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Research Note

Cellulose nanocrystals reduce cold damage to reproductive buds in fruit crops

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Every year tree fruit and grape growers suffer significant economic losses from cold damage to reproductive buds. A combination of pre-planned passive and active frost protection methods has been prevalently used to achieve some level of protection. Herein, we describe a new effective approach to protecting reproductive fruit buds from cold damage. Electrostatic application of cellulose nanocrystals (CNC) dispersion to fruit buds forms a thermal insulation layer with low thermal conductivity (0.061 W m^{-1} K^{-1}). The dispersion was applied to dormant grapevine (*Vitis vinifera*) buds, and hardiness was evaluated by differential thermal analysis (DTA). CNC-treated buds were more resistant to freezing temperatures than untreated buds. Cold hardiness was improved by 2–4 °C with CNC treatment. The hardiness of sweet cherry (*Prunus avium* L.) reproductive buds at the 'first white' stage of development was also tested comparing CNC-treated (2% mass) and non-treated clusters. Pistil mortality was evaluated 24 h after treatment. Untreated pistils were killed at ca. −1 °C while the CNC-treated buds were hardy to ca. −4 °C. The temperature at which ca. 10%, 50%, or 90% of untreated pistils were killed was ca. −1.5 °C, −2.8 °C and −5.5 °C, the temperature at which ca. 10%, 50%, or 90% of treated pistils were killed was ca. −4.4 °C, −6.5 °C and −7.7 °C. In general, CNC treatment improved cold-hardiness of grape and sweet cherry buds by about 2–4 °C. These results demonstrate great potential for field applications of CNC to improve speciality crop yield security by protecting reproductive buds from cold damage.

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1. Background

The tree fruit industry is an important component of the U.S. agricultural sector, representing about 18% of $24.5 billion annual crop production (Schnepf, 2017). Yield of these crops is determined largely by cross pollination during the brief, but critical, flowering period. During the transition from bud break to flowering, reproductive buds become increasingly susceptible to cold damage, a perennial threat that can cause severe economic losses (Warmund, Guinan, & Fernandez, 2008). The United Nations Food and Agriculture Organization reports that cold damage has caused more economic losses to crops in the U.S. than by any other weather hazard. A single freezing event can cause hundreds of millions of dollars in crop losses (Attaway, 1997). For example, nearly the entire crop (~90%) of apples and cherries in Michigan was lost due to cold damage in, 2012 (Smith, 2013).

Growers can utilise passive and/or active frost/freeze protection methods. Passive methods include site selection, cultivar selection, nutrient management, and cultural practices such as pruning and orchard floor preparation (Powell & Himelrick, 2000; Sigler, 2013; Snyder& De Melo-Abreu, 2005). These methods are less costly yet generally provide less protection than active methods (Jorgensen et al., 1996; Powell & Himelrick, 2000).

There are various active frost protection methods that growers can utilise during or prior to cold weather to reduce cold damage. These include wind machines (Battany, 2012), helicopters (Poling, 2008; Miles & Hinz, 1976), heaters (Evans, 2000), wind machine/heater combinations (Jorgensen et al., 1996) or sprinklers (Evans, 2000; Parsons, Wheaton, Faryna, & Jackson, 1991; Poling, 2008). Typically these methods can raise temperatures by 2.8–3.3 °C when the sky is clear, there is little wind (Jorgensen et al., 1996). More specifically, wind machines can raise temperatures by 1.1–2.8 °C (Poling, 2008; Powell & Himelrick, 2000), and helicopters increased orchard air temperature up to 2.8–4.4 °C when hovering over trees (Powell & Himelrick, 2000) during a radiative freeze. Oil- and propane-fuel heaters provide 2–2.5 °C protection, whereas wind machine and heater combinations can raise temperatures by 2.8–3.3 °C (Evans, 2000; Jorgensen et al., 1996). The use of over-tree sprinklers increased air temperatures of about −4 to −4.4 °C as long as the dew point in not less than −6 °C (Evans, 2000; Jorgensen et al., 1996; Sigler, 2013).

