Dealing with Frost Damage and Climate Change in Tree Fruit Crops

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Each year, the U.S. produces about 15 million tons of deciduous fruit crops that have a combined value of >$10 billion. Unpredictable cold damage to these nutritionally important crops is a major threat to industry profitability. Over the last 6 years, cold damage has accounted for almost half of the total value of insurance payments for crop loss to growers of the top five deciduous fruits (Table 1; www.rma.usda.gov). Recent events that occurred across large areas of North America include the "Easter freeze" (2007), "Mother's Day freeze" (2010), "killer frost" (2012), the "polar vortex" (2014), and the spring freeze events that occurred this year (2016). The scenario for these events generally included a period of unseasonably warm temperatures in early spring (March) inducing overwintering tree fruit crops to break bud and begin to grow, followed subsequently by low temperature events several weeks later (April) at a time when flowers and early vegetative growth had little to no frost tolerance. Unfortunately, the occurrence of erratic patterns of severe weather, including devastating spring frosts, is expected to increase as a direct result of global climate change, despite overall increases in mean temperatures (Gu et al. 2008). Finding approaches that will allow fruit growers to deal with this problem is a critical challenge.

> **"The overexpression of a CBF master regulator gene in apple can be used as a model system to better understand the genetic regulation of freezing tolerance, dormancy, and growth. Knowledge of these processes can lead to the development of strategies to adapt fruit trees to erratic weather patterns brought about by ongoing climate change."**

Recommendations for practices that mitigate cold damage sometimes lack much specificity and often vary in their proven effectiveness. Generally, they fall into two categories: passive and active. Passive methods include site, cultivar, and rootstock selection, and cultural practices that promote and/or prolong seasonal changes in the level of freezing tolerance. Knowledge of the response of different cultivars and rootstocks to fluctuating temperatures, however, is often lacking. Active methods to mitigate cold damage include the use of wind machines, sprinklers, and heaters. While various approaches to modify orchard temperatures are available, they are costly, sometimes impractical, and are often effective only during specific types of weather conditions, such as temperature inversions. The range of protection is also generally limited to two to three degrees below 0°C (32°F). The premise of passive and active approaches of mitigating cold damage can be summarized as trying to increase and/or prolong freezing tolerance or trying to prevent tree tissues from freezing, either by raising orchard temperatures or promoting supercooling (freeze avoidance - the ability of plant tissues to reach temperatures well below 0°C without freezing). Research at the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV has focused on both understanding the regulation and promotion of supercooling to avoid freezing and understanding the genetic mechanisms that regulate freezing tolerance and dormancy (Wisniewski et al. 2014a,b).

The ability to acclimate to freezing temperatures and undergo a period of dormancy is essential to the survival of temperate woody plants, including tree fruit crops. Seasonal regulation of growth and freezing tolerance is marked by developmental events such as growth cessation, bud set, the onset of dormancy, cold acclimation, deacclimation, and bud burst (Kalberer et al. 2006; Ibañez et al. 2010; Gusta and Wisniewski 2013) (Figure 1). Both cold acclimation and dormancy are regulated by a distinct set of changes in gene expression (Thomashow et al. 2001; Welling and

Table 1. Insurance payments for crop losses in U.S. apple, pear, peach, cherry, and grape.

Year	All causes (million $)	Cold damage	
		(million $)	(%)
2010	2,191	1,182	54
2011	131	43	32
2012	209	139	67
2013	114	56	49
2014	132	47	36
2015	131	41	31
Average	**485**	**251**	**45**

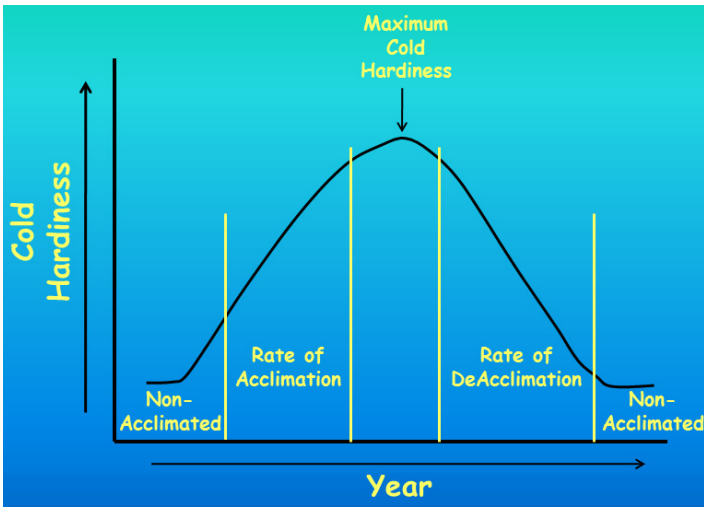

Figure 1. Seasonal changes in cold hardiness can be broken into different stages. Each one of the stages can be under different genetic control and uniquely inherited.

