100	Protandrous	100
Blackmore	Protandrous	100	Protandrous	100	Protandrous	100
Gobind	Homogamous	14.28	Protogynous	50	Homogamous	0
Hartley	Protandrous	100	Protandrous	100	Protandrous	100
Plant No. 10	Protandrous	100	Protandrous	83.33	Protandrous	83.33
Netar Akhrot	Protandrous	62.50	Slightly protandry	33.33	Protandrous	57.14
Roopa Akhrot	Protandrous	100	Protandrous	100	Protandrous	100
KX Giant	Slightly protandry	33.33	Homogamous	0	Homogamous	14.28
Rattan Akhrot	Protandrous	100	Protandrous	60	Protandrous	57.14
Lake English	Protandrous	100	Protandrous	100	Protandrous	100
Kandaghat Selection	Protandrous	71.43	Protandrous	100	Protandrous	100
Payne	Protandrous	80	Protandrous	60	Protandrous	50
Inder Akhrot	Protandrous	100	Protandrous	100	Protandrous	100
Plant No. 32	Slightly protandry	28.57	Protandrous	100	Protandrous	42.86
Xenia	Protandrous	83.33	Slightly protandry	33.33	Protandrous	50
Plant No. 45	Slightly protandry	16.67	Homogamous	0	Slightly protandry	14.28
Plant No. 46	Protandrous	66.67	Protandrous	100	Protandrous	71.42
Plant No. 47	Protandrous	100	Protandrous	100	Protandrous	100
Solding Selection	Protandrous	42.86	Protandrous	60	Protandrous	42.86
Luxmi Akhrot	Protandrous	100	Protandrous	100	Protandrous	100

20 French cultivars 16 protandrous, three protogynous and one homogamous. Among 100 cultivars studied by Yadrov (1982) the majority was protandrous (approximately 60%), while approximately 30% were protogynous and only 10 % were homogamous. Working on 10 cultivars of French and American origin Aleta and Ninot (1987) identified six absolutely protandrous cultivars, one protogynous and three partly homogamous. Korac *et al*. (1989) found among 14 cultivars, 11 protandrous, two protogynous and one partly homogamous. The occurrence of heterodichogamy in Persian walnut (i.e. protandry, protogyny and homogamy) as revealed here, is reported also by several other workers (Cheng, 1978; Radicati, *et al.,* 1983; Cerovic *et al.*, 1995).

The present investigation on the nature of dichogamy is primarily based upon the overlap between pollen shedding and stigma receptivity and not on the conventional method of classifying varieties on the basis of time of emergence of male and female flowers. The degree and nature of dichogamy is a varietal character, but it is also greatly affected by tree age, climate and geographic location (Wood, 1932).

Thus it is concluded that dichogamy is genotype-specific as shown from the considerable variation observed in the 20 cultivars/selections studied. To achieve optimum fruit set and consequent yield, near homogamous types ('Gobind' or 'KX Giant') may be planted in single cultivar walnut orchards, or a mixture of cultivars can be interplanted to allow sufficient overlap between pollen shedding and stigma receptivity. On the basis of the present study on protandrous-protogynous dimorphism, the mentioned possible pollinators in Table 2 are suggested as suitable cultivars/selections for growing in north-western India.

References

AKCA Y., SEN S.M., 1997 - *The relationship between dichogamy and yield-nut characteristics in* Juglans regia L. - Acta Horticulturae, 442: 215-216.

ALETA N., NINOT A., 1987 - *El Nogal. Un cultivo tradicional con futuro*. - Fruticultura professional, 11: 55-59.

BAUCKMANN M., 1974 - *The length of male flowers (catkins) on grafted walnut trees*. - Mitt. Rebe Wein, Obstbau Freuchterwert, 24: 463-466.

CEROVIC S., KORAC M., TODOROVIC J.N., 1995 - *Dichogamy in walnut (*Juglans regia L.*)*. - Jugoslovensko Vocarstvo, 29(3/4): 21-25.

CHENG W.C., 1978 - Juglans Regia L. - The Editorial Commission of Chinese Flora of Trees. The planting technology of the main forest trees in China, pp. 1342.

FORDE H.I., 1977 - *Walnuts*, pp. 439-455. - In: JANICK J. and J.N. MOORE (eds.). *Advances in fruit breeding*. Purdue University Press, West Lafayette Ind., USA.

GERMAIN E., JALINAT J., MARCHOU M., 1981 - *Divers aspects de la biologie florale de noyer*, pp. 13-27. - In: BERGOUGNOUX F., and P. GROSPIERRE(eds.) *Le Noyer*. INVUFLEC, Paris France.

GERMAIN E., JALINAT J., LEGLISE P., MASSERON A., TRONEL C., CHARTIER A., 1983 a - *Le noyer, resultants de 20 ans experimentation*. - Arb. Fruit., 356: 55-60.

Table 2 - Suitable pollinators for different walnut cultivars/selections

Main cultivars/selections	Suitable pollinators
ACO 38853	Plant No. 32
Blackmore	Gobind, Xenia
Gobind	Xenia, Plant No. 32
Hartley	Gobind, Xenia, Plant No. 32
Plant No. 10	Gobind, Xenia, Plant No. 32
Netar Akhrot	Roopa Akhrot, Inder Akhrot, Plant No. 45, Lake English
Roopa Akhrot	KX Giant, Plant No. 32, Plant No. 46
KX Giant	Kandaghat Selection, Plant No. 10
Rattan Akhrot	Plant No. 32, Plant No. 46
Kandaghat Selection	Plant No. 32, Xenia
Payne	Plant No. 32, Xenia
Inder Akhrot	Plant No. 32, KX Giant, Solding Selection
Plant No. 32	Gobind
Xenia	Gobind
Plant No. 45	Roopa Akhrot, Netar Akhrot
Plant No. 46	Gobind, Xenia
Plant No. 47	Solding Selection, Gobind, ACO 38853
Solding Selection	Plant No. 32, KX Giant
Luxmi Akhrot	Plant No. 32, Solding Selection, KX Giant, ACO 38853

GLEESON S.K., 1982 - *Heterodichogamy in walnuts. Inheritance and stable ratios*. - Evolution, 36(5): 892-902.

KORAC M., CEROVIC S., MITROVIAC M., KUZMANOVSKI I., JOVANEEVIAC D., SOLAR A., 1989- *Walnut production, population variability and breeding results achieved in Yugoslavia*. First Int. Symp. on Walnut Production, Budapest, Hungary, 25-29 Sept., pp. 9.

LUZA J.G., POLITO V.S., 1988 - *Microsporogenesis and anther differentiation in* Juglans regia L.*; a developmental basis for heterodichogamy in walnut*. - Bot. Gaz., 149(1): 30-36.

MAJACKAJA A.D., 1969 - *Dichogamy and fruiting in walnuts*. - Lesn. Hoz., 2: 32-35.

MANNING W.E., 1938 - *The morphology of the flowers of Juglandaceae. I. The inflorescence*. - American Journal of Botany, 25: 407-419.

McDANIEL J.C., 1957 - *The pollination of Juglandaceae varieties – Illinois observations and review of earlier studies*. - Nut Growers Assn. Annual Rep., 48: 89-93.

McGRANAHAN G.H., LESLIE C., 1991 - *Walnuts (*Juglans*)*, pp. 907-951. - In: BALLINGTON J.R., and J.N. MOORE(eds.) *Genetic resources of temperate fruit and nut crops*. ISHS Secretariat, The Netherlands, Vol. II, pp. 980.

RADICATI L., ME G., SACERDOTE S., VALLANIA R., 1983 - *Prime valutazioni di cultivars di noci Americana e francesi per le zone Montana*. - Frutticoltura, 45(3-4): 19-23.

SOLAR S., STAMPER F., SMOLE J., 1997 - *The degree of heterodichogamy of some walnut cultivars (*Juglans regia *L.) in Slovenia*. - Acta Horticulturae, 442: 217-224.

WOOD M.N., 1932 - *Dichogamy- An important factor affecting production in the Persian walnut*. - Proceedings of American Society for Horticultural Science, 34: 160-164.

YADROV, A.A. 1982. *Dichogamy and fruit production in walnut*. - Byulleten Gosudarstvennogo Nikitskogo Botanicheskogo Sada, 49: 68-72.

Adv. Hort. Sci., 2013 27(1-2): 67-72

Screening of herbicides for weed control, growth and yield of irrigated onion (*Allium cepa* L.) in tropical Savanna climate

D.I. Adekpe, J.A.Y. Shebayan, C.P. Shinggu, L. Aliyu

** Department of Agonomy, Faculty of Agriculture, Institute for Agricultural Research, Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria.*

Key words: herbicides, hoe weeding, onion, weed control.

Abstract: Field experiments were conducted during the 2006/2007 and 2007/2008 dry seasons at Sudan Savanna Nigeria to study the effects of herbicides on weeds and growth of irrigated onion (*Allium cepa* L.). The field was laid out in a randomized complete block design (RCBD) and the treatments included four rates (with and without supplementary hoe weeding - SHW) of oxadiazon 25 EC, butachlor 50 EC, pendimenthalin 50 EC, bullet 700 SC (atrazine + acetochlor + terbuthylazine), eight rates (with or without SHW) of oxyfluorfen 41 FW, hoe weeded at 3 and 6 weeks after transplanting (WAT) and untreated control. The treatments were replicated three times. The results indicated that application of oxadiazon 25 EC at 4 l/ha and oxyfluorfen 41FW at 6 l/ha both followed by SHW gave low weed dry weight, larger bulb diameter, higher mean bulb weight and bulb yield per hectare compared to the hoe-weeded control.

1. Introduction

Onion (*Allium Cepa* L.) belongs to the family Alliaceae in the genus *Allium* (Friensen, *et al*., 2006; Brewster, 2008). In Nigeria, onion is grown mainly for its bulb which is used in virtually every home on a daily basis to flavour and season a wide variety of dishes. As a constituent of a meal, both the green leaves and bulbs can be eaten raw or cooked in soups and salads. It is claimed to minimize high blood pressure and other heart diseases due to its favourable action on the elasticity of blood vessels. As an item of world trade, onion ranks second in importance after tomatoes among vegetables in Nigeria, where annual production stands at 621 000 t, making it the 36th highest producing country in the world. The genus *Allium* constitutes "bulb crops" and these are weak competitors with weeds which emerge among the crops (Boydston and Seymour, 2002). Weeds are known to reduce available moisture, nutrients, sunlight and growing space needed by crop plants. Subsequently, weeds can reduce growth, quality and yield of crops and make harvesting difficult. Onion seedlings are weak competitors with weeds because of their slow growth, small stature, shallow roots and thin canopy. In addition, their cylindrical upright leaves do not shade the soil enough to suppress weed growth. Weed interference was observed to be more devastating than insect (*Thrips tabaci*) infestations on onion dry bulb yield (Ghosheh and Al-Shannag, 2000). Herbicides may be applied before planting or after planting. Pre-plant application of a soil residual herbicide such as oxadiazon has proved effective for weed control in onion (Amrutkar *et al*., 2002). Oxyfluorfen and pendimenthalin were reported to have significantly reduced the weed population and increased onion yield to levels comparable to yields of weed control in a relay cabbage-onion cropping system (Sanjeev *et al.,* 2003). Onions are sown at densities that exceed 20 plants/m^2, and hand weeding operations are practiced with great care. On the other hand, high costs, lack of manpower and low economic returns are currently pushing onion farmers to consider herbicides as an alternative weed management option in onion production. The reliance on unstructured or non-sequenced weed management programs by farmers, especially in a country like Nigeria where agricultural commodities are still inexpensive, is also very common. Therefore, the objective of this study was to evaluate the efficacy of weed control using single herbicide applications. Although not all the herbicides used in this experiment were registered for onion production, producers might be tempted to use those available and at the lowest cost.

2. Materials and Methods

Field experiments were conducted during the 2006/2007 and 2007/2008 dry seasons at the Irrigation Research Station, Kadawa, of the Institute for Agricultural Research,

Corresponding author: idokodav@yahoo.com

Received for publication 18 February 2013
Accepted for publication 15 May 2013

Ahmadu Bello University, Zaria, Nigeria (11° 39'N, 08° 02'E and 500 m above sea level). Meteorological data on temperature, relative humidity and sunshine hours, as well as physical and chemical characteristics of the soil of the experimental site were collected December 2006-April 2007 and December 2007-April 2008 (Tables 1, 2 and 3).

Table 1 - Meteorological data showing mean minimum and maximum temperatures, relative humidity and sunshine hours during 2006/2007 dry season at Kadawa

Month	Days	Air temperature (°C)		Relative humidity (%)	Sunshine hours
		Min.	Max.		
December 2006	1-10	14.0	33.5	25.1	7.2
	11-20	13.2	32.6	27.2	7.4
	21-31	13.5	33.0	27.4	7.0
January 2007	1-10	11.7	31.6	28.3	6.8
	11-20	14.9	35.7	29.5	7.0
	21-31	13.4	34.3	29.8	6.1
February 2007	1-10	16.2	36.3	32.0	6.1
	11-20	19.7	35.9	34.1	5.8
	21-29	19.5	37.1	35.8	5.0
March 2007	1-10	19.2	35.8	37.1	6.2
	11-20	19.4	38.3	36.1	6.8
	21-3	20.4	41.4	36.7	5.7
April 2007	1-10	19.6	40.7	36.9	5.4
	11-20	18.6	39.1	37.5	5.6
	21-30	22.3	40.9	37.6	6.0
Total		255.6	546.2	491.1	95.1
Average		17.04	36.42	32.74	6.34

Source: Meteorological Unit of the Institute for Agricultural Research, Samaru, Zaria.

