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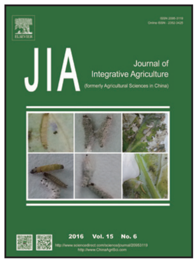

RESEARCH ARTICLE

Accumulated chilling hours during endodormancy impact blooming and fruit shape development in peach (*Prunus persica* L.)

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Abstract

Winter chill is essential for the growth and development of deciduous species. To understand the relationship between accumulated chilling hours during endodormancy and blooming and fruit shape development, we controlled chilling hours and investigated their effects on blooming date and fruit shape of peaches. The results showed that the number of days to full bloom date and the heat requirement for blooming were negatively correlated with accumulated chilling hours. Accumulated chilling hours were significantly negatively correlated with fruit shape index and fruit tip lengths, suggesting that the number of chilling hours affect the fruit shape development. Fewer accumulated chilling hours may be the major reason for longer fruit shape and protruding fruit tips. In conclusion, our results indicate specifically that decreased winter chilling hours can delay the bloom date and may lead to aberrant fruit shape development in peaches. Our study provides preliminary insights into the response of temperate fruit species to global climate change.

Keywords: accumulated chilling hours, blooming, fruit shape, endodormancy, *Prunus persica*

1. Introduction

Temperate tree species may establish dormancy to avoid suffering damage from cold temperatures in winter or early spring (Rohde and Bhalerao 2007; Fan *et al*. 2010). Dormancy is a typical adaptive trait of deciduous fruit species shaped by natural selection. The majority of deciduous species must meet a chilling requirement (CR) in order to break dormancy and a heat requirement (HR) in order to bloom (Campoy *et al*. 2012). The CR may differ by species, varieties, or growing regions (Wang *et al*. 2012). CR is the major factor that determines the bloom date (Egea *et al*. 2003; Ruiz *et al*. 2007; Alburquerque *et al*. 2008), which is an important agronomic trait affecting fruit development in temperate fruit trees. Cultivars with low CRs always bloom and ripen earlier, whereas those with high CRs bloom and ripen later (Scorza and Okie 1990). CR constrains the suitable areas of cultivation of many commercially important tree species and cultivars around the world. Previous studies have indicated that CR is a quantitative character controlled by at least one major gene (Hauagge *et al*. 1991). However, we know little about the molecular mechanism of CR regulation, and the genes that determine CR remain unknown. Some quantitative trait loci (QTLs) associated with CR have been found, including a major QTL at G1 in peach that explained 40.5–44.8% of the phenotypic variance (Fan *et al*. 2010).

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Although fruit shape development is determined primarily by genotype, it is also influenced by hormones and many climate factors. High temperature often induces long fruit shape, whereas low temperature induces round fruit shape (Sherman *et al*. 2003). Peach fruits that develop in high-temperature regions always show a higher incidence of protruding tips and pronounced sutures than do those that develop in low-temperature regions (Topp and Sherman 1989; Salvador *et al*. 1998). Topp and Sherman (1989) analyzed peach fruit quality at 13 locations from north to south Australia and confirmed that higher average temperatures during the coldest month are significantly correlated with increased fruit tip growth in most cultivars. A similar study reported that fruit shape index and fruit tip length of the same cultivars were greater in southern Florida than in northern Florida for the same varieties (Wert *et al*. 2007). Furthermore, the high-CR varieties showed much longer fruit tips than low-CR varieties (Wert *et al*. 2007). Rouse and Sherman (2002) indicated that inadequate chilling or prolonged endodormancy induced the elongation of the fruit tip. The fruits of some genotypes were round in cool locations (900 CU; CU, chill units, the unit used in the Utah model) but became significantly pointed when they were grown in warmer locations (200 CU) (Topp *et al*. 2008).

Similar fruit shape changes have also been observed in protected cultivation of peach and have seriously decreased the commercial quality of fruit. As global warming worsens, it poses a threat to deciduous fruits production. The number of accumulated chilling hours (CHs) in winter has gradually declined, which may seriously threaten the fulfillment of the CR among high-CR cultivars in some regions, subsequently affecting the fruit development (Campoy *et al*. 2011). To investigate the effects of declined winter chill on fruit development, chilling hours were controlled and the effects on fruit shape development and blooming were assessed. Our goal was to supply the theoretical basis for solving the potential threat posed to fruit development by global warming.