Palva 2006; Chinnusamy et al. 2010). A special class of genes called transcription factors regulate complex physiological and developmental processes in all living organisms through their ability to activate a large number of other genes that are responsible for controlling different aspects of metabolism and development. For example, transcription factors in plants can regulate growth, defense responses, ripening, flowering, and cold acclimation. The CBF transcription factor is a master regulator of cold responses in plants. It has been shown to be activated by low temperatures and then, in turn, activates a whole suite of genes that contribute in different ways to increasing freezing tolerance. A CBF gene from peach was isolated in our research program and overexpressed (constant expression) in M.26 apple rootstocks using apple transformation technologies in order to better understand the function of CBF genes in tree fruit crops. The transformed CBF transgenic apple line was designated as T166, while non-transformed control plants were designated simply as M.26 (Wisniewski et al. 2011).

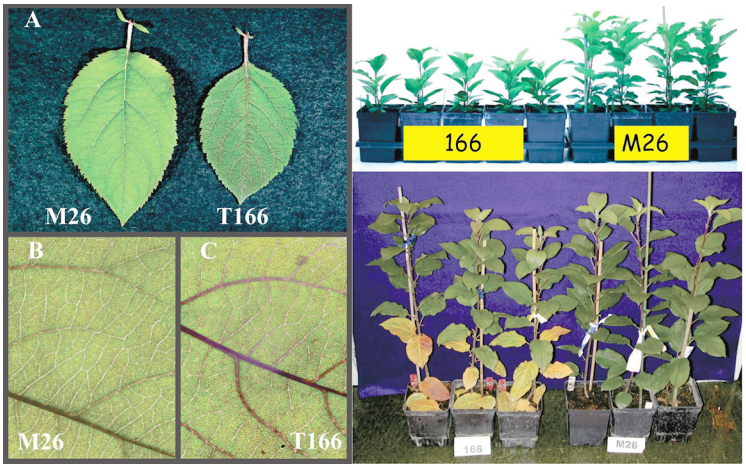

Figure 2. A comparison of the phenotype of non-transformed M.26 apple rootstocks and M.26 rootstocks transformed to overexpress a peach CBF gene, a master regulator of cold responses (T166). Left: CBF transgenic (T166) leaves accumulate higher levels of anthocyanins than non-transformed M.26 leaves in response to cold acclimation conditions. Top right: In general, CBF transgenic (T166) plants were smaller than M.26 plants. Bottom right: CBF transgenic (T166) trees exhibited terminal bud set and leaf senescence in response to exposure to cold acclimating conditions for two weeks (low, non-freezing temperatures and short photoperiod) and then returned to the normal growth conditions in the greenhouse, while non-transformed trees (M.26) returned to normal growth when they were returned to the greenhouse.

CBF Regulates Cold Hardiness, Growth and Dormancy in Apple

Overexpression of a peach CBF transcription factor gene in apple resulted in an increase in freezing tolerance in young trees whether they were in a non-acclimated or cold-acclimated state. The LT_{50} (the temperature at which 50% injury is observed as measured by electrolyte leakage) for leaves from non-acclimated CBF transgenic and non-transgenic M.26 trees was -9.5°C (14.9°F) and -5. 3°C (22.5°F), respectively, and -13°C (8.6°F) and -7.2°C (19°F), respectively, in acclimated trees. There was a higher accumulation of anthocyanins in CBF transgenic lines than in the untransformed M.26 trees, and the transformed trees were also initially smaller (Figure 2). While the impact on freezing tolerance and growth was expected based on earlier studies on the overexpression of *CBF* in herbaceous plants, the trees also exhibited an early onset of dormancy and leaf senescence (Figure 2). The early onset of dormancy could be triggered by short days without exposure to low temperatures, which was totally unexpected, since low temperatures rather than short days are required to induce dormancy in apple (Heide and Prestrud 2005).