Land preparation involved ploughing, harrowing and ridging (75 cm apart). The plot size was 12 m^2 (4 x 3 m) representing the planting basin. Planting was done in rows at spacing of 15 x 30 cm within and between rows. The onion variety used, D77, has a maturity period of 120 to 130 days and potential yield of 5-15 t/ha. Plots were irrigated by surface flooding before transplanting a day after herbicide application. Subsequent irrigation was applied once a week by surface irrigation of the basin to field capacity, resulting in approximately 15-20 applications to supply about 350-550 mm of moisture as needed by the plant per growing season. In the two seasons, Single Super Phosphate (SSP) fertilizer at a rate of 19.8kg P/ha and Muriate of Potash (Potassium) at 10.4 kg K/ha were incorporated into the basin as basal fertilizer before transplanting. Nitrogen (45 kg N/ha) was applied by broadcasting

Table 2 - Meteorological data showing mean minimum and maximum temperatures, relative humidity and sunshine hours during 2007/2008 dry season at Kadawa

Month	Days	Air temperature (°C)		Relative humidity (%)	Sunshine hours
		Min	Max		
Dececember 2007	1-10	13.6	35.0	26.9	6.0
	11-20	14.0	33.3	23.4	6.4
	21-31	14.5	32.0	27.2	5.4
January 2008	1-10	15.1	34.3	27.9	5.0
	11-20	13.6	32.7	28.0	5.7
	21-31	NA	NA	30.1	6.0
February 2008	1-10	17.3	38.3	34.3	5.0
	11-20	15.6	34.7	30.8	4.5
	21-29	14.8	35.9	32.0	4.5
March 2008	1-10	15.7	34.5	29.9	5.0
	11-20	18.9	32.3	32.3	6.1
	21-31	23.3	39.4	36.3	6.8
April 2008	1-10	25.7	42.8	38.3	5.4
	11-20	23.9	41.1	36.9	6.0
	21-30	25.7	41.2	37.6	6.0
Total		251.7	507.4	471.9	83.8
Average		18.00	36.24	31.46	5.59

Source: Meteorological Unit of the Institute for Agricultural Research, Samaru, Zaria.
NA= not available.

Table 3 - Physico-chemical properties of the 0-20 cm depth of soil at the experimental site during the 2006/2007 and 2007/2008 dry seasons at Kadawa

Composition	Dry season 2006/2007	Dry season 2007/2008
Physical properties		
Sand (%)	56.0	58.0
Silt (%)	30.0	30.0
Day (%)	14.0	12.0
Textural class	Sandy-loam	Sandy-loam
Chemical properties		
pH in H_2O	5.70	5.80
pH in 0.01 m $cacl_2$(1:2.5)	5.10	5.32
Organic carbon (g/kg)	0.58	0.56
Total nitrogen (g/kg)	1.20	1.30
Available phosphorus	0.90	1.20
Exchangeable cations (cmd/kg soil)		
Calcium	1.88	0.60
Sodium	0.81	0.73
Potassium	0.21	0.37
Magnesium	3.13	2.97
C.E.C.	6.70	6.90

using urea (46%W) as split doses at two and six weeks after transplanting (WAT).

Treatments consisted of the following rates, each applied with and without supplementary hoe weeding (SHW) for a total of four treatments per herbicide: oxadiazon 25 EC (4 and 6 l/ha), butachlor 50 EC (5 and 6 l/ha), pendimenthalin 50 EC (5 and 6 l/ha) and bullet 700 SC 9 (1.04 and 1.4 l/ha) (atrazine + acetochlor + terbuthylazine), eight treatments (4, 6, 8 and10 l/ha with and without SHW) of oxyfluorfen 41 flowable (FW), and an untreated control; hoe weeding was carried out at 3 and 6 weeks after transplanting (WAT). These treatments were laid out in a randomized complete block design (RCBD) with three replications. All herbicides were applied a day before transplanting using a conventional CP3 knapsack sprayer with a green deflector nozzle at a pressure of 2.1 kg/m^2 to deliver a spray volume of 200 l/ha. Supplementary hoe weeding was imposed at 6 WAT on some plots as indicated in the treatment because some herbicides have short persistence in the soil and therefore cannot exhibit long season weed control. In the Nigerian savanna, despite high labour requirements and cost of inputs, some workers have emphasized the need to supplement pre-emergence herbicide treatments with hoe weeding for long season weed control for increased yields in various crops (Adigun *et al*., 1987; Magani, 2008; Oluwafemi, 2013). Onion bulbs were harvested manually at 16 WAT.

Data on weed dry weight, mean bulb diameter, bulb weight and bulb yield per hectare were assessed and subjected to statistical analysis of variance (ANOVA) as described by Snedecor and Cochran (1967). Differences between treatment means were compared using Duncan Multiple Range Test (DMRT) (Duncan, 1955) at 5% level of probability.

3. Results

Weather and soil conditions

Meteorological data on temperature, relative humidity and sunshine hours during the study periods (2006/2007 and 2007/2008) are shown in Tables 1 and 2 respectively, while physical and chemical characteristics of the soil of the experimental sites for the same period are presented in Table 3. The average maximum temperature was 36.42 and 36.24°C in 2006/2007 and 2007/2008 respectively, while for the same periods the relative humidity was 32.74 and 31.46% and sunshine hours were 6.34 and 5.59. With regard to the soil of the experimental sites, it was classified as sandy loam in both study periods, and the organic carbon, total nitrogen and available phosphorus were, respectively, 0.58, 1.20 g/kg-^{1} and 0.90 mg/kg-^{1} in 2006/2007 and 0.56, 1.30 g/kg-^{1} and 1.20 mg/kg-^{1} in 2007/2008.

Weed dry weight

The untreated control had significantly (P≤M0.05) higher weed dry weight than all the other treatments except for oxadiazon at 4 and 6 l/ha in 2006/2007 and for the combined mean (Table 4). The hoe-weeded control in the 2006/2007 study period resulted in significantly lower weed dry weight, comparable to the application of oxadiazon at 4 l/ha followed by supplementary hoe weeding (fbSHW), oxyfluarfen at 10 l/ha fbSHW, butachlor at 6 l/ha fbSHW and pendimenthalin at 5 l/ha fbSHW (Table 4). In 2007/2008, all the herbicides and their rates, including the untreated control, gave statistically comparable weed dry weight. The application of oxadiazon at 6 l fbSHW had significantly (P≤0.05) higher weed dry weight than butachlor at 6 l/ha and the hoe-weeded control which gave statistically similar weed dry weights (Table 4).

Table 4 - Effects of rates of herbicides on weed dry weight in onion during dry seasons of 2006/2007, 2007/2008 and combine at Kadawa

Herbicides	Rates (l/ha)	Weed dry weight (kg/ha)		Combined mean
		2006/2007	2007/2008	
Oxadiazon	4	101.70 a	17.47 a-c	59.58 ab
	4 fbSHW	34.60 l-g	11.23 a-c	22.93 ef
	6	82.07 a	15.90 a-c	49.00 a-c
	6 fbSHW	38.60 c-f	21.00 a	29.85 d-f
Oxyfluorfen	4	56.37 b-e	8.57 a-c	30.08 c-f
	4 fbSHW	41.8 c-f	13.96 a-c	27.85 d-f
	6	57.70 b-e	14.90 a-c	36.32 c-f
	6 fbSHW	38.10 c-f	15.10 a-c	28.60 d-f
	8	45.97 l-f	13.96 a-c	29.97 d-f
	8 fbSHW	49.59 b-c	9.60 a-c	29.55 d-f
	10	62.33 b-e	16.37 a-c	39.35 c-e
	10 fbSHW	33.90 d-g	17.7 a-c	25.80 d-f
Butachlor	5	45.57 c-f	14.30 a-c	29.93 d-f
	5 fbSHW	38.40 c-f	14.46 a-c	26.43 d-f
	6	48.37 b-f	6.13 bc	27.25 d-f
	6 fbSHW	21.27 fg	14.97 a-c	18.12 fg
Bullet	1.04	57.20 b-e	13.03 a-c	35.12 c-f
	1.04 fbSHW	42.60 c-f	10.53 a-c	26.57 d-f
	1.4	46.80 c-f	14.20 a-c	30.50 d-f
	1.4 fbSHW	29.17 e-g	17.70 a-c	23.43 e-f
Pendimenthalin	5	67.70 b-d	19.43 a-c	43.57 b-d
	5 fbSHW	33.47 d-g	21.30 a-c	27.40 d-f
	6	69.27 bc	20.20 a-c	44.73 a-d
	6 fbSHW	62.33 b-e	14.46 a-c	38.55 c-e
Hoe weeded	3 + 6WAT	3.70 g	5.00 c	6.13 g
Untreated check		109.03 a	12.66 a-c	60.85 a
SE(±)		10.203	3.847	5.533

Means followed by same letter(s) within a column are not significantly different at 5% (P=0.05) using DMRT.
fbSHW= Followed by supplementary hoe weeding.
WAT= Weeks after transplanting.

Mean bulb diameter

Table 5 shows mean bulb diameter for the herbicide treatments. The results reveal a larger bulb size in the first study season compared to the second season. The application of oxyfluorfen at 4 l/ha produced larger bulbs for all treatments in 2006/2007 and for the combined mean (2006/2007-2007/2008); the values were comparable with the hoe-weeded control in both cases. In 2007/2008, the use of oxadiazon at 6 l/ha fbSHW resulted in bigger bulbs compared with those produced by oxadiazon at 4 l/ha (with or without SHW), pendimethalin at 6 l/ha without SHW and the hoe-weeded control. Oxadiazon at 6 l/ha and the untreated control gave comparable but smaller onion bulbs for the combined mean (Table 5).

Table 5 - Effects of rates of herbicides on mean bulb diameters in onion during dry seasons of 2006/2007, 2007/2008 and combine at Kadawa

Herbicides	Rates (l/ha)	Mean bulb diameter (cm)		Combined mean
		Dry season 2006/2007	Dry season 2007/2008	
Oxadiazon	4	19.52 ab	9.49 b-d	14.51 a-d
	4 fbSHW	19.13 ab	9.36 b-d	14.64 a-d
	6	14.85 b-f	7.99 d	11.42 d
	6fbSHW	17.68 a-e	16.40 a	17.04 ab
Oxyfluorfen	4	21.60 a	12.88 a-d	17.24 a
	4 fbSHW	16.93 a-f	15.75 ab	16.17 a-c
	6	14.43 b-f	11.87 a-d	13.15 a-d
	6fbSHW	15.75 b-f	10.65 a-d	13.20 a-d
	8	13.37 ef	13.12 a-d	12.75 b-d
	8fbSHW	13.53 l-f	10.77 a-d	12.15 cd
	10	15.79 b-f	10.95 a-d	13.37 a-d
	10fbSHW	17.99 a-c	14.13 a -d	16.06 a-c
Butachlor	5	13.19 d-f	13.22 a-d	13.23 a-d
	5 fbSHW	17.37 a-f	12.25 a-d	14.81 a-d
	6	14.76 b-f	9.97 a-d	12.36cd
	6 fbSHW	17.55 a-f	14.18 a-d	15.87 a-c
Bullet	1.04	15.67 b-f	8.49 cd	12.06 cd
	1.04 fbSHW	19.54 ab	13.23 a-d	16.38 a-c
	1.4	16.92 a-f	12.43 a-d	14.67 a-d
	1.4 fbSHW	16.60 a-f	13.00 a-d	14.80 a-d
Pendimenthalin	5	16.52 a-f	11.15 a-d	13.84 a-d
	5 fbSHW	19.15 a-c	14.64 a-c	16.90 ab
	6	17.02 a-f	9.76 b-d	13.34 a-d
	6 fbSHW	18.08 a-c	12.19 a-d	15.14 a-d
Hoe weeded	3 + 6WAT	18.96 a-d	9.78 b-d	14.37 a-d
Untreated check		11.87 f	10.05 a-d	10.90 d
SE (±)		1.673	1.903	1.269

Means followed by same letter(s) within a column are not significantly different at 5% (P=0.05) using DMRT.
fbSHW= Followed by supplementary hoe weeding.
WAT= Weeks after transplanting.

Mean bulb weight

Mean bulb weight appeared higher in the 2006/2007 dry season compared with the following year (Table 6). In the former, hoe weeded control yielded a significantly ($P \leq 0.05$) higher mean bulb weight, compared with oxadiazon at 4 l/ha (with or without SHW) and bullet (atrazine + acetochlor + terbuthylazine) at 1.04 l/ha fbSHW. The untreated control gave the lowest mean bulb weight, although it was comparable to some herbicide treatments.

Table 6 - Effects of rates of herbicides on mean bulb weight of onion during dry seasons of 2006/2007, 2007/2008 and combine at Kadawa

Herbicides	Rates (l/ha)	Mean bulb weight (g)		Combined mean
		Dry season 2006/2007	Dry season 2007/2008	
Oxadiazon	4	77.48 a-c	20.72 c	49.10 a-f
	4 fbSHW	86.03 ab	35.63 a-c	60.83 a-d
	6	48.34 c-h	19.38 c	33.89 fg
	6fbSHW	68.26 b-e	71.03 a	69.65 a
Oxyfluorfen	4	33.51 f-h	43.41 a-c	38.46 c-g
	4 fbSHW	58.97 b-g	64.45 ab	61.71 a-c
	6	37.38 e-h	33.08 a-c	35.23 fg
	6fbSHW	50.88 c-h	31.59 bc	41.23 c-g
	8	27.65 gh	43.70 a-c	35.68 e-g
	8fbSHW	35.77 f-h	27.61 bc	31.69 fg
	10	52.89 c-h	25.65 c	39.27 c-g
	10fbSHW	62.05 b-f	50.05 a-c	56.05 a-f
Butachlor	5	41.59 d-h	45.48 a-c	43.53 b-g
	5 fbSHW	48.60 c-h	48.27 a-c	48.44 a-f
	6	43.57 d-h	36.86 a-c	40.21 c-g
	6 fbSHW	47.36 c-h	45.87 a-c	46.62 a-f
Bullet	1.04	50.18 c-h	21.95 c	36.06 d-g
	1.04 fbSHW	77.25 a-c	43.19 a-c	60.22 a-e
	1.4	44.89 d-h	37.96 a-c	41.43 b-g
	1.4 fbSHW	68.58 b-e	38.24 a-c	53.41 a-f
Pendimenthalin	5	55.15 c-g	24.67 c	39.91 c-g
	5 fbSHW	52.96 c-h	55.03 a-c	53.00 a-f
	6	60.68 b-f	22.81 c	43.75 b-g
	6 fbSHW	69.19 b-d	33.68 a-c	51.44 a-f
Hoe weeded	3 + 6WAT	103.06 a	28.46 bc	65.77 ab
Untreated check		22.87 h	20.03 c	21.45 g
SE (±)		9.126	11.171	7.189

Means followed by same letter(s) within a column are not significantly different at 5% (P=0.05) using DMRT.
fbSHW= Followed by supplementary hoe weeding.
WAT= Weeks after transplanting.