2. Results

2.1. Low winter chilling hours delayed bloom date

To confirm how the winter accumulated chilling hours affected the spring events, we investigated the full bloom date after different numbers of controlled chilling hours. In each case, we used the number of days that elapsed between moving into the greenhouse (or forcing temperature conditions) and reaching full bloom, also known as the days to full bloom date, to verify whether the treatments affected blooming. Both the days to full bloom date and heat requirement were negatively correlated with chilling hours (Fig. 1-A). The days to full bloom date showed a decreased trend of 16 days per 200 CHs as accumulated chilling hours increased (P<0.01) (Fig. 1-B). However, the days to full bloom date no longer declined when the accumulated chilling hours reached approximately 950 CHs (Fig. 1-A). Meanwhile, heat requirement showed a similar negative trend with days to full bloom date which also became stable when chilling hours greater than 950 CHs (Fig. 1-A). This finding indicated that the lower number of accumulated chilling hours could lengthen the days to full bloom date and increase the heat requirement. Prolonging the accumulated chilling hours within a certain range (<950 CHs) was correlated with the

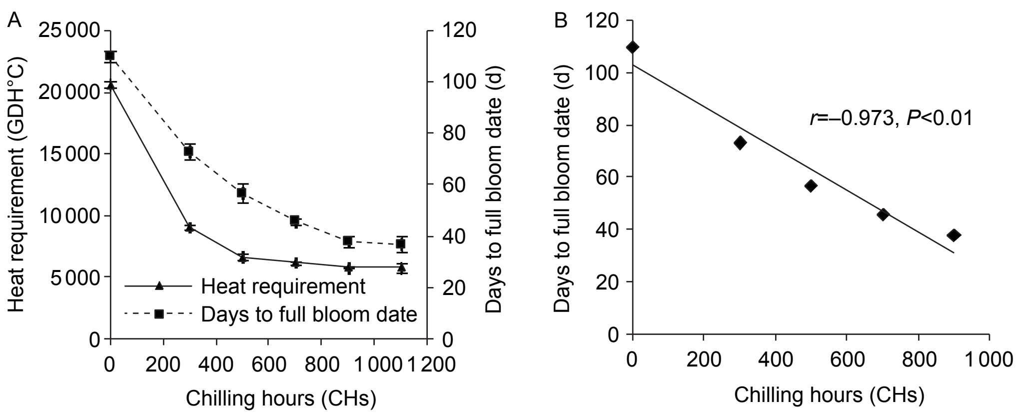

Fig. 1 Relationship between chilling hours and heat requirement and number of days to full bloom date. A, negative correlation between number of accumulated chilling hours (CHs) and number of days to full bloom date or heat requirement. The solid line indicates the number of days from moving into the greenhouse to full bloom date. The dotted line indicates the heat requirement. GDH°C indicates growing degree hours °C. Error bars indicate the standard deviation. The same as below. B, regression analysis between chilling hours and number of days to full bloom date. The regression analysis excluded the 1 100 CHs treatment because the days to full bloom date remained stable for accumulated chilling hours greater than approximately 950 CHs, as shown in Fig. 1-A. The same as below.

advance of blooming and the decrease of heat requirement.

2.2. Effects of accumulated chilling hours on fruit shape

We often use the number of days post full bloom date (dpb) to indicate the period of fruit development. As shown in Fig. 2, the fruits that developed in the 0, 300, and 500 CHs treatment groups were oblong at 20 dpb. The tips of fruits in those three treatments were longer than those of fruits in other treatment groups. Fruits in other treatment groups appeared ovate at the same period and were noticeably rounder than those from the 0, 300, and 500 CHs treatment groups. With the process of fruit development period (FDP), fruit shapes of every treatment became shorter gradually. Fruits in the 0, 300, and 500 CHs treatment groups were longer in shape and had longer tips than those of other groups, from 20 to 80 dpb. Fruits in the 700, 900, and 1 100 CHs treatment groups were rounder than those in the 0, 300, and 500 CHs treatment groups, from 60 to 80 dpb. These findings indicated that fruit shape was influenced by winter accumulated chilling hours. Lower accumulation of winter chilling hours generated a longer fruit shape than that of higher accumulation of winter chilling hours. Furthermore, fewer days of fruit development were associated with smaller differences between treatments with insufficient CR and other treatment groups.

