J. Amer. Soc. Hort. Sci. 127(3):333–336. 2002.

Chilling Requirement and Postrest Heat Accumulation in Peach Trees Inoculated with Peach Latent Mosaic Viroid

Philip G. Gibson[1] and Gregory L. Reighard
Department of Horticulture, Clemson University, Clemson, SC 29634

Additional index words. *Prunus persica,* bloom delay, chill hours, base temperature, Q_{10}, dormancy

Abstract. **Peach [*Prunus persica* (L.) Batsch (Peach Group)] trees bloom in response to chilling and postrest heat accumulation. The peach cultivar Coronet exposed to a graft-transmissible, infectious agent known as peach latent mosaic viroid (PLMVd) blooms at a different time than noninoculated trees of the same cultivar. To determine if chilling requirements differed between trees inoculated with PLMVd and noninoculated controls, fruiting shoots collected from the orchard and artificially chilled containerized trees were forced in a greenhouse. Additional artificially chilled containerized trees were forced under constant temperatures in growth chambers to determine if postrest heat accumulation requirements differed. There was no difference in the chilling requirement of the fruiting shoots collected from the field although the shoots exposed to PLMVd had a delayed response and fewer responded to greenhouse forcing conditions. The containerized trees also showed no differences in chilling requirements during winter 1999 or 2000. Trees inoculated with PLMVd had a significant delay in bloom. Growth chamber data revealed a significantly higher base temperature for heat accumulation in the PLMVd inoculated trees.**

Peach [*Prunus persica* (L.) Batsch (Peach Group)] growers in the southeastern United States frequently suffer crop losses due to late spring frosts, which kill the flower buds and fruitlets. Early-blooming cultivars are at the greatest risk of loss from late winter or early spring freezes. In peach, chilling and heat accumulation interact to control time of bloom (Richardson et al., 1975). Exposure to low temperatures or chilling of dormant peach buds is a physiological requirement to overcome endodormancy imposed by internal mechanisms within the bud (Faust et al., 1997). The requirement for postdormancy heat accumulation has been shown to influence the time of bloom in many temperate-zone fruit trees (Rattigan, 1986; Spiegel-Roy and Alston, 1979; Tabuenca, 1980; Tabuenca et al., 1972; Wilson et al., 1975).

Several metabolic processes associated with release from dormancy are temperature dependent (Petel et al., 1992; Siller-Cepeda et al., 1992). During release from dormancy, temperature is the dominant factor regulating growth (Fuchigami and Nee, 1987). Temperature and metabolic rate are closely related. The rate of enzyme-catalyzed reactions in relation to temperature can be characterized by the Arrhenius Equation, which describes the energy of activation (Segel, 1976). The temperature coefficient, Q_{10}, is the factor by which the rate of reaction increases with a 10 °C increase in temperature. The Q_{10} can be calculated from the Arrhenius Equation. A modification of the equation, known as Van't Hoff's reaction rate/temperature rule, has been adapted to describe reaction rates and Q_{10} values of physiological processes in plants (Larcher, 1980). Q_{10} values are valuable as a measure of a plant's rate of development above its base temperature, which can provide an explanation for the timing of bloom after rest completion (Werner et al., 1988). Consequently, an analysis of the chilling and postrest heat accumulation requirements can provide a mechanistic explanation for closely related tree genotypes with differing bloom times (Werner et al., 1988).

'Ta Tao 5' is infected with peach latent mosaic viroid (PLMVd) and the viroid is graft-transmissible (Diener, 1987). When buds from 'Ta Tao 5' are grafted onto the scaffold branches of mature peach trees, the time of bloom is delayed as compared to the nonexposed trees of the same cultivar (Reighard 1995, 1998). Such a delay may be beneficial in an effort to avoid crop losses due to freezing temperatures during bloom. The graft-transmissible effects of 'Ta Tao 5', which cause delay in bloom, could be related to variations in chilling requirement, postrest heat accumulation, or both. Alterations in dormancy physiology may be attributed to the presence of infectious agents like PLMVd. As a result, detection of PLMVd should be incorporated into studies involving graft-transmissible effects from 'Ta Tao 5'. Therefore, the objective of this research was to evaluate the chilling and heat accumulation requirements of 'Coronet' peach inoculated with PLMVd using 'Ta Tao 5' buds.