The application of oxadiazon at 6 l/ha followed by supplementary hoe weeding (fbSHW) in 2007/2008 and combined mean resulted in the highest mean bulb weight, but it was comparable to oxadiazon at 4 l/ha (with and without

supplementary hoe weeding), oxyfluorfen at 4 and 10 l/ha fbSHW, pendimenthalin at 5 and 6 l/ha fbSHW and the hoe-weeded control (Table 6). The untreated control consistently maintained the lowest mean bulb weight in both years of study and in the combine mean.

Onion bulb yield

In 2006/2007, the use of oxadiazon at 4 l/ha fbSHW, oxyfluorfen at 6 l/ha and pendimentalin at 5 and 6 l/ha fbSHW resulted in statistically similar but significantly (P≤0.05) higher bulb yield compared with oxyfluorfen at 4, 8 and 10 l/ha and the untreated control. The differences in bulb yield between all other herbicides and their rates including the untreated control were not significant (P≤0.05) statistically (Table 7). The effect of the herbicides and their rates on onion bulb yield was not significant in 2007/2008. The application of pendimenthalin at 5 l/ha fbSHW resulted in significantly (P≤0.05) higher bulb yield compared with oxyfluorfen at 8 and 10 l/ha and the untreated control for the combined mean. All the other herbicides and their rates gave statistically similar bulb yields (Table 7).

Table 7 - Effects of rates of herbicides on onion bulb yield during dry seasons of 2006/2007, 2007/2008 and combine at Kadawa

Herbicides	Rates (l/ha)	Onion bulb yield (t/ha)		Combined mean
		Dry season 2006/2007	Dry season 2007/2008	
Oxadiazon	4	6.00 a-c	0.73	3.37 a-d
	4 fbSHW	6.93 a	0.83	3.88 a-c
	6	3.78 a-d	0.66	2.22 a-d
	6fbSHW	4.30 a-d	2.66	3.44 a-d
Oxyfluorfen	4	2.17 b-d	2.27	2.22 a-d
	4 fbSHW	3.06 a-d	2.60	2.83 a-d
	6	7.05 a	1.13	4.09 ab
	6fbSHW	3.90 a-d	0.70	2.30 a-d
	8	1.78 cd	2.13	1.96 b-d
	8fbSHW	3.68 a-d	0.93	2.30 a-d
	10	2.05 cd	0.88	1.46 cd
	10fbSHW	5.22 a-d	1.90	3.58 a-c
Butachlor	5	3.22 a-d	2.07	2.65 a-d
	5 fbSHW	5.05 a-d	2.13	3.59 a-c
	6	3.22 a-d	1.90	2.58 a-d
	6 fbSHW	3.58 a-d	1.40	2.48 a-d
Bullet	1.04	3.45 a-d	0.92	2.19 a-d
	1.04 fbSHW	5.39 a-d	1.20	3.30 a-d
	1.4	2.83 a-d	2.13	2.48 a-d
	1.4 fbSHW	4.22 a-d	1.00	2.61 a-d
Pendimenthalin	5	4.61 a-d	0.47	2.54 a-d
	5 fbSHW	6.56 a	2.93	4.75 a
	6	4.00 a-d	0.53	2.27 a-d
	6 fbSHW	6.92 a	1.20	4.06 ab
Hoe weeded	3 + 6WAT	5.78 a-c	0.47	3.12 a-d
Untreated check		1.22 d	0.73	0.98 d
SE (±)		1.279	0.762	0.737

Means followed by same letter(s) within a column are not significantly different at 5% (P=0.05) using DMRT.
fbSHW= Followed by supplementary hoe weeding.
WAT= Weeks after transplanting.

4. Discussion and Conclusions

Weed dry weights were very high in the untreated control compared to the herbicide-treated plots in 2006/2007 and combined means, except for oxadiazon at 4 and 6 l/ha without fbSHW. This could be due to a higher weed intensity resulting from no hoe weeding and herbicide treatment. Lower weed dry weights and herbicide suppression of weeds in crops has been demonstrated in many studies (Ibrahim, 2001; Ishaya, 2004; Adekpe *et al*., 2007).

The statistically similar weed dry weight found in the present study for hoe weeded control and oxadiazon at 4 l/ha fbSHW, oxyfluorfen at 10 l/ha fbSHW, butachlor at 6 l/ha fbSHW and pendimnthalin (5 l/ha fbSHW) demonstrates herbicide competitiveness with conventional hoe weeding in weed management practices. Similar observations have been made in other related studies (Adekpe *et al*., 2007; Shinggu *et al*, 2009; Oluwafemi, 2013). Bulb size was higher with the application oxyfluorfen at 4 l/ha compared to to hoe-weeded control in both 2006/2007 and combined data. Numerical differences in bulb size (diameter) were observed and in some cases not statistically significant, reflecting the within treatment variability encountered in the study. The use of oxyfluorfen has been reported to increased onion performance (Ghosheh, 2004)

Onion mean bulb weight and bulb yield (t/ha) were observed to be better with oxyfluorfen (6 l/ha fbSHW) and pendimenthalin at 5 and 6 l/ha fbSHW. This observation may not be unconnected with the observed favourable weed control, higher bulb diameter influenced by these herbicides. The weed control must have helped in the reduction of competition for both the above and underground growth factors by the weeds, thereby providing an adequate environment for the growth and bulb yield of onion. The high total nitrogen and available phosphorus found in the soil of the experimental sites might have contributed to high bulb yield in these low weed-infested plots. The use of oxyfluorfen, oxadiazon and pendimethalim has been reported to improve performance in some bulb and root vegetable crops (Amrutkar *et al*., 2002; Sanjeev *et al*., 2003; Adekpe *et al*., 2007; Adekpe *et al*., 2012).

It can be concluded from the present study that the use of oxadiazon at 4 and 6 l/ha fbSHW and oxyfluorfen at 6 l/ha fbSHW gives the lowest weed dry weight, higher bulb diameter, higher mean bulb weight and bulb yield per hectare compared to the hoe-weeded control.

Acknowledgements

The authors wish to acknowledge the permission of the Director, Institute for Agricultural Research, Ahmadu Bello University, Zaria to use its facilities in carrying out this work.

References

ADEKPE D.I., ESSIEN J.E., SHEBAYAN J.A.Y., ISHAYA D.B., SHINGGU C.P., 2012 - *Evaluation of herbicides for weed management in irrigated carrot (*Daucus carota *L.) at Samaru, Zaria, Nigeria*. - Conference of the Weed Science Society of Nigeria (WSSN), Ahmadu Bello University, Zaria, 18-22 November.

ADEKPE D.I., SHEBAYAN J.A.Y., CHIEZEY U.F., MIKE S., TUNKU P., 2007 - *Effects of weed control, date of planting and intra-row spacing on weeds and bulb yield of garlic (*Allium sativum *L.) at Kadawa, Nigeria*. - Adv. Hort. Sci., 21(3): 165-171.

ADIGUN J.A, LAGOKE S.T.O., KARIKARI S.K., 1987 - *Herbicides evaluation studies in transplanted chilli pepper (*Capsicum frutescens *L.) in the Nigerian Savanna*. - Crop Protection, 6(4): 15-19.

AMRUTKAR S.D., PATIL B.M., KARUNAKAR A.P., JIOTODE D.J., 2002 - *Effect of various herbicides on yield and uptake of nutrients in onion (*Allium cepa *L.).* - Res. Crops, 3: 659-661.

BOYDSTON R.A., SEYMOUR M.D., 2002 - *Volunteer potato (*Solanum tuberosum *L.) control with herbicides and cultivation in onion (*Allium cepa *L.).* - Weed Technology, 16: 620-626.

BREWSTER J.L., 2008 - *Onions and other vegetable Allium.* - 2nd Edition, Bddles Ltd, King's Lynn, UK, pp. 432.

DUNCAN D.B., 1955 - *Duncan multiple range and multiple F. test*. - Biometris, 11: 1-42.

FRIENSEN N., FRITSCH R., BLATTNER F.R., 2006 - *Phylogeny and new intragenetic classification of* Allium (*Alliaceae) based on nuclear ribosomal DNA and Its sequences*, pp. 372-395. - In: COLUMBUS J.T. (ed.) *Monocots III*. - Proceedings of the Third International Conference on the Comparative Biology of Monocotyledons, Claremart, California, USA.

GHOSHEH H.Z., 2004 - *Single herbicide treatments for control of broadleaved weeds in onion (*Allium cepa *L.).* - Crop protection, 23: 539-542.

GHOSHEH H.Z., AL-SHANNAG H.K., 2000 - *Influence of weeds and onion thrips,* Thrips tabaci *(thysanoptera) on onion bulb yield in Jordan*. - Crop Protection, 19: 175-179.

IBRAHIM. D.D., 2001 - *Effects of rates of nitrogen, method of weed control and intra-row spacing on the growth and yield of irrigated paper (*Capsicum annuum *L.) at Samaru - Zaria, Nigeria*. - M.Sc Seminar Pepper, Department of Agronomy, Ahmadu Bello University, Zaria, p. 28.

ISHAYA D.B., 2004 - *Evaluation of weed control treatment and rice varieties on weeds, growth, and yield of rice/sorghum mixture*. - PhD Seminar Paper, Department of Agronomoy, Ahmadu Bello University, Zaria, pp. 34.

MAGANI I.E., 2008 - *Weed control in Sorghum - Groundnut mixture in the simultaneous farming system of Southern Guinea Savanna Zone of Nigeria*. - Journal of Animal and Plant Sciences, 1(1): 3-8.

OLUWAFEMI A.B., 2013 - *Evaluation of weed management strategies in cocoyam (*Colocasia esculentus *(L.) Schott) production in Ado-Ekiti, Ekiti State, Nigeria*. - International Research Journal of Agricultural Science and Soil Science, 3(2): 38-42.

SANJEEV A, SANDHU K.S., AHUJA S., 2003 - *Weed management through the use of herbicides in cabbage - onion relay cropping system*. - Annal of Biology, 19: 27-30.

SHINGGU C.P., DADARI S.A., SHEBAYAN J.A.Y., ADEKPE D.I., ISHAYA D.B., 2009 - *Effects of variety, crop arrangement and period of weed interference on the performance of maize grown in mixture in Northern Guinea Savannah of Nigeria*. - ARPN Jounrnal of Agricultural and Biological Science, 4(2): 47-51.

SNEDECOR G.W., COCHRAN W.G., 1967 - *Statistical Methods*. - 6th Edition, IOWA State University Press, USA.

Adv. Hort. Sci., 2013 27(1-2): 73-80

Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giombo) *D. kaki* Thunb. when stored under refrigerated conditions

M.R. De Moraes*, É.R. Daiuto*, R.L. Vietes*, N.C. Cardoso*, R.E. Smith (1)**
* *Faculty of Agronomic Sciences, UNESP Botucatu, C.P. 237, Botucatu 18610307, SP, Brazil.*
** *U.S. FDA, 11510 W 80st, Lenexa, KS 66224, USA.*

Key words: Caqui Giombo, *Diospyros kaki* L., firmness, persimmon, postharvest.

Abstract: The effects of active modified atmospheres were evaluated in 'Giombo' persimmons with tannins already removed (non-astringent) and stored at 0ºC and 85-90% relative humidity for 35 days. The goal was to maintain quality and delay ripening. The fruits were picked by hand when they were about 50% green, sanitized and subjected to different mixtures of CO_2 and O_2. Fruits were wrapped in thin film plastic made of nylon + polyethylene and analyzed every seven days for weight loss, respiratory activity, coloration, titratable acidity, soluble solids, ratio, pH, firmness, pectin methyl esterase and polygalacturonase enzyme activities, reducing sugars, ascorbic acid and astringency index. Refrigerated storage and active modified atmospheres were effective in conserving the quality of 'Giombo' persimmons. The fruits submitted to the highest CO_2 concentrations (7 and 8%) had the lowest weight loss and respiratory intensity with a delay in the climacteric peak.

1. Introduction

Persimmons are the edible fruits of deciduous trees in the genus *Diospyros*, many of which originated in China (Martínez-Calvo *et al*., 2012), even though some species are native to other parts of the world, including *D. lotus*, or date plum, which is native to southwest Asia and southeast Europe. The fruit was called "fire of the gods", or *Dios pyros* by the ancient Greeks, thus the name of the genus. Persimmons produce popular and nutritious fruits that have become traditional crops in Korea and Japan and are found throughout the world (Veberic *et al.,* 2010; Dembitsky *et al*., 2011; Giordani *et al.,* 2011). They are sweet and are relatively good sources of carotenoids, vitamin C, polyphenols and proanthocyanidins (Gu *et al*., 2008; Dembitsky *et al*., 2011; Giordani *et al*., 2011). Moreover, persimmons have been reported to have health benefits in both traditional and western medicine (Giordani *et al*., 2011). For example, persimmons of the Triumph variety improved lipid metabolism and atherosclerosis indices in rats that were fed a diet that was high in cholesterol (Gorinstein *et al*., 1998; 2011).