Subsequent field trials were conducted over a three-year period in order to characterize the seasonal growth and development of the transformed trees under field conditions (Artlip et al. 2014). Results confirmed that CBF transgenic trees had increased levels of cold hardiness, relative to untransformed M.26 trees, entered dormancy and exhibited leaf senescence earlier than non-transformed M.26 trees (Figure 3), exhibited later budbreak (Figure 4), and reduced growth (Figure 5). A subsequent study (Wisniewski et al. 2015) demonstrated that the overexpression of the peach CBF gene in apple affected the expression of several cold-regulated genes that contribute to freezing tolerance, dormancy, and early bud break, as well genes that regulate the level of bioactive forms of gibberellic acid, which plays an important role in shoot growth. Cold-regulated genes are associated with increased freezing tolerance in a wide array of plant species (Chinnusamy et al. 2010). Dormancy-related genes have been extensively studied in peach and linked to chill unit accumulation (Jiminez et al. 2010), while the early bud break gene was reported to regulate the time of bud break in poplar (Yordanov et al. 2014). CBF expression has also been associated with genes that regulate gibberellic acid synthesis and breakdown (Achard et al. 2008) in several plant species. Based on the findings of our study, and the existing literature,

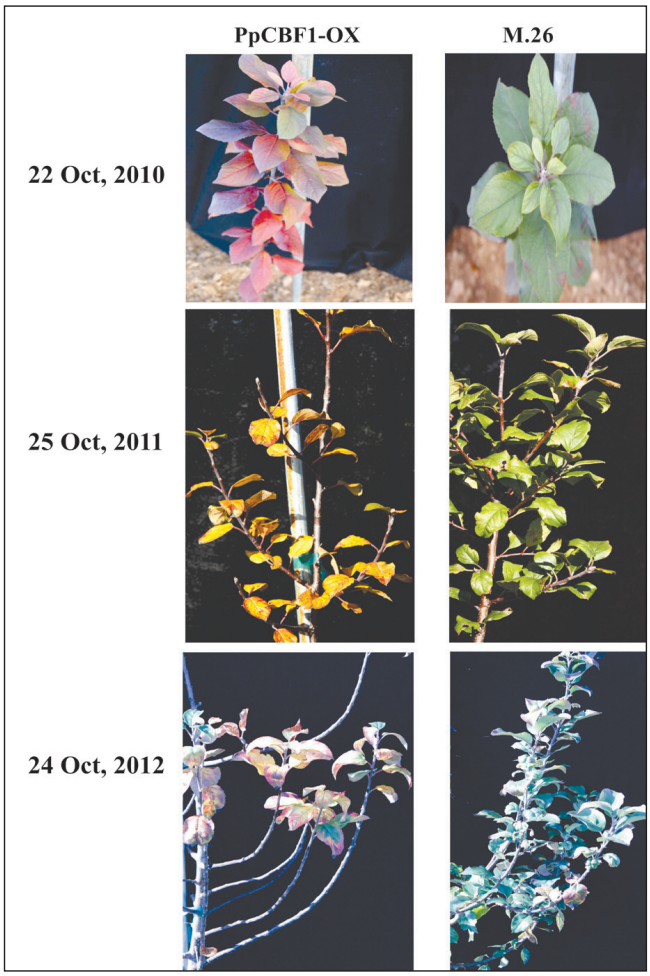

Figure 3. Early leaf senescence and leaf drop in transgenic M.26 apple rootstocks overexpressing a peach CBF gene (PpCBF1-Ox), relative to non-transformed M.26 rootstock trees. Photographs of field-grown trees.

a model was developed for apple suggesting how the CBF transcription factor could act as a master regulator of cold acclimation, dormancy, and growth (Figure 6). We are currently using the CBF transgenic line as a model system to investigate the regulation of seasonal changes in cold hardiness, dormancy, and growth in fruit crops.

An extension of this research was to determine if a transgenic rootstock could affect the physiology and development of a scion grafted onto the transgenic rootstock. It was hoped that the desirable attributes, such as the delayed bud break and increased freezing tolerance, would be expressed in any scion cultivar that was grafted on to that transgenic rootstock. A three-year, reciprocal grafting study was conducted using the CBF transgenic and non-transgenic M.26 rootstocks, and Royal Gala as a scion (Artlip et al. 2016). Unfortunately, the only statistically significant effect that was observed for the rootstock was growth suppression and a slight prolonging of the juvenile period prior to flowering. The onset and release from dormancy, and freezing tolerance were unaffected. This indicates that each cultivar would need to be independently transformed in order to see an effect on freezing tolerance and dormancy. Further studies, using the CBF transgenic line as an intergraft are being conducted.