Materials and Methods

Chilling requirement. Greenhouse forcing of field-collected shoots was used to determine the chilling requirement of 'Coronet' peach trees inoculated with PLMVd using 'Ta Tao 5' chip buds (inoculated) in Aug. 1997. The chilling requirement for 'Coronet' peach is 750 to 900 h (Werner and Williams, 1985). Terminal shoots 25 to 35 cm in length were collected at 7 d intervals for 7 weeks beginning 8 Jan. 1999, from 5-year-old, dormant, noninoculated control 'Coronet' trees and inoculated 'Coronet' trees. The trees were planted in a high-density, Y-trained orchard system at the Musser Fruit Research Center near Clemson, S.C. Two shoots were removed from two trees in each of the 12 inoculated blocks and 12 noninoculated control blocks. Six shoots were placed in 250 mL glass beakers with 180 mL of Floralife solution (Floralife, Inc., Walterboro, S.C.) at 9 $g \cdot L^{-1}$ and forced in a greenhouse at days/nights of 25/13 °C with natural photoperiod and irradiance. Basal ends of the shoots were recut and the solution changed weekly. Bloom stage was recorded every other day. Chilling was considered satisfied when 50% of the flower buds had opened after 28 d of forcing. Days to bloom was recorded as the number of days required for 80% of the flower buds to reach full bloom stage.

Received for publication 27 Dec. 2000. Accepted for publication 8 Feb. 2002. We thank David Ouellette, Kathy Brock, and William Newall, Jr., for technical assistance.
[1]Current address: Gwinnett Technical College, 5150 Sugarloaf Parkway, Lawrenceville, GA 30046; e-mail Pgibson@gwinnett.tec.ga.us.

Artificially chilled, containerized trees of noninoculated control 'Coronet' and 'Coronet' inoculated with PLMVd using 'Ta Tao 5' T-buds were forced in a greenhouse during Winter 1998–99 and Winter 1999–2000 to determine chilling requirements. One-year-old 'Coronet' peach trees on 'Lovell' seedling rootstocks were grown in 11.4 L, black plastic containers with a medium of 4 Fafard peat-lite mix (Fafard, Anderson, S.C.) : 1 river sand (by volume) with pelletized, dolomitic lime at 2.4 kg·m^{-3}. The trees were grown outdoors on gravel under drip irrigation with 30 mg of 14N–4.2P–11.6K Osmocote slow-release fertilizer (Scott's, Inc., Marysville, Ohio) applied per container as topdressing on 1 May, 15 June, and 1 Aug. of each growing season. On 15 June 1998, half of the trees were randomly selected to receive PLMVd inoculation consisting of two 'Ta Tao 5' T-buds grafted onto the stem near the base of the 'Coronet' trunk.

Trees were removed after leaf drop and chilled artificially in a dark, walk-in cooler at a constant temperature of 6 °C. Four of the inoculated trees and four of the controls were removed from chilling at 100-h intervals ranging from 400 to 1000 h in 1999 and 100 to 1100 h in 2000. The first year's chilling treatments, with the exception of the 400 chilling hour treatment, constituted a sufficient amount of the chilling requirement to allow normal growth during the following growing season. Trees were forced in a greenhouse maintained at days/nights of 25/13 °C with natural photoperiod and irradiance. Bloom stage was recorded every other day until all trees reached post bloom. Days to bloom was recorded as the number of days required for 80% of the flower buds to reach full bloom stage.