2.3. Effects of accumulated chilling hours on fruit shape indexes

The fruit shape indexes of all treatments peaked at 20 dpb. Fruit shape indexes decreased as the FDP progressed and stabilized around 70 dpb (Fig. 3). As shown in Fig. 3, throughout the FDP, the fruit shape indexes of the 0 and 300 CHs treatment groups were significantly greater than those of other treatments. The maximum value of fruit shape index at 20 dpb occurred in the 0 CH treatment group. At 30 dpb, the fruit shape indexes of the 0, 300, and 500 CHs treatment groups were greater than those of other treatments. At 40 dpb, the fruit shape indexes of the 0, 300, 500, and 700 CHs treatment groups were greater than those of other treatments. From 50 to 80 dpb, the fruit shape indexes of the 0, 300, and 500 CHs treatment groups were all greater than those of other treatments (Fig. 2).

Our results indicated that insufficient CR was correlated with an increase in the fruit shape index. We confirmed that a negative correlation existed between accumulated chilling hours and fruit shape index (r=–0.962, P<0.01) at harvest maturity stage (80 dpb) (Fig. 4-A). Fewer accumulated

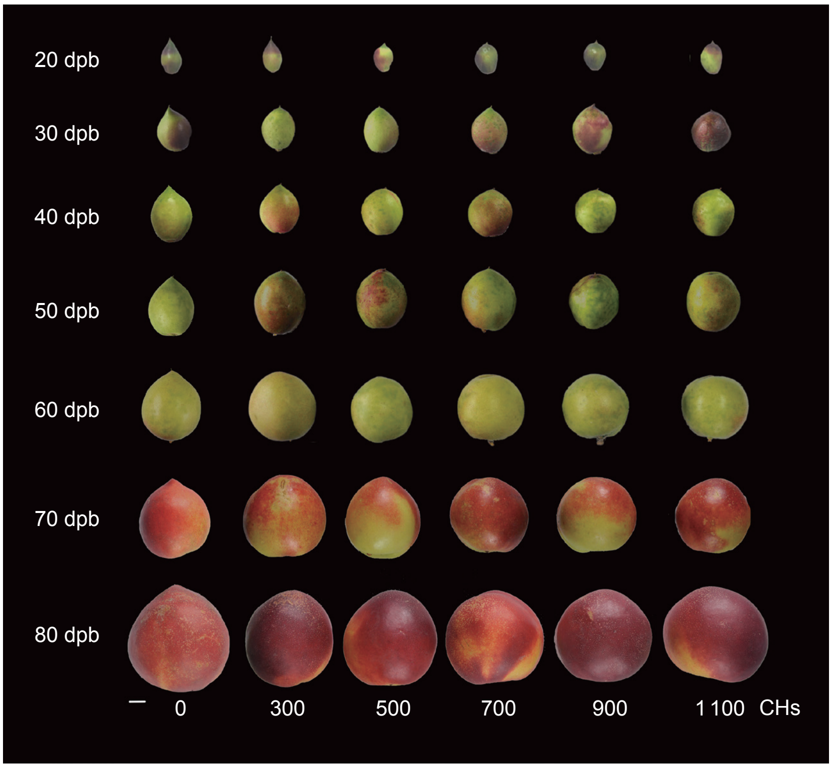

Fig. 2 Fruit shape of peaches in different treatment groups with different fruit development periods. dpb, days post full bloom date. The same as below.