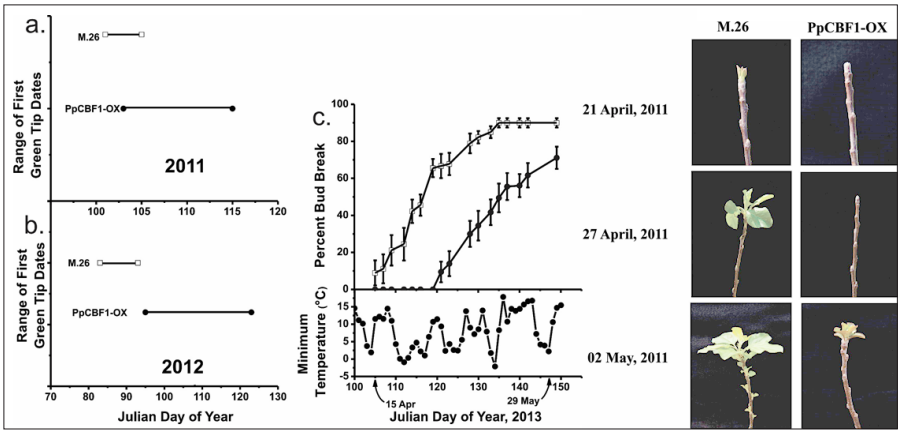

Figure 4. Delayed bud break in CBF transgenic M.26 apple rootstocks (PpCBF1-Ox) relative to non-transformed M.26 trees. Graphs represent data from 2011(a), 2012(b), and 2013(c): open symbols = M.26 trees and closed symbols = PpCBF1-Ox trees. Photo of representative shoots in 2011.

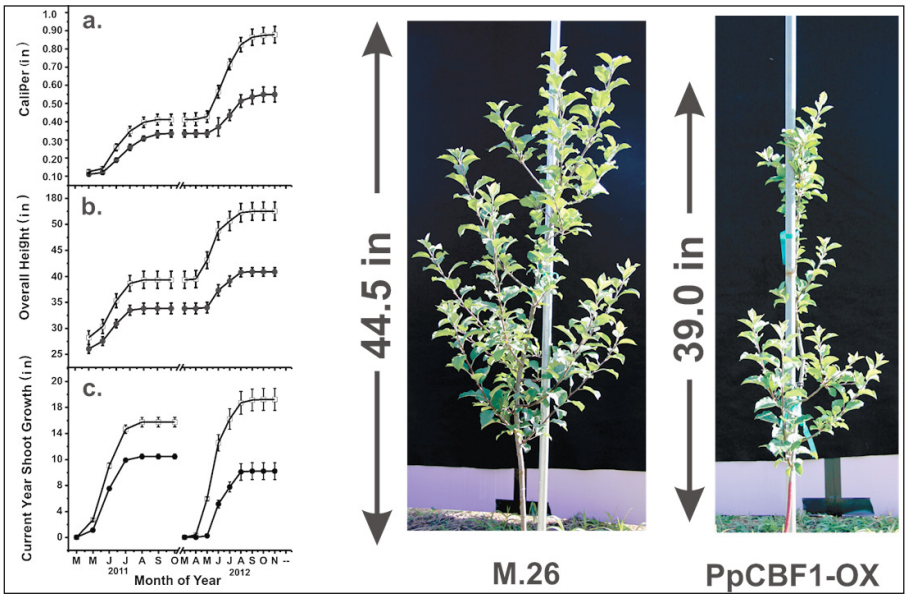

Figure 5. Overexpression of a peach CBF gene in transgenic M.26 reduces growth in field-planted trees compared with growth in non-transformed M.26 trees. Overall height, stem diameter (caliper), and current-year shoot growth were all reduced in the transgenic trees. Photos taken in spring, 2012.

Adapting Fruit Trees to Erratic Weather Patterns

Freeze damage has been a perennial concern to tree fruit growers. While climate warming, and the resulting trend of milder winters, appears to have reduced the threat of midwinter freeze damage in many fruit growing areas, it has brought with it a new set of potential problems. It is expected that climate change and the onset of higher temperatures and higher levels of carbon dioxide may change the life cycles and distribution of many insects and disease-causing microorganisms, and also affect the timing of seasonal developmental events, such as the onset and release of dormancy, and the ability to cold acclimate. Despite these pending problems, little information is directly available about how different fruit cultivars will react to these changes or what can be done to adapt trees to these changing conditions.

Erratic weather patterns, such as mild winters, early warming in the spring, followed by more seasonable or extreme low temperatures, can be expected to increase in frequency. These conditions will result in early bud break in many cultivars and the exposure of very frost-tender tissues (flowers and young shoots) to devastating freezing events, such as was experienced in the Mid-Atlantic States in the spring of 2016. What can be done to address this problem on a short-term and a long-term basis is a serious question.