Heat accumulation requirement. In Feb. 1999, containerized trees grown and chilled artificially as described previously for 1000 h in a dark, walk-in cooler at a constant temperature of 6 °C were forced in (CMP 3244; CONVIRON, Pembina, N.Dak.) growth chambers. Trees were forced under a 9-h photoperiod at 10.0, 15.5, 21.0, or 26.5 °C, with four inoculated and four noninoculated control trees at each temperature. Photosynthetic photon flux (*PPF*) provided by cool-white fluorescent and incandescent lamps was 400 μmol·m^{-2}·s^{-1} at plant level as measured with a line quantum sensor (LI-191SA; LI-COR, Lincoln, Nebr.) Bloom stage was recorded every other day until 100% of the flower buds reached petal fall.

The number of days necessary to reach 80% budbreak (W) was determined for each tree at each temperature. The reciprocal transformation, 100/W was used in data analyses. Arnold (1959) and Campbell and Sugano (1975) have used this transformation, which represents the daily average rate of development (DARD) toward budbreak. For example, if it takes 20 d to reach 80% budbreak at a given temperature, the DARD = 100/20 = 5% per day = 5 DARD. DARD values were calculated for inoculated and noninoculated control trees at each forcing temperature. The base temperature for postrest heat accumulation could then be determined using the X-intercept method of Arnold (1959). Q_{10} values were determined to verify the response to temperature over the range of temperatures applied in this experiment (Larcher, 1980).

Table 1. Chilling requirement, the number of days required to bloom, and the proportion of shoots of 'Coronet' peach responding to forcing conditions of Winter 1999, field-collected shoots.[z]

Treatment	Chilling hours	Days to bloom	Shoots responding (%)
Control	812	20 a[y]	88 a
Inoculated	812	23 b	25 b

[z]All values are based on 24 shoots from each treatment.
[y]Mean separation within columns by unpaired *t* test at $P < 0.05$.

PLMVd detection. To determine the incidence of PLMVd in the trees, two leaf petioles from each tree were blotted onto Hybond N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.). The blotted membrane was sent to the National Research Support Project-5 Plant Virus Laboratory in Prosser, Wash., where a [^{32}P]-labeled cRNA probe was used to detect the presence of PLMVd in the blots (Shamloul, 1995).

Results

All trees used in this study inoculated with 'Ta Tao 5' T-buds tested positive for the presence of PLMVd, whereas none of the noninoculated controls had PLMVd.

Chilling requirement. The chilling requirement of the field grown trees was fulfilled on 5 Feb. 1999 for both the inoculated and noninoculated control trees based on greenhouse forcing of the field-collected shoots (Table 1). Chill hours, as determined by a modification of the Auburn chill hour model (Linvill, 1990) were 812 on 5 Feb. 1999. The number of days required for the field-collected shoots to respond to greenhouse forcing conditions was significantly longer for the inoculated trees when compared to the controls (Table 1). Additionally, the proportion of shoots responding to greenhouse forcing conditions was significantly reduced in the treated trees (Table 1).

The container forcing study conducted in Winter 1998–99 resulted in no significant variations in the response to chilling treatments between inoculated trees and the noninoculated controls (Fig. 1A). All trees bloomed in response to greenhouse forcing conditions with only the trees given 400 artificial chilling hours showing signs of low chilling. The inoculated trees and the noninoculated control trees removed from the cooler after accumulating 400 chilling hours had <5% of the flower buds breaking dormancy after 60 d of greenhouse forcing.

The container forcing study conducted in Winter 1999–2000 with the same trees, except the trees receiving <400 artificial chilling hours as described previously, showed completion of the chilling requirement corresponded to ≈700 artificial chilling hours with no significant difference between the two treatments (Fig. 1B). However, the inoculated trees required a significantly longer period of time to bloom after 700 artificial chilling hours than the controls (Fig. 1B). The delay in flower development continued through the remaining chilling treatments with the exception of the 1100 chilling hour treatment where the controls were delayed longer than the inoculated trees.