The most cultivated species is the *D. kaki* Thunb. (Martínez-Calvo *et al*., 2012; USDA, 2012), also known as Chinese persimmon, Japanese persimmon, kaki, caqui and *Diospyros kaki* L. (Martínez-Calvo *et al*., 2012; USDA, 2012). There is also a species (*D. virginiana*) that is native to eastern North America (Celik and Ercisli, 2008). Persimmons are climacteric fruits (Veberic *et al*., 2010). That is, the amount of sugars (particularly sucrose) and total carotenoids increase in the final stages of ripeness and firmness, while soluble tannins and titratable acidity levels decrease, resulting in improved flavor (Candir *et al*., 2009). In the northern hemisphere, they are harvested between September and December (Dembitsky *et al*., 2011). On the other hand, the Brazilian caqui cultivar called 'Giombo' is very productive and matures late, with fruits being picked from March to the end of May (Martins and Pereira, 1989). This relatively short harvest season, coupled with the lack of information about storage, limits its expansion and causes losses in the final processing and marketing of the fruit (Donazzolo and Brackman, 2002).

Persimmon cultivars are classified into four groups: pollination-constant astringent, pollination-variant astringent, pollination-constant non-astringent, and pollination-variant non-astringent (Campo-Dall'Orto *et al*., 1996; Celik and Ercisli, 2008). Persimmons have also been divided into a volatile-independent group (VIG, corresponding to the pollination-constant non-astringent group) and the volatile dependent group (VDG, consisting of the pollination-constant astringent, pollination-variant astringent and pollination variant non-astringent types) (Giordani *et al*., 2011). Tannins in the VIG type are usually

(1) Corresponding author: robert.smith@fda.hhs.gov

Received for publication 13 March 2013
Accepted for publication 25 May 2013

relatively high in molecular weight and soluble in water. Moreover, their concentrations are maximum at an early stage of development and are <1% of the fresh weight. On the other hand, the VDG types contain tannins that are usually soluble in water, have a lower molecular weight and are not palatable at harvesting time. The seeds of pollination-variant non-astringent cultivars can exude ethanol, which makes the water-soluble tannins insoluble (Giordani *et al*., 2011). All persimmons are edible when soft, but they can be astringent at harvest time (Giordani *et al*., 2011). Parthenocarpic fruits of pollination-variant non-astringent cultivars, and both seeded and parthenocarpic fruits of astringent cultivars are edible only after removing the astringency artificially or when soft, overripe or dried. This happens when low molecular weight, soluble tannins are made water-insoluble, probably by binding with pectins (Giordani *et al*., 2011). Astringency can be removed by storing persimmons in a modified atmosphere containing elevated amounts of CO_2 or ethanol (Del Bubba *et al*., 2009; Edagi *et al*., 2009) and refrigeration can delay the ripening of persimmons picked when only half-ripe (Vieites *et al*., 2012).

The heart-shaped 'Hachiya' cultivar is the most popular pollination-constant astringent persimmon, while the 'Fuyu' is a popular pollination-constant non-astringent cultivar in Japan (Celik and Ercisli, 2008).

The Brazilian 'Giombo' cultivar is in the variable denomination which includes fruits that are yellow and contain tannins. Seedless fruits keep their astringency even when ripe, so the tannins must be removed artificially. The biggest inconvenience in accelerating the ripening process to remove tannins is that it diminishes the shelf life (Edagi *et al*., 2009). According to Antoniolli *et al.* (2000), this can compromise the firmness of the pulp when stored for a long period.

Refrigerated storage is among the practices used to maintain the quality of fruits for a short length of time (Vieites *et al*., 2012); it can prolong the useful storage time, but the majority of workers show that it should not exceed 35 days to remain safe (Ben-Aire and Zutkhi, 1992; Brackmann and Saquet, 1995; Chitarra and Chitarra, 2005).

Other methods have been tested to extend the shelf life of fruits, with modified atmospheres standing out. Also plastic films can increase CO_2 and decrease O_2 however the concentrations of these gases are not controlled and vary with time, temperature, type of plastic and respiratory rate (Sargent *et al*., 1993). According to Ferri *et al*. (2004) storing 'Fuyu' persimmons at 0ºC maintains the firmness of the pulp for 90 days, but when only using refrigerated storage the shelf life is less than 30 days.

Another approach is to use a modified active atmosphere in which the initial concentration of gases inside the packaging is controlled. 'Giombo' persimmons have not yet been tested under these conditions, therefore the objective of this study was to test the effects of a modified active atmosphere on the cold storage of 'Giombo' persimmons that have had the tannins removed, making them non-astringent.

2. Materials and Methods

'Giombo' persimmons were from the Sacramento Agropastoril Ltda, Avaré (SP), located at a latitude of 23°05'56"S, longitude 48º55'33"W and altitude of 780 m, with an annual precipitation of 1500-1700 mm yr^{-1}, annual temperature between 20 and 24°C and soil classified as purple oxysoil (structured earth, purple, oxidized). The fruits were collected by hand when they were at stage 3 of maturity, medium-ripe, about 50% green. To remove the tannins, fruits were collected in plastic boxes and exposed to ethanol fumes at a concentration of 6.6 ml kg^{-1}, in chambers at 25°C for 48 h. Fruits were then submitted to the following gas mixtures: 0.03% CO_2 and 21% O_2 (T1= control; 5% CO_2 and 4% O_2 (T2); 6% CO_2 and 4% O_2 (T3); 7% CO_2 and 4% O_2 (T4) and 8% CO_2 and 4% O_2 (T5). The fruits were wrapped in plastic wrappers made of nylon and polyethylene and stored refrigerated at 0±0.5ºC and 85-90±5% relative humidity for 35 days, and were analyzed every seven days. For the control group (non-destructive), two whole fruits were analyzed five times and for the destructive group, two fruits were cut into pieces and then analyzed in triplicate.

Loss of mass (%)

The weight of fruits was measured with an analytical balance and the results expressed as a percentage.

Respiratory activity

The liberation of CO_2 was measured following the method of Bleinroth *et al*. (1976), using a saturated solution of barium hydroxide and 0.1 N potassium hydroxide. The respiratory rate was calculated using the equation:

$TCO_2 = 2.2\ (Vo-V1).\ 10/P.T.$

where T CO_2 = respiratory rate (ml of CO_2. $kg^{-1}.h^{-1}$);
Vo= volume of HCl needed to titrate the potassium hydroxide solution before and V1, after absorbing CO_2 (ml);
P= mass of the fruits; T = time of respiration;
2.2= equivalent weight of CO_2 (44/2), multiplied by the concentration of HCl;
10= adjustment for the total amount of KOH used.
Analyses were carried out in triplicate.

Titratable acidity

Expressed as gram equivalents of malic acid per 100 g of pulp (g of malic acid 100 g^{-1}), obtained by titrating 5 g of homogenized pulp diluted to 100 ml with distilled water with 0.1 N NaOH, using a phenolphthalein indicator, in conformance with Odair *et al*. (2008).

Soluble solids

Made using a Palette ATAGO PR-32 refractometer and expressed as (ºBrix), in conformance with Odair *et al.* (2008).

Maturity index (ratio)

Determined from the ratio of soluble solids to titratable acidity (2008).

pH

Measured using a model 300 pH meter in conformance with Odair *et al*. (2008).

Sugars

Measured using the methods of Somogy (1945) and Nelson (1944) and a Micronal B 382 spectrophotometer to measure the absorbance at 535 nm.

Ascorbic acid

Determined by adding 30 ml of 4% oxalic acid to 30 g of pulp and titrating with 0.5% DPI-2,6-diclorophenolindophenol with results expressed as ml of ascorbic acid 100 ml^{-1} of pulp (MAPA, 2011).

Astringency index

Determined using the method of Gazit and Levy (1963) and modified by Vitti (2009) in which one of the sides of the cut fruit is placed on a piece of filter paper that has been impregnated with a solution of 5% $FeCl_3$. Soluble tannins react and turn the paper dark, which is then analyzed visually on a scale of 1 to 5, with 5 being the darkest and most astringent.

Coloration

Measured in a Konica Minolta (Chroma meter, CR 400/410) colorimeter over the spectral region of 380 to 780 nm. The reflectance reading was obtained with an angle of observation of 2° and illumination C. The color was expressed by a system of rectangular coordinates: L* a* and b* in conformance with the CIE (*Comission Internatinale de E'clairage*), where L* is the percent luminosity (0% = black and 100% = white), a* represents the colors red (+) or green (-) and b* the colors yellow (+) or blue (-).

Firmness

Measured using a Texture Analyzer (Stevens – LFRA texture analyzer) with a penetration distance of 10 mm and a velocity of 2.0 mm sec^{-1}, and using a TA 9/1000 fixture and a pressure of 15 gram of force per cm^2 ($gf\ cm^{-2}$).

Enzyme activity

Activities of polygalacturonase (PG) and pectin methylesterase (PME) were determined by the methods of Albershein *et al*. (1967) and Ahmed and Labavitch (1980).

The Tukey test at a 5% probability level was used to compare results, as recommended by Gomes (2000) and by linear regression analysis for weight loss.

3. Results and Discussion

The respiratory activity of 'Giombo' persimmon fruits increased during the storage periods, as shown in Figure 1. Concomitantly, there was a steady weight loss, depending on the treatment (Table 1). The fruits exposed to 7 and 8% CO_2 showed less weight loss than the other treatments. In order to have acceptable surface shrinkage for fresh fruits (Finger and Vieira, 2002) the maximum tolerated weight loss should be between 5 and 10%, thus the weight losses of 'Giombo' persimmons found in this study are acceptable.

It was also observed that the respiratory rate was less at the highest concentrations of CO_2 (6, 7 and 8%) compared

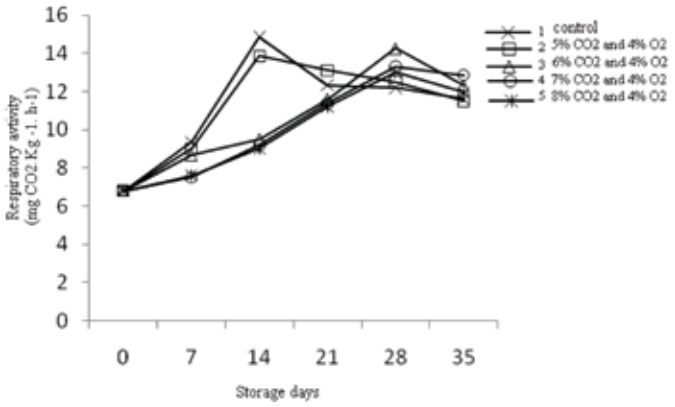


Fig. 1 - Respiratory activity (mg CO_2.Kg^{-1}. h^{-1}) of 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0ºC, 85-90% relative humidity for 35 days at different concentrations.

Table 1 - Weight loss (%) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	control	Treatment				
		5%CO_2 4%O_2	6%CO_2 4%O_2	7%CO_2 4%O_2	8%CO_2 4%O_2	Averages
7	0.105±0.024 Aa	0.236±0.189 Ca	0.136±0.184 Aa	0.038±0.011 Aa	0.024±0.093 Aa	0.108±0.028
14	0.144±0.09 Aa	0.323±0.127 a	0.216±0.112 Aa	0.098±0.019 Aa	0.093±0.047 Aa	0.175±0.053
21	0.186±0.104 Aa	0.353±0.226 Ca	0.261±0.108 Aa	0.123±0.026 Aa	0.118±0.059 Aa	0.208±0.155
28	0.190±0.023 Ab	0.695±0.068 Ba	0.270±0.120 Ab	0.143±0.046 Ab	0.130±0.068 Ab	0.286±0.141
35	0.268±0.119 Ab	1.041±0.262 Aa	0.355±0.220 Ab	0.221±0,017 Ab	0.222±0.063 Ab	0.421±0.353
Averages	0.149±0.115	0.441±0.281	0.206±0.098	0.104±0.057	0.098±0.015	

Small letters compare averages of different treatments on each day.
Upper case letters compare averages between different days.
Averages followed by at least one letter in common do not differ statistically.

to fruits exposed to 5% CO_2 and 4% O_2 which reached a peak on the 14th day of storage. The fruits exposed to 6, 7 and 8% CO_2 had maximum respiratory activity after 28 days. The elevated concentrations of CO_2 inhibited the respiratory activity of the fruits.

The color of the fruits was not statistically different in fruits treated differently, as shown in Tables 2-4. No darkening was seen on the surface of the fruits. The values of a* in the apical region of the fruits was less than that of the median or basal regions. Negative a* values were found only on the first day, indicating the presence of a green color and verifying that the fruits did ripen during storage. This is in agreement with data reported by Chitarra and Chitarra (2005) who reported that the change in color is associated with ripening, which is a standard attribute for determining fruit quality. The increase in a* and b* color indices each reflected changes from yellowish-green to orangish-red.

Brackmann *et al*. (1997) reported that persimmons stored refrigerated at 5ºC did not show an appreciable change in color since low temperatures inhibit the biosynthesis of carotenoids. The differences in color seen in the present study were due to differences at the time of collection. Danieli *et al*. (2002) reported that 'Fuyu' persimmons that were collected when still yellowish-green eventually changed to red. Still, there were no such changes in the treatments in the current study. This is commercially important since coloration is a primary quality standard.

The amounts of soluble solids, titratable acidity and the ratio between the two are reported in Table 5, which shows that there is no significant change, regardless of dose of CO_2 and storage time.

Working with the same cultivar, Antoniolli *et al.* (2000) found that there was little change in the amount of soluble solids, as confirmed also by the current study. Murray and Valentini (1998) reported that limits in the precision of the method and the many factors that affect soluble solids make it difficult at times to establish interactions between the process of maturation and the content of soluble solids. Thus, the amount of soluble solids serves best as a standard of quality rather than an index that measures the effects of storage.

The present study also verified that there is a slow increase in the titratable acidity of the fruits during storage, with values ranging from 0.07 to 0.10 grams equivalents of malic acid per 100 g of pulp. According to Costa and Balbino (2002), the increase in titratable acidity is due to the formation of galacturonic acid during the process of breaking down cell walls, which occurs during fruit storage. The ratios of soluble solids to titratable acidity varied little during storage.

The pH of the samples oscillated between 5.49 and 5.87, as shown in Table 6. This is similar to the results reported by Blum *et al*. (2008) who found no change in pH or acidity in 'Giombo' persimmons that were covered with carnuba wax during cold storage. Therefore, the modified atmospheres used in the current study did not affect the pH very much.