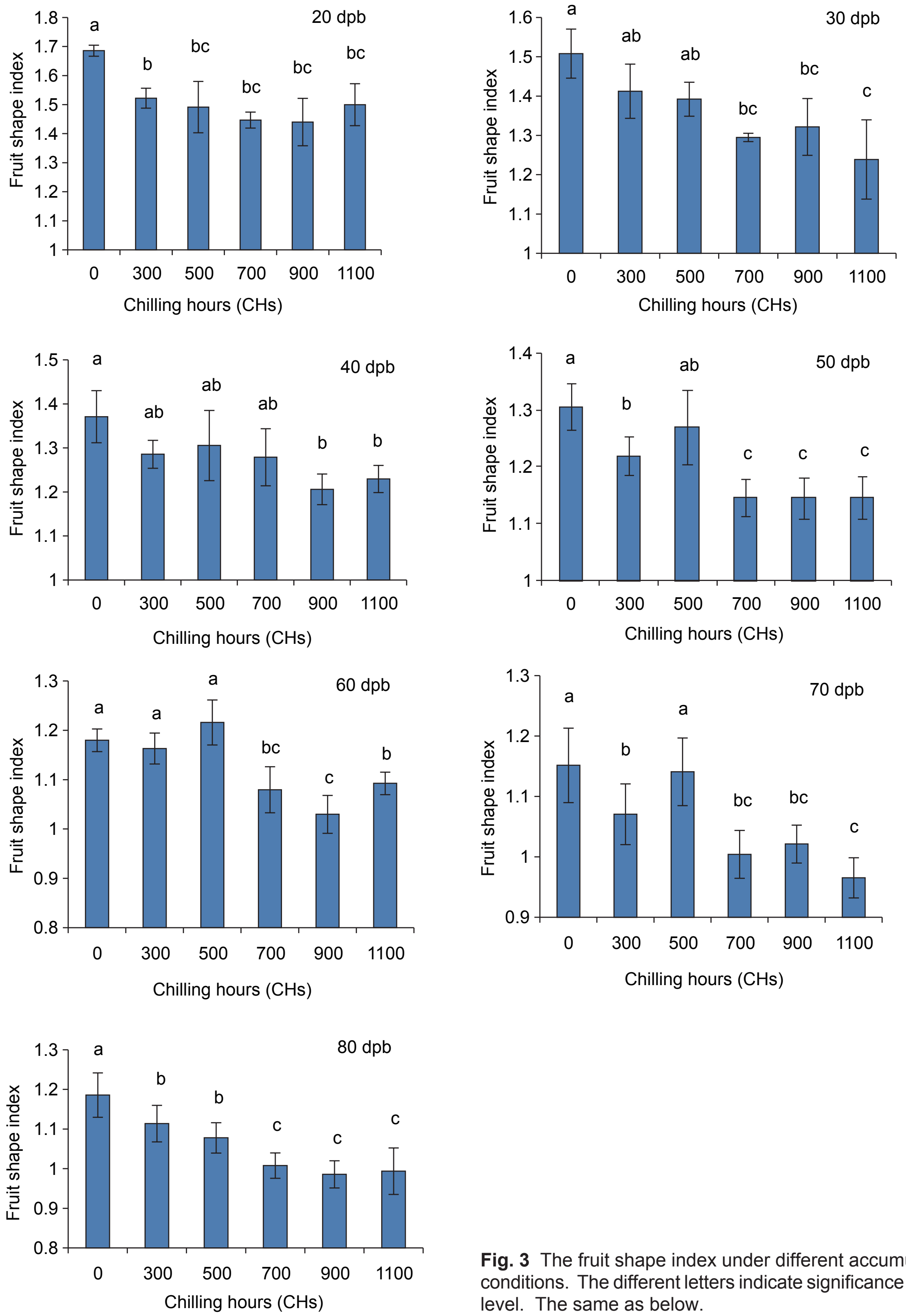

Fig. 3 The fruit shape index under different accumulated chill conditions. The different letters indicate significance at the 0.05 level. The same as below.

chilling hours were associated with longer fruit shapes. The significant correlation between accumulated chilling hours and fruit shape indexes indicated that a low accumulation of chilling hours was correlated with longer fruit and increased fruit shape indexes. Insufficient CR may be one of the causes of longer fruit shape.