Heat accumulation. Inoculated trees were delayed significantly in the number of days to bloom at the 10 °C forcing temperature, but were not significantly different from the controls at 15.5, 21.0, or 26.5 °C (Fig. 2). Average DARD values for the treated trees ranged from 2.6 at 10 °C to 8.0 at 26.5 °C (Fig. 3). The DARD value at the x-intercept reveals a base temperature for heat accumulation of 2.1 °C for the inoculated trees. Average DARD values for the controls ranged from 3.0 at 10 °C to 7.7 at 26.5 °C (Fig. 3). The DARD value at the x-intercept revealed a base temperature for heat accumulation of –0.2 °C for the controls. Regression lines were analyzed and the slopes of the two lines were significantly different ($P < 0.001$). The lines intersect at a forcing temperature of 17.3 °C. Q_{10} values determined over the range of temperatures applied in this experiment indicate a significant and consistently higher rate of response to temperature for the inoculated trees (Fig. 4).

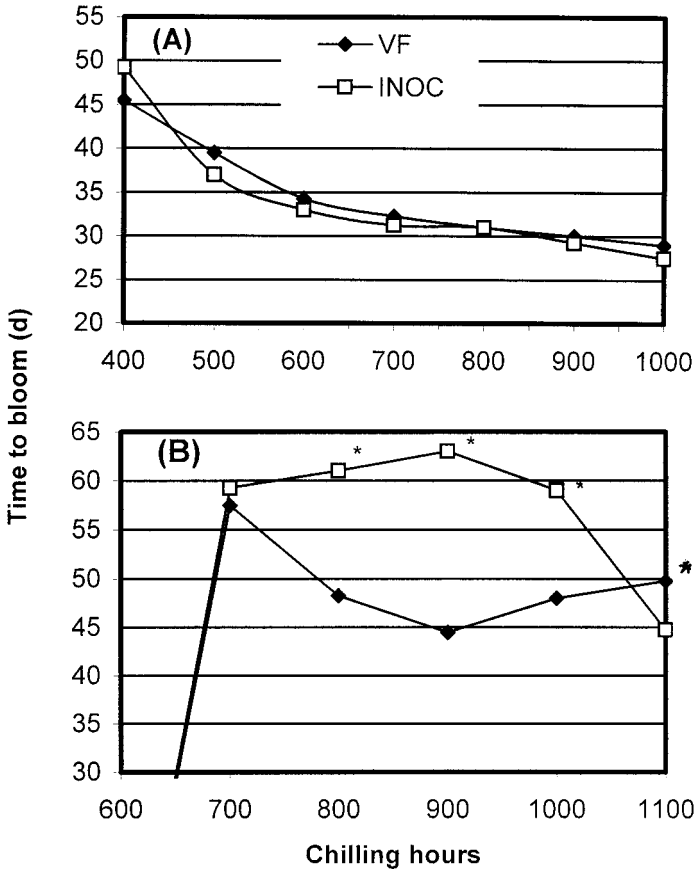

Fig. 1. (**A**) Time required for 'Coronet' peach to reach 80% full bloom in the container forcing experiment conducted Winter 1998–99. Chilling hours were artificial chilling hours accumulating 24 h each day in a cooler at 6 °C. VF = noninoculated controls and INOC = inoculated trees. All means (n = 4) were nonsignificant by an unpaired *t* test at $P < 0.05$. (**B**) Time required for 'Coronet' peach to reach 80% full bloom in the container forcing experiment conducted Winter 1999–2000. All data points (n = 4) >700 chilling hours were significant at $P < 0.05$ by an unpaired *t* test.