One of the main concerns about storage is the rapid loss of firmness of the pulp, which makes fruits commercially unacceptable. Thus, the fact that the present study shows only small variations in firmness, as shown in Table 7, is important, and can be explained as a combination of cold storage in a modified atmosphere, each of which decrease metabolism and prolong shelf life.

Table 2 - Luminosity (%) of caquis 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Days of storage	Luminosity		
	Reg. apical	Reg. mediana	Reg. basal
0	45.3 ab	48.9 ab	46.6 a
7	44.3 b	48.4 b	45.6 ab
14	44.8 b	48.2 b	45.2 ab
21	45.3 ab	48.8 ab	44.7 b
28	46.2 a	49.3 a	45.2 ab
35	45.5 ab	49.3 a	46.0 ab

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 3 - Color a* in 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	Color a*		
	Reg. apical	Reg. media	Reg. basal
0	-0.1 d	0.5 d	1.4 d
7	2.5 cd	3.0 c	7.5 c
14	2.8 cd	3.1 c	7.3 c
21	6.7 b	9.8 b	11.8 b
28	12.5 a	15.8 ab	15.8 ab
35	15.6 a	18.9 a	18.3 a

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 4 - Color b* in 'Giombo' persimmons with tannins removed, submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Storage days	Color b*		
	Apical	Medium	Basal
0	34.5 a	39.0 a	32.9 a
7	31.2 b	37.7 abc	32.1 a
14	32.6 b	36.7 bc	31.9 a
21	29.0 c	35.0 c	26.7 b
28	32.6 b	37.4 bc	28.1 b
35	32.1 b	38.9 abc	32.3 a

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 5 - Amounts of soluble solids (ºBrix), titratable acidity (g malic acid per 100 g of pulp) and ratio in 'Giombo' persimmons with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatments	Soluble Solids (SS)						Averages
	Storage days						
	0	7	14	21	28	35	
Control	17±0.3	15.7±0.2	16.9±1.8	17.4±1.5	14.4±1.9	17.4±0.5	16.5±1.6
5%CO_2 4%O_2	17±0.3	16.5±0.9	15.8±0.9	16.6±2.0	15.7±1.0	17.0±0.8	16.5±1.1
6%CO_2 4%O_2	17±0.3	16.8±0.2	15.9±0.9	16.4±0.7	15.3±0.5	15.6±0.2	16.2±0.8
7%CO_2 4%O_2	17±0.3	18.1±1.0	16.8±0.7	16.8±0.3	15.2±0.3	17.0±0.8	16.8±1.0
8%CO_2 4%O_2	17±0.3	15.8±0.6	16.9±0.4	16.9±1.6	15.4±1.0	16.1±0.6	16.4±1.0
Average	17A±0.3	16.6A±1.1	16.5A±1.03	16.8A±1.2	15.2B±1.0	16.6A±0.9	
	Titratable Acidity (TA)						
Control	0.07±0.01	0.09±0.01	0.07±0.01	0.08±0.05	0.08±0.03	0.08±0.01	0.08±0.02
5%CO_2 4%O_2	0.07±0.01	0.06±0.01	0.08±0.01	0.08±0.05	0.10±0.04	0.09±0.01	0.08±0.03
6%CO_2 4%O_2	0.07±0.01	0.08±0.01	0.07±0.02	0.07±0.05	0.06±0.02	0.09±0.01	0.07±0.02
7%CO_2 4%O_2	0.07±0.01	0.07±0.01	0.06±0.01	0.08±0.03	0.10±0.02	0.14±0.04	0.09±0.03
8%CO_2 4%O_2	0.07±0.01	0.07±0.01	0.06±0.01	0.10±0.04	0.08±0.03	0.09±0.01	0.08±0.02
Average	0.07B±0.01	0.07B±0.01	0.07B±0.01	0.08AB±0.04	0.08AB±0.03	0.10A±0.03	
	Ratio						
Testemunha	234±27	183±17	250±34	260±114	195±77	230.5±45	225.3±60
5%CO_2 4%O_2	234±27	267±48	199±10	279±142	183±75	188.2±27	225.0±71
6%CO_2 4%O_2	234±27	221±26	224±32	278±125	265±72	176.4±14	233.2±63
7%CO_2 4%O_2	234±27	283±53	300±63	239±73	155±22	131.8±40	223.9±76
8%CO_2 4%O_2	234±27	222±24	269±7	220±152	215±77	179.6±29	223.2±66
Averages	234AB±23	235AB±48	248.7AB±47.1	255.1A±107.6	202.4AB±68.8	181.3B±42.8	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 6 - pH of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatments	Days of storage						Averages
	0	7	14	21	28	35	
Control	5.87±0.28	5.50±0.03	5.52±0.16	5.63±0.13	5.83±0.12	5.91±0.24	5.71a±0.23
5%CO_2 4%O_2	5.87±0.28	5.49±0.16	5.44±0.04	5.49±0.08	5.48±0.11	5.60±0.08	5.56b±0.20
6%CO_2 4%O_2	5.87±0.28	5.50±0.07	5.48±0.08	5.60±0.12	5.52±0.05	5.68±0.14	5.61ab±0.19
7%CO_2 4%O_2	5.87±0.28	5.52±0.11	5.46±0.13	5.44±0.09	5.44±0.02	5.54±0.06	5.55b±0.19
8%CO_2 4%O_2	5.87±0.28	5.48±0.07	5,57±0.02	5.45±0.06	5.54±0.10	5.53±0.06	5.58ab±0.18
Averages	5.87A±0.24	5.50B±0.08	5.49B±0.01	5.52B±0.12	5.56B±0.16	5.65B±0.18	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 7 - Firmness (gf.cm^{-2}) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0ºC and 85-90% relative humidity for 35 days

Treatment	Storage days						Averages
	0	7	14	21	28	35	
Control	671±48	497±26	515±295	568±207	378±204	378±320	501 b±209
5%CO_2 4%O_2	671±48	579±86	651±75	786±43	627±76	732±253	674 ab±123
6%CO_2 4%O_2	671±47	652±49	780±65	766±38	627±89	691±199	698 a±100
7%CO_2 4%O_2	671±47	607±84	634±47	673±169	551±108	647±179	630 ab±1084
8%CO_2 4%O_2	671±47	555±13	661±142	684±170	503±91	694±126	628 ab±1203
Averages	671AB±404	578AB±735	648AB±157	695A±145	537B±141	628AB±232	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

The activities of the enzymes PME and PG exhibited different tendencies during the 35 days of storage, as shown in Tables 8 and 9. In a previous study, enzyme activities decreased, remained constant or increased during maturation, depending on the fruit and method of analysis (Lima *et al.*, 2006) For the fruits tested in this study, there was a nearly linear increase in PME activity with storage time. However, there was little difference in the PME activities of fruits that were exposed to 6, 7 and 8% CO_2. A reduction in PG activity was also seen. It has been suggested that this can be explained by the lack of substrate for the enzyme or the existence of other multi-enzyme complexes (Abeles and Takeda, 1989). Enzymes such as β-galatosidase and other cellular proteins could be acting in the destruction of the cell walls of the fruits, causing the extravasation of cellular fluids. It is also possible that proteases could have catalyzed the hydrolysis of PG.

Antunes *et al.* (Antunes *et al.*, 2006) measured the activities of PG and PME in blackberries (*Rubus spp*.) that were stored in different environments for different times. They concluded that the activity of PME increased during storage for all cultivars and storage conditions, while the activity of PG decreased. This was confirmed in the present study on persimmons.

There was a slow decrease in the amount of reducing sugars beginning on the 28th day of storage, as shown in Table 10.

The fruits had ascorbic acid levels ranging from 15.6 to 40.4 mg per 100 ml, as shown in Table 11. The amount of ascorbic acid decreased with time. Silva *et al.,* (2011) evaluated the quality of 'Fuyu' persimmons and verified that covering them with wax did not affect the levels of ascorbic acid. According to Chitarra and Chitarra (2005) vitamin C tends to decrease during the ripening and stor-

Table 8 - Pectin methylesterase (UE.min^{-1}.g^{-1} of fresh fruit) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0ºC and 85-90% relative humidity for 35 days

Treatment	Days of Storage					
	0	7	14	21	28	35
Control	1343aF±85	3782aC±145	3320cE±140	3490aD±155	10898aA±385	10675aB±375
5%CO_2 4%O_2	1343aF±85	2766bD±130	6081aA±415	2814cC±133	2057dE±125	4830bB±225
6%CO_2 4%O_2	1343aF±85	2446dB±115	2520dA±130	1881eD±114	2074cC±125	1811eE±100
7%CO_2 4%O_2	1343aF±85	2607cA±125	22389eB±128	1913dE±123	2056eC±125	1945dD±105
8%CO_2 4%O_2	1343aF±85	1751eE±102	3879bA±149	3150bB±150	2174bD±130	2189cC±185

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 9 - Polygalacturonase (min^{-1}g^{-1} of fresh fruit) of 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0ºC and 85-90% relative humidity for 35 days

Treatment	Storage days					
	0	7	14	21	28	35
Control	890 aA±143	548±95 aA	591±55 aB	341±55 bC	213±40 aC	206±86 aB
5%CO_2 4%O_2	890 aA±143	453±60 aB	239±37 bC	473±71 bB	243±72 aB	143±84 bC
6%CO_2 4%O_2	890 aA±143	89±4 bC	200±39 bC	648±14 aA	257±88 aA	394±15 aA
7%CO_2 4%O_2	890 aA±143	432±268 aB	257±40 bC	374±30 bB	80±33 dD	175±43 cC
8%CO_2 4%O_2	890 aA±143	333±25 aB	673±24 aA	241±83 cC	166±132 bC	102±47 bC

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

Table 10 - Reducing sugars (%) in 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0ºC and 85-90% relative humidity for 35 days

Treatment	Storage days						Average
	0	7	14	21	28	35	
Control	13.3±1.8	12.1±2.1	13.9±1.6	13.5±1.1	10.8±0.5	11.1±0.4	12.4±1.7
5%CO_2 4%O_2	13.3±1.8	13.5±1.3	13.7±0.9	13.9±2.0	12.4±0.6	10.9±0.6	13.0±1.5
6%CO_2 4%O_2	13.3±1.8	15.2±1.2	13.8±0.9	12.7±0.5	11.6±0.4	10.0±1.4	12.8±2.0
7%CO_2 4%O_2	13.3±1.8	16.0±1.5	13.6±0.7	13.1±0.8	12.0±0.5	11.1±0.5	13.2±1.8
8%CO_2 4%O_2	13.3±1.8	14.1±0.6	14.1±1.1	13.0±1.6	12.2±0.9	10.6±0.4	12.9±1.6
Average	13.3±1.8	14.2A±1.9	13.8A±0.93	13.2A±1.2	11.8B±0.8	10.7B±0.8	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

age of many fruits, due to the action of ascorbic acid oxidase, other oxidative enzymes and/or peroxidases.

The astringency index of 'Giombo' persimmons that had their tannins removed and were exposed to modified atmospheres and were stored at 0°C had an index of 1 (no tannins) until the end of storage, which is in agreement with results reported by Núñez-Delicado *et al.* (2003) who reported that ethanol dissolves the tannins.

Refrigerated storage in active modified atmospheres is effective in preserving 'Giombo' persimmons. Fruits exposed to 7 or 8% CO_2 gave the best results. This work should not be taken as reflecting FDA policy or regulations.

References

ABELES F.B., TAKEDA F., 1989 - *Increased cellulase activity during blackberry fruit ripening*. - HortSci., 24(5): 851.

AHMED E.A., LABAVITCH J.M., 1980 - *Cell wall metabolism in ripening. I cell wall changes in ripening "Bartlett" pears*. - Plant Physiol., 65: 1009-1013.

ALBERSHEIM P., NEVINS D.J., ENGLISH P.D., KARR A., 1967 - *A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography*. - Carbohyd. Res.; 5(3): 340-345.

ANTONIOLLI L.R., CASTRO P.R.C., KLUGE R.A., SCARPARE F.J.A., 2000 - *Remoção da adstringência de frutos de caquizeiro 'Giombo' sob diferentes períodos de exposição ao vapor de álcool etílico.* - Pesq. Agropec. Bras., 35(10): 2083-2091.

ANTUNES L.E.C., GONÇALVES E.D., TREVISAN R., 2006 - *Alterações da atividade da poligalacturonase e pectinametilesterase em amora-preta (*Rubus *spp.) durante o armazenamento.* - Rev. Bras. Agroci., 12(1): 63-66.

BEN-AIRE R., ZUTKHI Y., 1992 - *Extending the storage life of 'Fuyu' persimmon by modified-atmosphere packaging*. - Hort Sci., 27: 811-813.

BLEINROTH E.W., ZUCHINI A.G., POMPEO R.M., 1976 - *Determinação das características físicas e mecânicas de variedade de abacate e sua conservação pelo frio.* - Coletânea ITAL, Campinas, *7*(1): 29-81.

BLUM J., HOFFMANN F.B., AYUB R.A., JUNG D.L., MALGARIM M.B., 2008 - *Uso de cera na conservação pós-colheita do caqui cv. Giombo*. - Rev. Bras. Fruticult. Jaboticabal, SP, 30(3): 830-833.

BRACKMANN A., MAZARO S.M., SAQUET A.A., 1997 - *Frigoconservação de caquis (*Diospyrus kaki*, L.) das cultivares Fuyu e Rama Forte.* - Ciência Rural, Santa Maria, 27(4): 561- 565.

BRACKMANN A., SAQUET A.A., 1995 - *Efeito da temperatura e condições de atmosfera controlada sobre a conservação de caqui (*Diospyrus kaki *L).* - Rev. Ciência Rural, Santa Maria, 25: 375-378.

CAMPO-DALL'ORTO F.A., OJIMA M., BARBOSA W., ZULLO M.A.T., 1996 - *Novo processo de avaliação da adstringência dos frutos no melhoramento do caquizeiro.* - Bragantia Campinas, 55: 237-243.