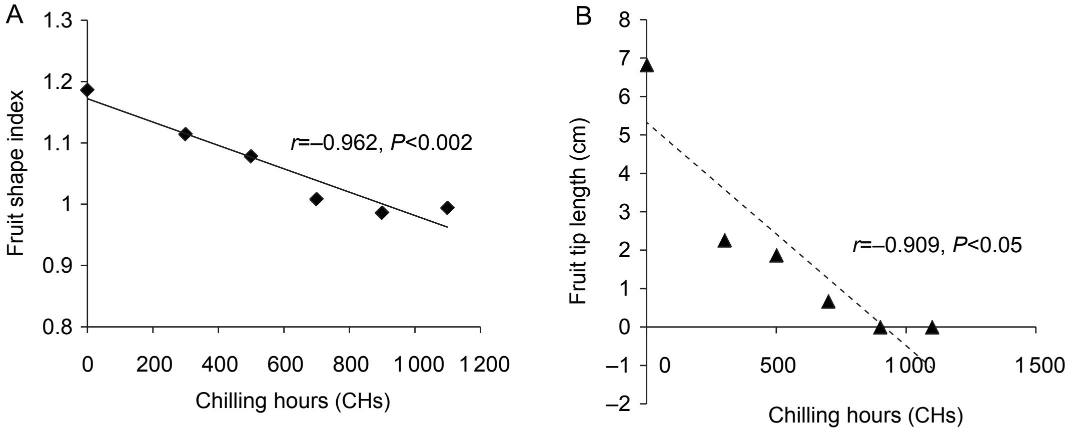

Fig. 4 Significant negative trend according to regression analysis between fruit shape index and accumulated chilling hours (A, $P<0.01$) and between fruit tip length and accumulated chilling hours (B, $P<0.05$).

2.4. Effects of accumulated chilling hours on fruit tip length

As shown in Fig. 5, the fruit tip lengths differed in different treatments. Notably, the 0 CH treatment produced the longest fruit tip throughout the fruit development period. During the process of fruit development period, the tip lengths of fruits in the 700, 900, and 1 100 CHs treatment groups decreased rapidly, especially from 50 to 80 dpb. Eventually, the tips of fruits in the 700, 900, and 1 100 CHs treatment groups were barely visible, especially at fruit maturity. A significant negative correlation was established between accumulated chilling hours and fruit tip lengths ($r=-0.909$, $P<0.05$) (Fig. 4-B). These results indicated that insufficient CR or fewer accumulated chilling hours was correlated with an increase in fruit tip lengths and reduced quality of appearance in fruit, which are serious problems for long-distance transport and sale.

3. Discussion

CR is a quantitative character that is controlled by at least one major gene (Hauagge and Cummins 1991; Tzonev and Erez 2003). It is difficult to determine an appropriate criterion to evaluate whether the CR is satisfied. For instance, a widely used method for determining the peach CR is to record the chilling hours at which the bud break rate reaches 50% or the bud break weighted average is equal to or greater than 2.5 after about 3 weeks of forcing temperature. The accumulated chilling hours to meet this standard are defined as the CR of a given peach variety. However, the basis of this determined criterion is the expected production quantity and economic value of the fruit. In order to maintain high efficiency of production and yield an economic profit, it is important to determine the minimum CR, which may be less than the physiological CR. Although the minimum CR is able to break endodormancy, promote normal growth and development, and achieve the expected yield, generally the intrinsic fruit quality is not optimal. We could define this CR as the minimum critical CR, but it is not the optimal CR for peach and does not meet the physiological chilling requirement. Therefore, the minimum CR is sufficient to meet expected production, but with a trade-off in terms of poor fruit quality, such as protruding fruit tips. Peach fruits were normal in open cultivation systems in temperate regions because the CR was excessive there.

Determining the CR accurately remains a challenge. First, it is difficult to differentiate between paradormancy and endodormancy (Faust *et al*. 1997). Secondly, the beginning of the accumulated chilling hours cannot be determined precisely. Thirdly, different species show different sensitivities to chilling temperature. In addition, endodormancy is affected by many factors, such as tree nutritional level, that also affect the CR (Fan *et al*. 2010).