To date, there are no reliable and effective management tools available to a fruit grower that can be easily applied to mitigate potential frost injury shortly prior to or after a significant freezing event. Even active approaches, such as the use of wind machines, sprinklers, heaters, etc. would have little impact if temperatures drop below -5°C (23°F) or below. One approach that could be used to address the problem would be to make trees more resilient to losses in cold hardiness and early bud break brought about by warm temperatures in mid-winter and early spring. Our investigations into the genetic regulation of freezing tolerance and dormancy reflect that approach. A more comprehensive understanding of how these processes are regulated will enable the development of strategies to control them in a precise manner as needed, without having a detrimental effect on other economically-important traits such as yield and fruit quality.

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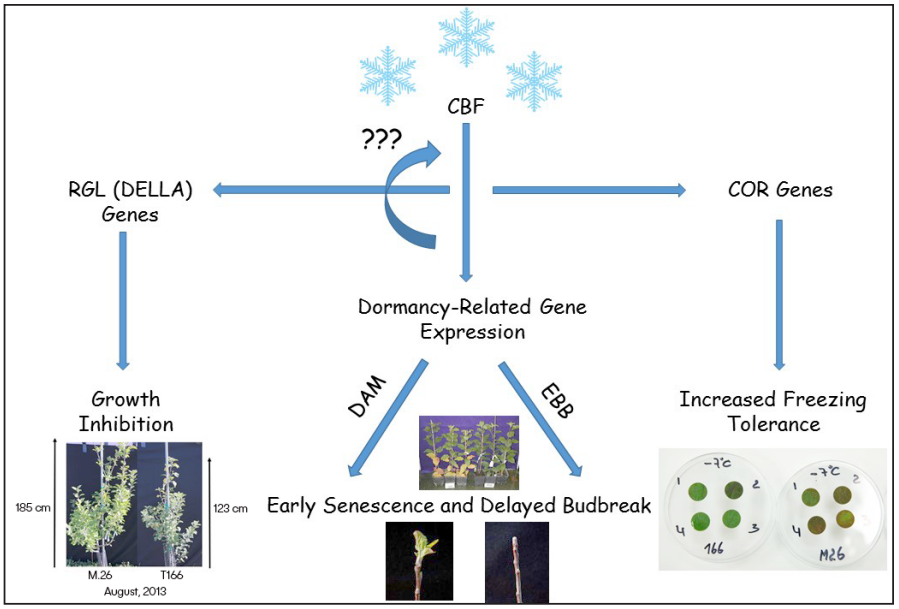

Figure 6. Hypothetical model of how the CBF transcription factor gene could serve as a master regulator of freezing tolerance through the regulation of cold-regulated (COR) genes, the onset and release of dormancy through dormancy-related (DAM) and early-budbreak (EEB) genes, and growth through (RGL and DELLA) genes that regulate the activity of gibberellic acid (GA).

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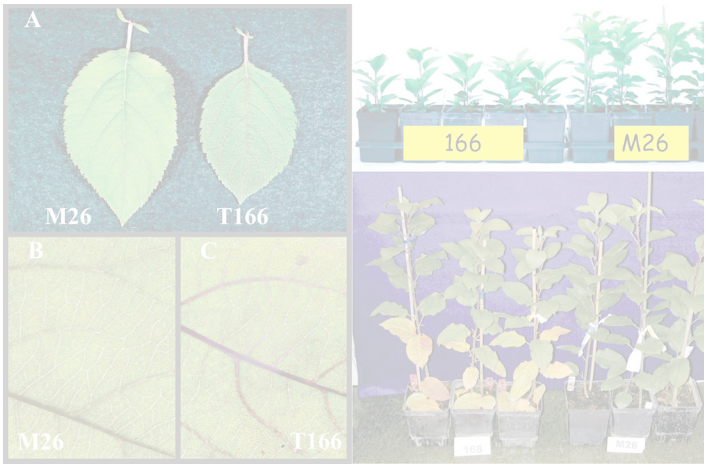
Michael Wisniewski [email: michael.wisniewski@ars.usda.gov], **John Norelli**, and **Timothy Artlip** are USDA-ARS research scientists located at the Appalachian Fruit Research Station in Kearneysville, WV where they are members of a project entitled, "Improving Stress and Disease Resistance in Tree Fruit Crops," (CRIS Project Number, 8080-21000-022-00D) focused on improving cold hardiness and fire blight resistance