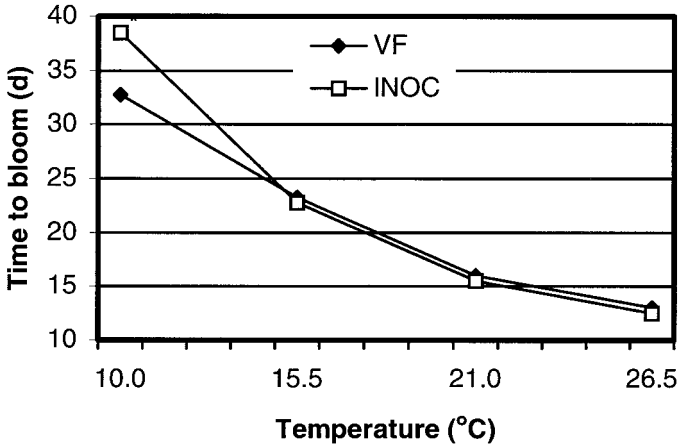

Fig. 2. Time required for 'Coronet' peach to reach 80% full bloom in the growth chamber. Trees received 1000 artificial chilling hours accumulating 24 h each day in a cooler at 6 °C. VF = noninoculated controls and INOC = inoculated trees. Differences at the 10 °C forcing temperature were significant at $P < 0.05$ by an unpaired *t* test. Data points (n = 4) for 15.5, 21.0, and 26.5 °C were nonsignificant.

Discussion

Results of these experiments suggest that the chilling requirement of 'Coronet' peach trees inoculated with PLMVd is not significantly different from that of the noninoculated control trees. The greenhouse forcing experiment on containerized 'Coronet' trees indicated the delay in bloom from inoculation with PLMVd does not occur until the second season after treatment. This suggests that a full growing season is required for PLMVd to influence the physiological processes controlling the time of breaking dormancy. A growing season delay in the appearance of effects from PLMVd is plausible because the incubation period of viroids have been shown to vary considerably with temperature (Diener, 1987), mineral nutrition (Weathers, 1960), and viroid strain (Raymer and Diener, 1969; Wallace, 1978).

Peach trees set flower buds in mid to late summer and growth control mechanisms begin to develop during paradormancy (Faust et al., 1997). The progress of bud development in the fall and the continued bud development postrest contribute to the timing of budbreak and full bloom. Lack of bud development in the fall may contribute to a greater requirement for bud development under forcing conditions. Additionally, breaking dormancy requires heat unit accumulation (Arnold, 1959) above a base temperature. Accumulation of heat units necessary to release buds is the growth-limiting factor during ecodormancy (Ashcroft et al., 1977; Richardson et al., 1975; Spiegel-Roy and Alston, 1979). Several models have been proposed to estimate the accumulation of the heat necessary to release peach buds from dormancy (Erez and Lavee, 1971; Richardson et al., 1975; Schwartz et al., 1997). The heat requirement is most often expressed as growing degree days (GDD) or growing degree hours (GDH) using 4.5 °C as the base temperature. Werner et al. (1988) recommends caution in making comparisons and interpretations using the base temperature 4.5 °C, since the temperature has not been verified experimentally. Significant differences in the base temperature for heat-unit accumulation have been documented in pear (*Pyrus communis* L.) (Spiegel-Roy and Alston, 1979), plum (*Prunus cerasifera* Ehrh.) (Tabuenca, 1980; Wilson et al., 1975), almond [*Prunus dulcis* (Mill.) D.A. Webb] (Rattigan and Hill, 1986; Tabuenca and Herrero, 1972), and western sand cherry (*Prunus besseyi* Bailey) (Werner et al., 1988). These studies indicate that variability