In the present study, we found that the fewer winter chilling hours were correlated with longer fruit shape and protruding fruit tips, which was also noted by previous similar studies in regions with warm winters (Topp and Sherman 1989; Wert *et al*. 2007; Topp *et al*. 2008). We confirmed that the fewer number of chilling hours was the potential major reason for longer fruit shape. In protected cultivation systems, farmers always elevate the temperature in the greenhouse early in order to get early ripening. Although the conditions in the greenhouse might satisfy the minimum CR for peach, they do not meet the physiological CR and subsequently cause a longer fruit shape.

In addition, farmers always harvest the fruits when they are 70% of maturity, in order to bring them to market earlier and reap higher profits. Our data show that the earlier the

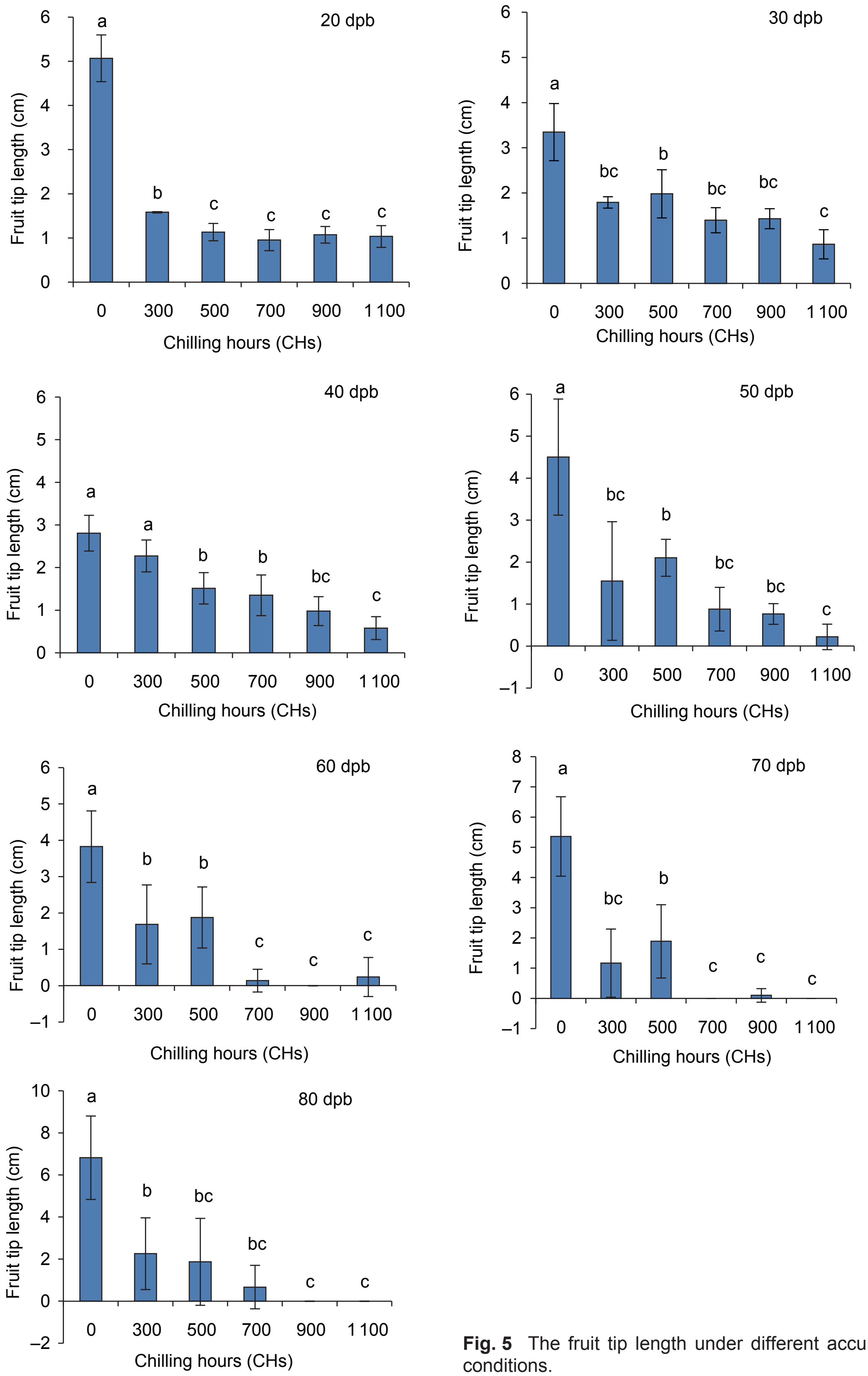

Fig. 5 The fruit tip length under different accumulated chill conditions.