CANDIR E.E., OZDEMIR A.E., KAPLANKIRAN M., TOPLU C., 2009 - *Physico-chemical changes during growth of persimmon fruits in the east Mediterranean region*. - Scientia Horticulturae, 121: 42-48.

CELIK A., ERCISLI S., 2008 - *Persimmon cv. Hachiya (*Diospyros kaki *Thunb.) fruit: some physical, chemical and nutritional properties*. - Intl. J. Food Sci. Nutr., 59: 599-606.

CHITARRA M.I.F., CHITARRA A.B., 2005 - *Pós-colheita de frutos e hortaliças: fisiologia e manuseio*. - Ed. UFLA, Lavras, Minas Gerais, Brasil, pp. 785.

COSTA A.F.S., BALBINO J.M.S., 2002 - *Características da fruta para exportação e normas de qualidade*, pp. 12-18. - In: FOLEGATTI M.I.S., and F.C.A.U. MATSUURA (eds.) *Mamão: pós–colheita. Papaya: Post-harvest Embrapa Informação Tecnológica*. Brasília, DF, Série Frutas do Brasil, 21.

DANIELI R., GIRARDI C.L., PARUSSOLO A., FERRI V., ROMBALDI C., 2002 - *Efeito da aplicação de ácido giberélico e cloreto de cálcio no retardamento da colheita e na conservabilidade de caqui.* - Fuyu. Rev. Bras. Fruticult. Jaboticabal, SP, 24(1): 44-48.

DEL BUBBA M., GIORDANI E., PIPPUCCI L., CINCINELLI A., CHECCHINI L., GALVAN P., 2009 - *Changes in tannins, ascorbic acid and sugar content in astringent persimmons during on-tree growth and ripening and in response to different postharvest treatments*. - J. Food Comp. Anal., 22: 668-677.

DEMBITSKY V.M. POOV., DEMBITSKYA V.M., POOVARODOM S., LEONTOWICZ H., LEONTOWICZ M., VEARASILP SU., TRAKHTENBERG S., GORINSTEIN S., 2011 - *The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites*. - Food Res. Intl., 44(7): 1671-1701.

Table 11 - Ascorbic Acid (mg 100 ml^{-1}) in 'Giombo' persimmons, with tannins removed and submitted to modified atmospheres and stored at 0°C and 85-90% relative humidity for 35 days

Treatment	Storage days						Averages
	0	7	14	21	28	35	
Control	38±11	44±4	33±7	34±15	20±9	18±7	31±13
5%CO_2 4%O_2	38±11	38±2	37±9	37±19	24±11	13±3	31±13
6%CO_2 4%O_2	38±11	36±3	34±7	33±18	21±2	18±2	30±11
7%CO_2 4%O_2	38±11	46±13	30±6	43±22	36±20	13±0	34±16
8%$CO_2$4%O_2	38±11	39±2	41±2	19±2	41±11	16±2	32±12
Averages	38AB±9	40A±7	35AB±67	33AB±16	28BC±13	16C±4	

Averages followed by the same letter, lower case in columns and capital letters in rows do not differ significantly by the Tukey test at 5% probability.

DONAZZOLO J., BRACKMANN A., 2002 - *Efeito do CO_2 em atmosfera controlada na qualidade de caqui (*Diospyros kaki*, L.) Cv. Fuyu*. - Rev Bras. Agroci. Pelotas, 8: 241-245.

EDAGI F.K., CHIOU D.G., TERRA F.A.T., SESTARI I., KLUGE R.A., 2009 - *Remoção da adstringência de caquis 'Giombo' com subdosagens de etanol*. - Ciênc. Rural, Santa Maria, 39: 2022-2028.

FERRI V.C., RINALDI M.M., DANIELLI R., LUCCHETTA L., ROMBALDI C.V., 2004 - *Atmosfera modificada na conservação de caquis (*Diospyrus kaki, *L.). cultivar Fuyu*. - Rev. Bras. Agroci., 10: 111-115.

FINGER F.L., VIEIRA G., 2002 - *Controle da perda pós colheita de água em produtos hortícolas*. - UFV, Viçosa, pp. 29.

GAZIT S., LEVY Y., 1963 - *Astringency and its removal in persimmon*. - Israel J. Agr. Res., 13(3): 125-132.

GIORDANI E., DOUMETT S., NIN S., DEL BUBBA M., 2011 - *Selected primary and secondary metabolites in fresh persimmon (*Diospyros kaki *Thunb.): A review of analytical methods and current knowledge of fruit composition and health benefits*. - Food Res. Intl., 44: 1752-1767.

GOMES F.P., 2000 - *Curso de estatística experimental. 14. ed*. - Fundação de Estudos Agrários Luiz de Queiroz FEALQ, Piracicaba, Brazil.

GORINSTEIN S., BARTINIKOWSKA E., KULASEK G., ZEMSER M., TRAKHTENBERG S., 1998 - *Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol*. - J. Nutr., 128: 2023-2027.

GORINSTEIN S. LEONTOWICZ H., LEONTOWICZ M., JESION I., NAMIESNIK J., DRZEWIECKI J., PARK Y.S., HAM K.S., GIORDANI E., TRAKHTENBERG S., 2011 - *Influence of two cultivars of persimmon on atherosclerosis indices in rats fed cholesterol-containing diets: Investigation* in vitro *and* in vivo. - Nutr., 27(7-8): 838-846.

GU H.-F., LI C.-M., XU Y.-J., HU W.-F., CHEN M.-H., WAN Q,-H., 2008 - *Structural features and antioxidant activity of tannin from persimmon pulp.* - Food Res. Intl., 41: 208-217.

LIMA M.A.C., ALVES R.E., FILGUEIRAS, H.A.C. 2006 - *Mudanças relacionadas ao amaciamento da graviola durante a maturação pós-colheita*. - Pesq. Agropec. Bras., 41(12): 1707-1713.

MAPA, 2011 - *Modified Tillman's Method*. - Ministério da Agricultura, Pecuária e Abastecimento http://www.agricultura.gov.br .

MARTÍNEZ-CALVO J., NAVAL M., ZURIAGA E., LLÁCER G., BADENES M.L., 2012. Genet. Resour. Crop. Evol. Published on-line.

MARTINS F.P., PEREIRA F.M., 1989 - *Cultura do caquizeiro. Jaboticabal*. - FUNEP, pp. 71.

MURRAY R., VALENTINI G., 1998 - *Storage and quality of peach fruit harvest at different stages of maturity*. - Acta Horticulturae, 465: 455-463.

NELSON N.A., 1944 - *A photometric adaptation of Somogy method for the determination of Glucose*. - J. Biol. Chem., 153: 375-380.

NÚÑEZ-DELICADO E., SOJO M.M., GARCÍA-CARMONA F., SÁNCHEZ-FERRER A., 2003 - *Partial Purification of latent persimmon fruit polyphenol oxidase*. - J. Agric. Food Chem., 51: 2058-2063.

ODAIR Z., NEUS S.P., TIGLEA P., 2008 - *Métodos físico-químicos para análise de alimentos. Physical-chemical methods for analyzing foods*. - Instituto Adolfo Lutz, São Paulo, Brasil.

SARGENT S.A., CROCKER T.E., ZOELLNER J.J., 1993 - *Storage characteristics of 'Fuyu' persimmons*. - Proc. Florida State Hort. Soc., 106: 131-134.

SILVA M.C., ATARASSI M.E., FERREIRA M.D., MOSCA M.A., 2011 - *Qualidade pós –colheita de caqui 'Fuyu' com utilização de diferentes concentrações de cobertura comestível. Postharvest quality of 'Fuyu'*. - Ciênc, Agrotec. Lavras, 35(1): 144-151.

SOMOGY M., 1945 - *Determination of blood sugar*. - J. Biol. Chem., 160: 69-73.

USDA, 2012 - *Germplasm Resources Information Network* (GRIN). - Website: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?14293

VEBERIC R., JURHAR J., MIKULIC-PETKOVSEK M., STAMPAR F., SCHMITZER V., 2010 - *Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki *L.)*. - Food Chem., 119: 477-483.

VIEITES R.L., PICANÇO N.F.M., DAIUTO É.R., MORAES M.R., 2012 - *Optimum temperature and state of maturity for storing persimmons,* Diospyros kaki *L., caqui 'Giombo'*. - Nat. Prod. J., 2: 180-187.

VITTI D.C.C., 2009 - *Destanização e armazenamento refrigerado de caqui «Rama Forte» em função da época de colheita*. - PhD, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brasil.

Adv. Hort. Sci., 2013 27(1-2): 81-87

Comparison of tall fescue (*Festuca arundinacea* Schreb.) and common bermudagrass (*Cynodon dactylon* [L.] Pers.) turfgrasses and their seed mixtures

M.R. Salehi, H. Salehi [(1)]
Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran.

Key words: combined seeds, lawn, management practices, visual quality.

Abstract: With dense shoots above ground, a well-developed root system and large amounts of biomass underground, turfgrass provides many environmental benefits, including moderating soil erosion, water runoff and leaching, contributing to carbon sequestration, moderating temperatures, and reducing noise, glare, and visual pollution. In this investigation, (*Cynodon dactylon* [L.] Pers.) tall fescue (*Festuca arundinacea* Schreb.) and common bermudagrass were compared in monoculture and different mixtures of 0 to 100%, based on the number of seeds used. Perennial ryegrass (*Lolium perenne* L.), a common lawn in Shiraz, was used as control. The experiment was conducted in a split block design (season as main plot and turfgrass types as subplot) and each treatment had four replications. Data were analyzed with MSTATC software and means were compared using Tukey's test at 5% level. Turfgrasses were compared by measuring visual quality after winter and summer, rooting depth, verdure and/ or root fresh and dry weight, tiller density, and clippings fresh and dry weight. Results showed that, with the exception of mean rooting depth and chlorophyll index after summer, spring sowing is better than fall sowing. However, it can be concluded that the 80% tall fescue and 20% bermudagrass treatment is the best treatment, or has not significant differences with the other good treatments, except with regard to tiller density. This type can be used alternatively in overseeding programs in areas with soil and environmental conditions similar to the present investigation site.

1. Introduction

There are four main methods to establish turfgrasses from seeds: monoculture, seed blend, seed mixture and overseeding. In seed mixture, the species or genera of turfs are mixed together (Salehi, 2008). Mixture of species has long been used for turf seedlings (Newell *et al.*, 1996). Turf mixture, as opposed to a single species, broadens the genetic base, and thus increases the probability of providing pest resistance and tolerance to environmental extremes (Newell *et al.*, 1996). A turf consisting of a mixture may not be as uniform as single species in appearance or texture. However, when disease or other injuries affect one species in the mixture, the resulting injury is generally not as severe as when affecting a lawn with a single species (Corman, 1955; Daniel *et al.*, 1955; DeFrance, 1951).This method consists of three types of mixing including cool-cool season turf, cold-warm season turf and rarely warm-warm season turf seed mixtures. Mixture of cool-warm season turf may be useful for areas with inconsistent environmental conditions because each component in the mixture excels in a specific environment (Davis, 1958), especially in a transition zone (Akbari *et al.*, 2011; Salehi, 2008). For example, in Missouri (classified as a transition zone) bermudagrass and Kentucky bluegrass are grown together (Dunn *et al.*, 1994). Bermudagrass dominates in the summer, while Kentucky bluegrass in the cooler months of fall and spring (Daniel *et al.*, 1955). Early investigations to mix bermudagrass with cool season species were often unsuccessful because of the dominance of bermudagrass during summer months (Beard, 1973; Davis, 1958). This problem could be solved by:

i) Using warm season grasses with lower dominance in order to get a lower competition between the species. There is no known report on this method (Salehi, 2008).
ii) Using more aggressive cool-season turf may lessen the advantage for bermudagrass in summer and may make a permanent, balanced, cool-warm season mixture more practical than in past years (Davis, 1958; Turgeon, 1991).
iii) Using growth inhibitors for warm-season turfs in the first summer after planting to prevent exceeding growth or using accelerator hormones in the first spring and fall after planting for more growth of cool season turfs (Salehi, 2008).

[(1)] Corresponding author: hsalehi@shirazu.ac.ir

Received for publication 11 April 2013
Accepted for publication 3 May 2013

Misiha (1991) compared different turfgrass seed mixtures and demonstrated that the mixture of *Poa pratensis* L. 'Entopper' with *Festuca rubra* L. 'Hareld' had the best establishment rate and highest chlorophyll content, plant density, and clipping dry weight. Newell *et al.* (1996) indicated that *Lolium perenne* L. and *Festuca rubra* L. seed mixture had the best wear tolerance. Salehi and Khosh-Khui (2004) used *Lolium perenne* L., *Cynodon dactylon* (L.) Pers., *Poa pratensis* L. and *Festuca rubra* L. in mixtures of 1:1 (by weight) and a 1:1:1:1 (by weight) and two sport turfgrass cultivars, BAR11 (Barenbrug Co.) and MM (Mommersteeg Co.). The seeds were sown in March and October in two years. The turfgrasses were compared by measuring visual quality, chlorophyll index after winter and summer, rooting depth, verdure and/or root fresh and dry weight, tiller density, and clippings fresh and dry weight. They showed that fall sowing was superior to spring sowing and resulted in greater root growth, clippings yield, and chlorophyll content. *Poa+Cynodon* seed mixture was the best treatment and showed high tiller density, root growth, and chlorophyll content. They concluded that the cool-warm season seed mixture (*Poa+Cynodon*) can be used alternatively in overseeding programs in transition zone areas similar to Shiraz, Iran.