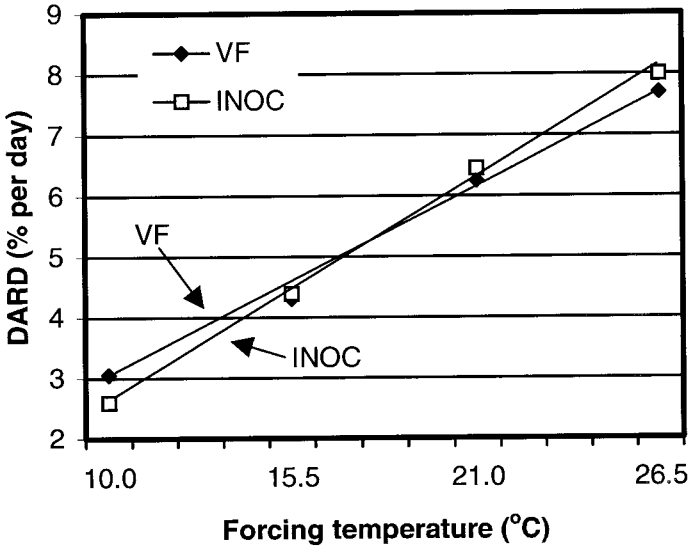

Fig. 3. Daily average rate of development (DARD) vs. forcing temperature (°C) after 1000 h of artificial chilling of inoculated trees (INOC) and noninoculated controls (VF) of 'Coronet' peach. Linear regression lines shown were generated from the formulas: INOC 80% DARD = –0.69 + 0.33 (forcing temperature), $r^2 = 0.99$. VF 80% DARD = 0.057 + 0.29(forcing temperature), $r^2 = 0.99$.

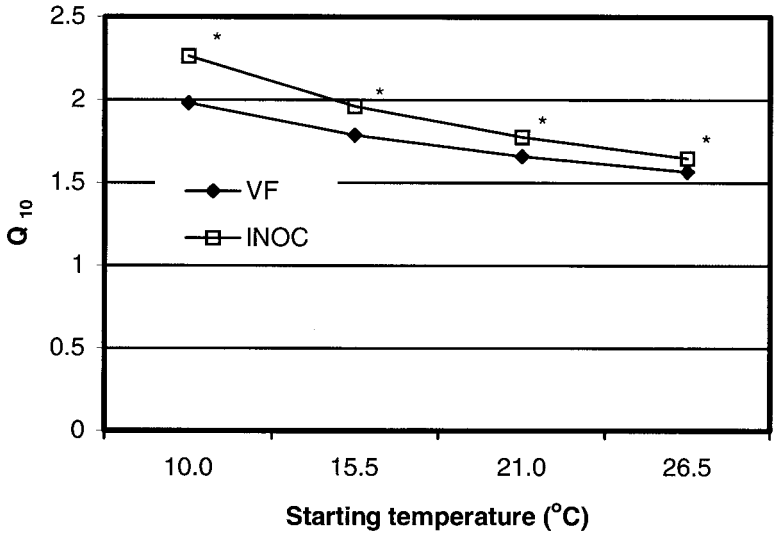

Fig. 4. Calculated Q_{10} values for the range of temperatures applied in this experiment. VF = noninoculated control and INOC = inoculated trees. All values (n = 4) were significant at $P < 0.003$ by an unpaired t test.

in heat-unit base temperature correlates positively with variations in time of bloom. Fewer heat units accumulate as the base temperature is increased (Thomson and Moncrieff, 1982). The calculated higher base temperature of the inoculated trees at 2.1 °C relative to the –0.2 °C base temperature established for 'Coronet' may explain the difference in response to forcing conditions.

The bloom delay in the field-collected shoot forcing study of 3 d, the average bloom delay of 8 d on the container forcing experiment in Winter 1999–2000, and the bloom delay in the growth chamber study being limited to the lowest temperature may be explained by differences in the Q_{10} values. The consistently higher Q_{10} value for the inoculated trees indicates a more rapid response to higher temperatures. Consequently, the amount of bloom delay observed in trees inoculated with PLMVd will increase under cooler temperatures, while the delay will decrease under higher temperature conditions following completion of rest. Scalabrelli and Couvillon (1986) found that increased chilling did not reduce the heat requirement, but increased the uniformity of chilling requirement among buds. The accelerated response to forcing by the PLMVd inoculated trees after 1100 h of artificial chilling is consistent with an increased uniformity among floral buds with a higher Q_{10} value.

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