harvest time or FDP, the longer the fruit shape (Fig. 2). For these two reasons, peaches grown in protected cultivation systems are longer in shape and have protruding fruit tips than peaches grown in a natural environment. As China is a big consumer of peaches, long-distance transport of these fruits is of great importance. However, longer fruit shape and protruding fruit tips complicate long-distance transport. To overcome this problem, prolonging the accumulated chilling hours and rationalizing the harvesting time may be two available solutions to gain the normal fruit shapes.

The global climate has warmed by approximately 0.6°C over the past 100 years with two main periods of warming, between 1910 and 1945 and from 1976 onwards (Walther *et al*. 2002). Previous studies have observed the decrease in winter chill and the resulting changes in phenological events (Menzel *et al*. 2001, 2006). Not only phenological events but also the plant growth and development are also sensitive to temperature changes. The present study reports the responses of peach to fewer winter chilling hours to supply evidence for combating climate changes through an environmentally controlled experiment. The physiological and molecular mechanisms by which chilling hours affect fruit shape remain unclear and need to be studied further.

4. Conclusion

Winter accumulated chill plays an important in dormancy breaking in deciduous species. If the CR is not satisfied, growth and development may be impaired. Here, we found that a lower number of accumulated chilling hours could be the possible reason for longer fruit shape and protruding tips in peaches.

5. Materials and methods

5.1. Plant materials and growth conditions

We used potted Zhong Nong Jin Hui peach (*Prunus persica* L.) as the plant materials in the present study. The peach trees were grafted using the *Prunus davidiana* as rootstocks in 2010 at Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences (ZFRI) (N34.71°, E113.70°, 74 m a.s.l.). The study was completed at the ZFRI from 2012 to 2014. The cultivar CR of the Zhong Nong Jin Hui peach is approximately 600 CHs (Wang *et al*. 2012). The period of fruit development, also known as the number of days from full bloom date to fruit ripeness, was 80 d (Wang *et al*. 2012). The pots we used were 60 cm in diameter and 80 cm in height. The trees were planted in a mixed matrix of peat:vermiculite:perlite:sand:cow dung=4:1:1:2:1. A total of six treatments were prepared with different numbers of accumulated chilling hours: 0, 300, 500, 700, 900, and 1 100 CHs. Every treatment contained 15 trees. All treatments adopted the same management pattern. All treatments were moved into the intelligent greenhouse (maintained at 10–25°C, 16:8 h light:dark) when they had satisfied the expected accumulated chilling hours.

5.2. Accumulated chilling hours and heat requirement tests

The plant materials were exposed to natural cold to satisfy the expected accumulated chilling hours. Accumulated chilling hours were recorded using the temperature automatic recorder (LOGGER 95-4, zjlogger.testmart.cn). The chilling hours were detected using a 0–7.2°C model (excluding 0°C) that was demonstrated to be suitable at ZFRI (Weinberger 1950; Wang *et al*. 2003). The heat requirement was estimated using a growth degree hours model (Anderson *et al*. 1986).

5.3. Bloom date tests

We investigated the full bloom date of all treatments. The full bloom date was defined as the date on which 25% of flowers were completely open. The days to full bloom date referred to the number of days from moving into the greenhouse to the full bloom date.

5.4. Morphological phenotype tests

We measured the fruit vertical diameter, fruit cheek diameter, and fruit tip length using a Vernier caliper from 20 to 80 dpb. A total of 10 peach fruits at the periphery and the middle of the tree crown were randomly selected for measurement from every treatment group. We also took pictures of peach fruit every 10 days from 20 to 80 dpb. Fruit shape index was calculated using the following formula.

$$\text{Fruit shape index}=\frac{\text{Fruit vertical diameter}}{\text{Fruit cheek diameter}}$$

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