Following the study by Salehi and Khosh-Khui (2004), Akbari *et al.* (2011) compared *Poa* and *Cynodon* turfgrasses and their seed mixtures. In this research turfgrasses Kentucky bluegrass (*Poa pratensis* L. 'Merion') and common bermudagrass (*Cynodon dactylon* [L.] Pers.), in monoculture or in mixtures of 0 to 100%, based on number of seeds, were used. Perennial ryegrass (*Lolium perenne* L. 'Barball'), a common turf in the campus of Shiraz University, was used as control. The seeds were sown in October in two years. The turfgrasses were compared by measuring visual quality after winter and summer, chlorophyll index after winter and summer, rooting depth, verdure and/or root fresh and dry weight, tiller density, and clippings fresh and dry weight. *Poa* monoculture showed high tiller density, root fresh and dry weight and total fresh and dry weight. *Lolium* monoculture showed high rooting depth after winter and clippings fresh and dry weights. *Cynodon* monoculture quality was poor with regard to many characteristics, mainly due to fall sowing. The seed mixture composed of 20% *Cynodon* + 80% *Poa* was the best treatment and resulted in the highest rooting depth after summer, verdure fresh and dry weights, chlorophyll index after winter and summer, visual quality after winter and summer, and a good turf according to the other characteristics. Based on good characteristics of recently coming cool season *Festuca arundinacea* Schreb. cultivars to Shiraz, we proposed to test the performance of cool-warm season seed mixtures of a cultivar of this species with common bermudagrass in the same location as previous report. To the best of our knowledge there is no report of testing the seed mixture of this species with bermudagrass.

2. Materials and Methods

Characteristics of experimental location

Studies were conducted at the experimental farm of the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran, at Bajgah, 1810 m above mean sea level, 52°32' E and 29°36' N, with Daneshkadeh soil series (fine, mixed, mesic, Calcixerollic Xerochrepts, pH=8), from 2007 to 2008. The meteorological data for the experimental site is shown in Table 1. Long-term averages of maximum and minimum temperatures are 38°C and -9°C, respectively and yearly precipitation at this site is 400 mm.

Treatments

The species used in this study were *Festuca arundinacea* Schreb. 'Starlet' and *Cynodon dactylon* [L.] Pers. (California origin) in monoculture or in different mixture (by number of seed) and *Lolium perenne* L. cultivar Squire in monostand. In this investigation perennial ryegrass was used as control (Table 2). Turfgrass plots were established by directly sowing the seeds at two times, April and October, in 2007.

Management practices during investigation

Turfgrasses were irrigated every week in spring, every four days in summer, and every ten days in fall and every month in winter. It was enough to moisten the soil without overwatering. Turfgrasses were mowed at 5 cm height with an electrical mower. Weeds were manually removed. In this study no fertilizer was used.

Table 1 - Monthly average of temperature and precipitation (from April 2007 to August 2008)

Year	Month	Precipitation (mm)	Minimum T (°C)	Maximum T (°C)	Average T (°C)
2007	April	138.5	-3	24.5	11.56
	May	3	2	30.5	17.37
	June	0	7.8	38	22.07
	July	2.5	12.4	38	25.86
	August	0	9	36	24.16
	September	0	6	36	21.26
	October	0	3	29.5	15.79
	November	0	-5	26.5	11.34
	December	0	-12.3	18.5	3.4
2008	January	34	-14.8	12.6	0.5
	February	35	-13.7	20	3.5
	March	0	-9.6	22.2	7.5
	April	3.5	-3	29	14
	May	0	1.4	32.5	17.3
	June	0	5	38	22.4
	July	0	10.6	39	25.1
	August	0	13	38	24.2

Table 2 - Seeding rate of turfgrasses used (by percentage and weight)

Treatment number	*Cynodon* (%)	*Lolium* (%)	*Festuca* (%)	*Cynodon* (g m^{-2})	*Lolium* (g m^{-2})	*Festuca* (g m^{-2})
1	0	0	100	0.0	0	46.0
2	10	0	90	0.7	0	41.4
3	20	0	80	1.4	0	36.8
4	30	0	70	2.1	0	32.2
5	40	0	60	2.8	0	27.6
6	50	0	50	3.5	0	23.0
7	60	0	40	4.2	0	18.4
8	70	0	30	4.9	0	13.8
9	80	0	20	5.6	0	9.2
10	90	0	10	6.3	0	4.6
11	100	0	0	7.0	0	0.0
12	0	100	0	0.0	50	0.0

Measurements

Tiller density, verdure fresh and dry weight, clippings fresh and dry weight, root fresh and dry weight and total fresh and dry weight were measured after summer (October). Chlorophyll index and mean root depth and visual quality were measured after summer and winter (October and March, respectively).

Visual quality was assessed periodically throughout and after each growing season using a ranking scale from 0 to 9, 0= no live turf; 9= ideal shoot density, winter and summer color, and uniformity. Clippings and verdures were collected 20 days after mowing from 5 cm above the ground.

To measure dry weight, the materials were dried out at 60°C for 48 h. All data were measured for 100 cm^2 subsamples in each plot. A random subsample of each plot was collected using a 10×10×50 cm metal block inserted into the soil. Then, the samples were soaked in tap water and soil was removed. After cleaning and air drying, plant samples were transferred to the laboratory for further measurements. Chlorophyll index (mg 100 cm^{-2}) was measured by spectrophotometric methods (by Spectronic 20D device) at 645 and 663 nm wavelengths (Salehi and Khosh-Khui, 2004).

Experimental design and data analysis

Experiments were conducted in 2×12 split block design with factorial arrangements, sowing season (spring and fall) as main plot and seed mixtures as subplot, and four replications in each treatment. Individual plots measured 4 m^2 (1×4 m). Data were analyzed using MSTAT-C program and the mean comparisons were made following Tukey's test at $p \leq 0.05$.

3. Results

Average root depth after summer

Results show that with spring sowing, there was no significant difference among all the seed mixtures' average root depth after summer. However, with fall sowing, the increase of tall fescue percentage led to greater root depth in the soil. The deepest root system was observed in treatment 1 with fall sowing composed of 100% seeds of tall fescue. Comparing averages of spring and fall sowing data, it is clear that the average root depth with fall sowing is greater than spring sowing (Table 3).

Average root depth after winter

With spring sowing the deepest root system belonged to the treatment composed of 100% tall fescue seed; decreasing the percentage of tall fescue seed, the root depth decreased. The deepest root system with fall sowing was found in *Lolium* monoculture, followed by the mixture with 90% tall fescue and 10% bermudagrass. By decreasing the percentage of tall fescue seeds, root depth decreased, except between treatments 4 and 5, in fall sowing and after winter. Averages of spring and fall sowing data indicate that the highest proportion of the root system grows near the soil surface with fall sowing (Table 3).

Tiller density

The average tiller density with spring sowing was found to be higher than with fall sowing. In spring, the mixture composed of 60% tall fescue and 40% bermudagrass had the highest tiller density and bermudagrass monoculture had the lowest; in fall, *Lolium* monoculture had the highest and bermudagrass monoculture had the lowest (Table 3).

Root fresh and dry weight

According to the results obtained, with the spring sowing, the mixture composed of 70% tall fescue and 30% bermudagrass produced the heaviest root system. With a decreasing percentage of tall fescue seeds, root system weights decreased. Therefore, the lowest root weight with spring sowing was observed in bermudagrass monoculture. With the fall sowing, the mixture composed of 80% tall fescue and 20% bermudagrass produced the heaviest and the 10% tall fescue and 90% bermudagrass mixture produced the lightest root systems (Table 3).

Clippings fresh and dry weight

Maximum values for clippings fresh and dry weights were found with the spring sowing of 60% tall fescue and 40% bermudagrass mixture. However, there was no significant difference ($p \leq 0.05$) for treatments 1 to 6. Furthermore, increasing the percentage of bermudagrass seeds (up to 40%), clippings weight increased and then decreased. With the fall sowing, the maximum clippings weight belonged to the mixture with 90% tall fescue and 10% bermudagrass. However, by decreasing the percentage of tall fescue seeds the weight decreased. Indeed, the lowest clippings weight was found with bermudagrass monoculture. Averages of spring and fall sowings data

showed that clipping weights (both fresh and dry) of fall sowing was more than spring sowing (Table 4).

Verdure fresh and dry weight

Spring sowing results reveal that the maximum verdure fresh and dry weights belonging to the mixture composed of 60% tall fescue and 40% bermudagrass. However, it was not significantly different in treatments 4 and 6. With fall sowing, the 70% tall fescue and 30% bermudagrass mixture produced the maximum verdure weight. However, it was not significantly different in treatments 2 to 7 and 12 and tall fescue monoculture. Furthermore, averages of spring and fall sowing data show that verdure fresh and dry weights were greater in spring sowing than fall sowing (Table 4).

Total fresh and dry weight

The maximum total fresh and dry weights with spring sowing were found in treatment 5 (60% tall fescue and 40% bermudagrass). However, the values were not significantly different from treatment 4. The minimum total weight with spring sowing was observed in bermudagrass monoculture. With fall sowing, maximum and minimum total weights were noted in treatments composed of 80% tall fescue and 20% bermudagrass and bermudagrass monoculture, respectively. Furthermore, the average total weight of spring sowing was more than fall sowing (Tables 4 and 5).

Chlorophyll index after winter

With regard to chlorophyll, results showed that with spring sowing, the maximum index belonged to the 70% tall fescue and 30% bermudagrass mixture. A decrease in tall fescue seeds percentage led to a decrease in chlorophyll index. With fall sowing, the maximum index was found with the 90% tall fescue and 10% bermudagrass mixture (treatment 2). However, there was no significant difference between this treatment and treatments 3 and 4 (Table 5).

Chlorophyll index after summer

As for the chlorophyll index after summer, with spring sowing, treatment 3 gave the maximum chlorophyll index, however there was no significant difference between this treatment and treatments 1 and 2; the minimum index was

Table 3 - Effects of different seed mixtures and sowing times on some biological characteristics of the turfgrasses used

Sowing season	Treatment number	Average root depth after summer (cm)	Average root depth after winter (cm)	Tiller density (no 100 cm^{-2})	Root fresh weight (g)	Root dry weight (g)
Spring	1	26.25 c-f*	33.37 a	175 de	53.75 cd	22.40 bc
	2	25.37 c-f	33.25 a	180 cd	52.50 cd	21.00 c
	3	23.75 e-h	31.50 ab	186 bcd	51.80 cde	20.34 cd
	4	23.25 e-h	29.62 bc	193 ab	59.75 bc	22.98 bc
	5	22.50 fgh	29.00 bcd	194 ab	57.87 bc	22.26 bc
	6	25.00 c-g	28.75 bcd	179 cde	44.60 def	16.86 de
	7	23.25 e-h	28.75 bcd	155 fg	42.50 efg	15.74 ef
	8	24.75 d-g	26.87 c-f	141 hi	40.75 fg	14.87 efg
	9	24.62 d-g	27.12 cde	129 ij	34.25 gh	12.32 fgh
	10	24.50 d-g	25.87 def	124 jk	22.75 ij	7.84 ij
	11	23.75 e-h	24.12 efg	116 kl	19.87 ij	6.56 j
	12	26.75 cde	32.25 ab	178 cde	35.70 fgh	11.57 gh
	Average	24.48 B	29.21 A	162.8 A	43.02 A	16.22 A
Fall	1	33.62 a	23.50 fg	183 bcd	66.00 ab	27.50 a
	2	33.25 a	24.50 efg	185 bcd	66.87 ab	26.96 a
	3	31.75 ab	24.12 efg	190 abc	69.87 a	27.95 a
	4	31.75 ab	22.12 gh	177 de	64.12 ab	24.85 ab
	5	28.75 bc	24.00 efg	167 ef	57.62 bc	21.83 bc
	6	27.75 cd	19.62 hi	152 gh	35.60 fgh	13.49 e-h
	7	25.00 c-g	19.37 hi	148 gh	34.37 gh	12.88 fgh
	8	24.00 c-g	16.87 ij	130 ij	29.12 hi	10.48 hi
	9	23.37 e-h	14.12 jk	105 lm	19.00 j	6.78 j
	10	21.12 gh	12.75 k	96 m	13.75 j	4.79 j
	11	20.00 h	11.62 k	77 n	14.37 j	4.79 j
	12	31.75 ab	25.12 efg	198 a	42.32 fg	12.81 fgh
	Average	27.68 A	19.81 B	151 B	42.75 B	15.27 B

* In each column, means followed by the same letter(s) (small letters for means and capital letters for main averages) are not significantly different at 5% level according to Tukey's test.

found with treatment 11. With fall sowing, maximum and minimum chlorophyll indices were found with treatments 2 and 11, respectively. Averages of spring and fall sowing data showed that the chlorophyll index for spring sowing is significantly greater than fall sowing (Table 5).

Visual quality after summer

With spring sowing, treatment 5 gave grasses with the best visual quality. However, there was no significant difference between treatments 2 to 7, and tall fescue, bermudagrass and *Lolium* monocultures. With fall sowing, treatment 3 gave the best visual quality; there was no significant difference between this treatment and treatments 2 to 5, and tall fescue and *Lolium* monocultures. Furthermore, the average visual quality of spring sowing was greater than fall sowing (Table 5).

Visual quality after winter

The best average of visual quality with spring sowing was found with the treatment composed of 100% *Lolium* and, generally, by decreasing the percentage of tall fescue seeds the visual quality decreased. However, visual quality after winter increased between treatments 2 and 3, and also between 4 and 5. With fall sowing, *Lolium* monoculture gave the best average visual quality. In the mixtures, the 80% tall fescue and 20% bermudagrass mixture gave the best visual quality and the worst was found with bermudagrass monoculture. Averages of spring and fall sowings data showed that visual quality with spring sowing is more than fall sowing (Table 5).