Despite varying degrees of efficacy in reducing cold damage, each of these methods has drawbacks. For example, wind machines are noisy, expensive, and they do not provide protection if winds are greater than 2.2 m s^{-1} (5 mph), nor if there is a convective freeze. Environmental pollution from fuel-burning heaters can be problematic due to air pollution, and use of helicopters is expensive.

Cellulose nanocrystals (CNC) represent a new generation of renewable nano-biomaterials with unique physical, chemical, and optical properties with vast potential applications. CNC can be obtained from a variety of sources including plants, sea animals such as tunicates, and microorganisms (Siqueira, Bras, & Dufresne, 2010). While the dimensions of the CNC crystal structures differ among sources, CNC typically has dimensions of 2–50 nm lateral and 100–2000 nm length (Habibi, Lucia, & Rojas, 2010; Hamad, 2006). CNC has a strength-to-weight ratio higher than steel, can be drawn into a thin film like layer, and be produced in a various colours (Fernandes et al., 2013). The unique properties of CNC have led to its being used in many industrial sectors including paints and coating, polymer composites, catalysis, cosmetics, biosensors, drug delivery, and medical devices (Lam, Male, Chong, Leung, & Luong, 2012). However, despite significant advances toward large-scale production of CNC from forest biomass, there has been little success in identifying a marketable product that justifies this new renewable nanomaterial (Durán, Lemes, & Seabra, 2012; Eichhorn et al., 2010; Jonoobi et al., 2015; Wadas, 2016).

We hypothesise that CNC will be useful for reducing cold damage in fruit buds due to its low thermal conductivity (0.061 W m^{-1} K^{-1}). This is lower than conductivity of other materials developed for frost protection such as aqueous foam that exhibits thermal conductivity of ca. 0.11 W m^{-1} K^{-1} for lettuce (Choi, Zimmt, & Giacomelli, 1999) and polypropylene insulation of ca. 0.225 W m^{-1} K^{-1} for potatoes (Bhullar, 2012; Wadas, 2016) and strawberries (Hochmuth, Locascio, Kostewicz, & Martin, 1993). Polyethylene terephthalate was effective as a thermal insulator, reducing frost damage in citrus trees and grape with a thermal conductivity about 0.29 W m^{-1} K^{-1} (Kipnees & Raszewski, 1991). Low-density polyethylene (LDPE) with a thermal conductivity of about 0.31 W m^{-1} K^{-1} was moderately effective for protecting grape from cold damage, but it allows about 60–80% of radiant heat to pass (Willwerth, Ker, & Inglis, 2014). CNC has potential as a thermal barrier since materials with thermal conductivities of ca. 0.2 W m^{-1} K^{-1} at room temperature are commonly considered as thermal barriers or insulators (Singh et al., 2014).

The current research was conducted to evaluate the potential of CNC as a thermal insulation material, protecting sweet cherry (*Prunus avium* L.) and grape (*Vitis vinifera*) reproductive buds from cold damage. A differential thermal analysis system was used to determine the lethal temperatures for plant tissue using a programmable freezer (Gutiérrez, Chaves, Anothai, Whiting, & Hoogenboom, 2014; Gutiérrez, Chaves, & Hoogenboom, 2016; Mills, Ferguson, & Keller, 2006).

2. Materials and methods

2.1. Materials

The raw CNC material was obtained from both FPInnovations, and USDA Forest Products Laboratory (FPL) and the CNC suspension was prepared in the Bioproducts, Sciences, and Engineering Laboratory (BSEL) at Washington State University (WSU).

2.2. CNC film and spray solution preparation

Pure CNC solution was prepared by dispersing freeze dried CNC in deionised water (2% mass) under sonication for 60 min at room temperature. CNC spray solution was prepared by the addition of a bromide based surfactant during the sonication. CNC film was prepared by casting the solution on a petri dish