4. Discussion and Conclusions

Average root depth after summer

There were no significant differences among different seed mixtures' root depth with spring sowing, maybe because their growth took place at the same time in spring. Maximum root depth in spring belonged to *Lolium*, based on its powerful germination and growth at the beginning of culture (Christians 2004; Salehi 2008; Akbari *et al.*, 2011). With fall sowing, by increasing the tall fescue seed per-

Table 4 - Effects of different seed mixtures and sowing times on some biological characteristics of the turfgrasses used

Sowing season	Treatment number	Clippings fresh weight (g)	Clippings dry weight (g)	Verdure fresh weight (g)	Verdure dry weight (g)	Total fresh weight (g)
Spring	1	8.00 de*	1.68 efg	16.75 d-g	5.46 def	78.50 de
	2	7.95 def	1.69 efg	23.37 b	7.30 ab	83.82 cd
	3	7.95 def	1.69 efg	21.62 bc	6.36 bcd	81.45 d
	4	8.17 de	1.77 def	24.87 ab	7.23 ab	92.80 abc
	5	8.60 cd	1.91 cde	27.00 a	7.71 a	93.47 abc
	6	7.90 def	1.76 d-g	23.75 ab	6.74 abc	76.27 de
	7	6.97 efg	1.58 fgh	19.12 cde	5.31 def	68.60 efg
	8	6.42 gh	1.48 gh	16.87 d-g	4.60 f-i	64.05 fgh
	9	5.00 ij	1.18 ij	15.50 fgh	4.17 g-j	54.75 hi
	10	4.17 ijk	1.01 jk	14.50 ghi	3.87 h-k	41.42 jk
	11	3.20 kl	0.80 kl	10.77 jk	2.83 kl	33.85 kl
	12	6.90 efg	1.37 hi	17.37 d-g	4.96 efg	60.02 fgh
	Average	6.77 B	1.49 B	19.29 A	5.54 A	69.08 A
Fall	1	10.65 ab	2.26 ab	16.25 e-h	5.41 def	92.60 abc
	2	11.20 a	2.38 a	17.12 d-g	5.38 def	95.20 ab
	3	11.15 a	2.36 a	18.62 c-f	5.85 cde	99.65 a
	4	9.75 bc	2.13 abc	19.62 cde	5.85 cde	93.50 abc
	5	9.12 cd	1.99 bcd	18.62 c-f	5.46 def	85.37 bcd
	6	8.37 d	1.88 cde	18.62 c-f	5.46 def	62.62 fgh
	7	6.60 fg	1.50 fgh	17.00 d-g	4.83 e-h	57.97 ghi
	8	5.12 hi	1.19 ij	13.12 hij	3.70 ijk	47.37 ij
	9	3.75 jkl	0.88 kl	11.75 ij	3.25 jk	34.50 kl
	10	2.52 lm	0.61 lm	10.87 jk	2.93 kl	27.15 l
	11	1.72 m	0.43 m	8.10 k	2.16 l	24.20 l
	12	7.95 def	1.59 fgh	19.77 cd	5.63 def	70.05 ef
	Average	7.32 A	1.60 A	15.79 B	4.58 B	65.87 B

* In each column, means followed by the same letter(s) (small letters for means and capital letters for main averages) are not significantly different at 5% level according to Tukey's test.

centage, the average root depth increased because tall fescue had good growth in early autumn while at the same time bermudagrass growth was very weak as it is a warm-season turfgrass.

Average root depth after winter

With spring sowing, maximum root depth belonged to tall fescue monoculture. Moreover, with increased tall fescue seed percentage in spring sowing, the growth of bermudagrass became slow with the beginning of the fall season, however *Lolium* and tall fescue grew continuously. With fall sowing, maximum root depth was found with *Lolium* because of its power to germinate. An increase in tall fescue seed percentage led to root depth increases because tall fescue goes into dormancy slowly and with increased temperature growth starts early.

Tiller density

With both sowing times, minimum tiller density was found with bermudagrass, which confirms the results of Akbari *et al.* (2011). Furthermore, with increasing tall fescue seed percentage, tiller density increased, which may refer to its power of tiller production.

Root fresh and dry weight

Increasing bermudagrass seed percentage led to decreases in root weight for both the sowing times, confirming the results of Akbari *et al.* (2011).

Clippings and verdure fresh and dry weight

With spring and fall sowing times, maximum weight was found in treatment 5 (60% tall fescue) and treatment 2 (90% tall fescue), respectively for clippings; and treatment 5 60% (tall fescue) and treatment 4 (70% tall fescue), respectively for verdure weight. Because tall fescue has good shoot growth, increasing its seed percentage increases clippings weight. However, this character is not suitable because it means increased mowing.

Total fresh and dry weight

Maximum total weights were found among treatments composed of higher percentages of tall fescue seeds; with

Table 5 - Effects of different seed mixtures and sowing times on some biological characteristics of the turfgrasses used

Sowing season	Treatment number	Total dry weight (g)	Chloropyll index after winter**	Chlorophyll index after summer**	Visual quality after summer	Visual quality after winter
Spring	1	29.53 cd*	2.10 cd	9.38 ab	8.25 ab	8.00 a-d
	2	29.99 cd	2.07 cd	9.41 a	7.75 a-d	7.75 a-e
	3	28.40 de	2.07 cd	9.47 a	8.37 ab	8.12 a-d
	4	31.99 bcd	2.28 abc	9.05 b	8.37 ab	8.12 a-d
	5	31.89 bcd	1.94 def	8.60 c	8.87 a	8.37 abc
	6	25.35 ef	1.74 fg	8.27 cde	8.37 ab	7.00 b-f
	7	22.64 fg	1.41 h	8.00 ef	8.00 ab	6.37 def
	8	20.97 gh	1.07 i	7.79 fg	7.12 b-e	6.00 efg
	9	17.67 hi	0.74 j	7.54 gh	6.62 cde	5.25 fg
	10	12.73 hi	0.45 k	7.29 hij	7.12 b-e	2.87 hi
	11	10.19 kl	0.09 l	7.04 j	7.75 a-d	0.00 k
	12	17.90 hi	1.61 gh	8.19 de	8.62 a	8.87 a
	Average	23.27 A	1.47 B	8.34 A	7.93 A	6.39 A
Fall	1	35.17 ab	2.24 bc	9.32 ab	8.50 a	8.37 abc
	2	34.74 ab	2.47 a	9.36 ab	8.50 a	8.50 abc
	3	36.12 a	2.37 ab	9.30 ab	8.87 a	8.75 ab
	4	32.84 abc	2.32 ab	8.60 c	8.50 a	8.12 a-d
	5	29.84 cd	2.00 de	8.41 cd	7.87 abc	7.87 a-d
	6	20.74 gh	1.79 efg	8.06 def	6.25 ef	6.75 c-f
	7	19.20 ghi	1.52 h	7.83 fg	5.87 efg	5.37 fg
	8	15.38 ij	1.05 i	7.53 gh	5.00 fgh	4.50 gh
	9	10.39 kl	0.84 j	7.39 hi	4.87 gh	1.87 ij
	10	8.34 l	0.35 k	7.16 ij	4.37 h	1.00 jk
	11	7.38 l	0.03 l	6.54 k	6.50 de	0.00 k
	12	20.03 gh	1.82 ef	8.22 de	8.78 a	9.00 a
	Average	22.51 B	1.57 A	8.14 B	6.99 B	5.84 B

* In each column, means followed by the same letter(s) (small letters for means and capital letters for main averages) are not significantly different at 5% level according to Tukey's test. **mg 100 cm^{-2}.

increasing bermudagrass seed percentage, total weight decreases, especially with fall sowing.

Chlorophyll index after winter and summer

After winter, the chlorophyll index in bermudagrass was very low because it is a warm season turf and in winter its shoots become chlorotic. However, tall fescue and *Lolium* shoots remain green during winter. After summer, all the mixtures increased the chlorophyll index of shoots in all the seed mixtures. Furthermore, by lowering the plant density of tall fescue, light can penetrate more easily and thus the chlorophyll index becomes greater than tall fescue monoculture.

Visual quality after summer and winter

Visual quality has a direct correlation with chlorophyll index: as chlorophyll index increases, so does visual quality. Furthermore, visual quality depends on weed density and in mixtures with more bermudagrass the number of weeds increases, particularly with fall sowing.

Among *Festuca* and *Cynodon* seed mixtures, 80%F + 20%C was selected as an excellent turfgrass mixture in this study, with regard to all the positive characteristics except tiller density. This seed mixture established a fine, green color throughout the year. Furthermore it can be used alternatively in overseeding programs in areas with soil and environmental conditions similar to the present investigation site. Additional studies are needed to investigate the best cultural conditions of this selected seed mixture.

References

AKBARI M., SALEHI H., KHOSH-KHUI M., 2011 - *Cool-warm season* Poa-Cynodon *seed mixtures and their turf growth and quality*. - Acta Agric. Scand., Section B - Soil Plant Sci., 61(6): 559-564.

BEARD J.B., 1973 - *Turfgrass: Science and culture*. - Prentice-Hall, Englewood Cliffs, NJ, USA, pp. 704.

CHRISTIANS N., 2004 - *Fundamentals of turfgrass management*. - John Wiley and Sons Inc., NJ, USA, pp. 359.

CORMAN J.F., 1955 - *Home lawns*. - New York Agr. Exp. Sta. Ext. Bul., No. 922.

DANIEL W.H., HULL R.B., LEE O.C., LEHKER G.E., SHARVELLE E.G., 1955 - *The lawn-how to establish and maintain*. - Indiana Agr. Exp. Sta. Ext. Bul., No. 245.

DAVIS R.R., 1958 - *The effect of other spices and mowing height on the persistence of lawn grasses*. - Agron. J., 50: 671-673.

DEFRANCE J.A., 1951 - *How to make a lawn*. - Quart. Rev., Rhode Island Agr. Exp. Sta.

DUNN J.H., MINNER D.D., FRESENBURG B.F., BUGHRARA S.S., 1994 - *Bermudagrass and cool season turfgrass mixtures: Response to simulated traffic*. - Agron. J., 86: 10-16.

MISIHA A., 1991 - *Effect of cool season turfgrasses seed mixtures on lawn characteristics*. - Bul. Facult. Agr. Univ. Cairo, 42: 401-414.

NEWELL A.J., CROSSLEY F.E.M., JONES A.C., 1996 - S*election of grass species, cultivars and mixtures for lawn tennis courts*. - J. Sports Turf. Res. Inst., 72: 42-60.

NIEHAUS M.H., 1976 - *Effect of cultivar, seeding rate, and nitrogen fertilization on Kentucky bluegrass-perennial ryegrass turf mixtures*. - Agron. J., 68: 955-977.

SALEHI H., KHOSH-KHUI M., 2004 - *Turfgrass monoculture, cool-cool, and cool-warm season seed mixture establishment and growth responses*. - HortScience, 39: 1732- 1735.

SALEHI M.R., 2008 - *Comparison between tall fescue and common bermudagrass turfgrasses and their seed mixtures*. - MSc Thesis, Shiraz University, Iran.

TURGEON A.J., 1991 - *Turfgrass management*. - Prentice-Hall, Englewood Clifs, NJ, USA, pp. 392.

BOOK REVIEWS

IL TEMPIO DELLA NOTTE. ARCHITETTURA IPOGEA NEI GIARDINI PAESAGGISTICI. *Breda M.A.* Giardini e Paesaggio, Vol. 35. Leo S. Olschki, Florence (Italy), 2012, pp. 112. ISBN 978-88-222-6194-6. € 23.00.

In this 35th volume in the "Giardini e Paesaggio" series Maria Chiara Zerbi, in her presentation, underlines that the book is the result of many years of enthusiastic work and that through it the reader can appreciate these "architecturally secret places that were found in what were once private gardens but are now part of public lands, neglected and in need of restoratotion and consolidation to restore the original quality".

The author brings to this work many years of experience as a historian of garden architecture and history and treats in this book a unique theme: the *Temple of the Night*, an underground architectural element, or rather an environment within a grotto found in English-style gardens in Italy between the end of the 18th and beginning of the 19th centuries, and probably influenced by Germany models.

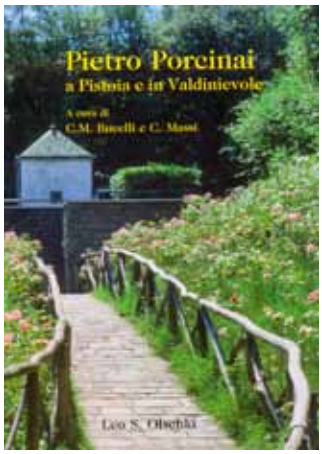
The book deals with two cases studied in depth by the author and highlights her ability to observe and compare, to recognize different situations for what they are, and to bring these aspects together through systematic study. She successfully presents the data through drawings and photographs, which make the book pleasant to read and easy to understand.

The first of the two cases, the temple created within one of the artificial grottoes in the park of the Conte Ambrogio Uboldo at Cernusco sul Naviglio, is known to specialists but has not been studied before. The second is a previously-unknown temple uncovered by chance in the park of Villa Batthyany near Gorla (in the province of Milan).

This is an interesting contribution – both scientifically and historically-culturally – to the vast literature in landscape studies. The reader will discover new and significant knowledge through this unique book that escapes easy definition. Indeed, its originality renders the book special for the specific topic, and especially as a way to know more about hidden architecture that until a few years ago essentially unknown.

Francesco Ferrini

PIETRO PORCINAI A PISTOIA E IN VALDINIEVOLE. *Bucelli C.M.*, and *C. Massi* (eds.). Giardini e Paesaggio, Vol. 34. Leo S. Olschki, Florence (Italy), 2012, pp. XIV+376. ISBN 978-8822-6162-5. € 39.00.



Pietro Porcinai, one of the great landscapists, worked in Pistoia and the Valdinievole area from the early 1930s to the 1980s. As explained in the presentation of this volume, his concern for the social aspect of gardens - as a healthy environment for recreation and sport, as a place to play, as well as for urban living and as an extension for the suburbs - is particularly evident around Pistoia. Examination of his masterpieces in the province of Pistoia is fundamental in the study of his vast and important production. This volume aims to gain greater insights into the link between the artist and the Pistoia landscape, with the idea that the matrices and points of reference for many of his projects in the area come from the atmosphere around him, and is a further testament to the role Porcinai's work has played, and continues to play in garden design.

Supported by the experience and careful attention of the two editors, the book is a valuable guide to the work of this landscapist. Through its interesting text, personal stories, drawings, past and present images, the volume is also a fascinating work of art in itself and will be appreciated as an enrichment of the current literature on this topic, meeting the needs of technicians and students and an ever-increasing number of enthusiasts. Clearly, the meticulous genius of Porcinai shines through, demonstrating that there is still much to know about him and his important work.

Francesco Ferrini