FINAL PROJECT REPORT

Project Title: Reducing cold damage with cellulose nanocrystals

PI: Matthew Whiting
Organization: WSU-IAREC/CPAAS
Telephone: 509-786-9260
Email: mdwhiting@wsu.edu
Address: IAREC
Address 2: 24106 N. Bunn Road
City/State/Zip: Prosser/WA/99350

Co-PI (2): Changki Mo
Organization: WSU-Tricities School of MME
Telephone: 509-372-7296
Email: changki.mo@tricity.wsu.edu
Address: WSU-Tricities
Address 2:2710 Crimson Way
City/State/Zip: Richland/WA/99354

Co-PI(3): Xiao Zhang
Organization: Chem. Eng., WSU
Telephone: 509-372-7647
Email: xiao.zhang@tricity.wsu.edu
Address: Ctr for Bioproducts & Bioenergy
Address 2: 2710 Crimson Way
City/State/Zip: Richland/WA/99354

Co-PI (4): Bernardita Sallato
Organization: WSU-Extension
Telephone: 509-439-8542
Email: b.sallato@wsu.edu
Address: IAREC
Address 2: 24106 N. Bunn Road
City/State/Zip: Prosser/WA/99350

Cooperators: Brent Arnoldussen, Jassim Alhamid, Zirkle Fruit Co.

Percentage time per crop: Apple:50 Pear: Cherry:50 Stone Fruit:

Other funding sources

Agency Name: USDA NIFA AFRI Bioenergy
Amt. requested/awarded: $404,030
Notes: Funded for 2018-2021 to investigate cellulose nanocrystal suspension preparation, thermal properties, field trials with CNC

Agency Name: WSU Commercialization Gap Fund
Amt. requested/awarded: $50,000
Notes: this funding is to evaluate plant-based dispersions for reducing cold damage, protect the IP, and develop a business plan for the commercialization of the IP

Total Project Funding: $92,736

Budget 1

Organization Name:	**WSU**	**Contract Administrator: Katy Roberts**
Telephone:	**509-335-2885**	**Email address: arcgrants@wsu.edu**

Item	**2018**	**2019**
Salaries	31,471	32,730
Benefits	2,360	2,455
Wages	4,800	4,992
Benefits	455	473
Equipment		
Supplies	10,000	1,000
Travel	1,000	1,000
Plot Fees		
Miscellaneous		
Total	50,086	42,650

Footnotes: salary for graduate research assistant to work out of WSU-IAREC; wages are to support hourly employees to assist with field trials and hardiness assessments; supplies in year 1 include materials to build 'polar pod' system (datalogger, thermocouples, heating elements, power supply) for hardiness evaluation in spring (i.e., when DTA is ineffective)

OBJECTIVES

1. Evaluate the utility of cellulose nanocrystals (CNC) applications for reducing cold damage
2. Summarize and disseminate key findings with stakeholders

SIGNIFICANT FINDINGS

- We have developed a reliable process for creating dispersions of cellulose nanocrystals (CNC)
- CNC treatments via handheld electrostatic sprayer provided resistance to cold damage in apple and sweet cherry
- Benefits of CNC coatings are related to rate of CNC
- Cold hardiness could be improved by 0 to 2 F at 1 % CNC treatment and by 3.6 to 7 F from 2 % CNC treatment
- CNC treatment may advance flowering in sweet cherry
- CNC treatments do not appear to be effective at full bloom (though they do not interfere with pollination)
- CNC coatings can be 'washed' off during rain
- CNC can be effective up to 7 days post application
- We have verified that these films work through an insulative effect by showing and decrease in thermal emissivity and increase in thermal capacity

Single application results:

Applications made to sweet cherry on 19 March were effective at improving tolerance to cold temperatures. At -2 C and -3 C, 30% of the untreated buds were killed. Nearly 90% of collected buds from the control trees were dead at -5°C, and none of the floral buds survived when temperature reached -7 °C. In contrast, CNC-treated buds were undamaged until -6 °C when pistil mortality was 70%. At the lowest temperature tested, - 9 °C, there were still 10% of CNC-treated buds that survived. A close analysis (Table 1) of mortality data suggested that the LT10 (temperature at which 10% buds are killed) of the control and CNC-treated buds were -1.4 °C and -5.1 °C respectively, whereas the LT90 (lethal temperature at 90% mortality) of the control and treated buds were -5 °C and -8 °C. The LT50 (lethal temperature at 50% mortality) of the control and treated buds were estimated as -4 °C and -5.7 °C.

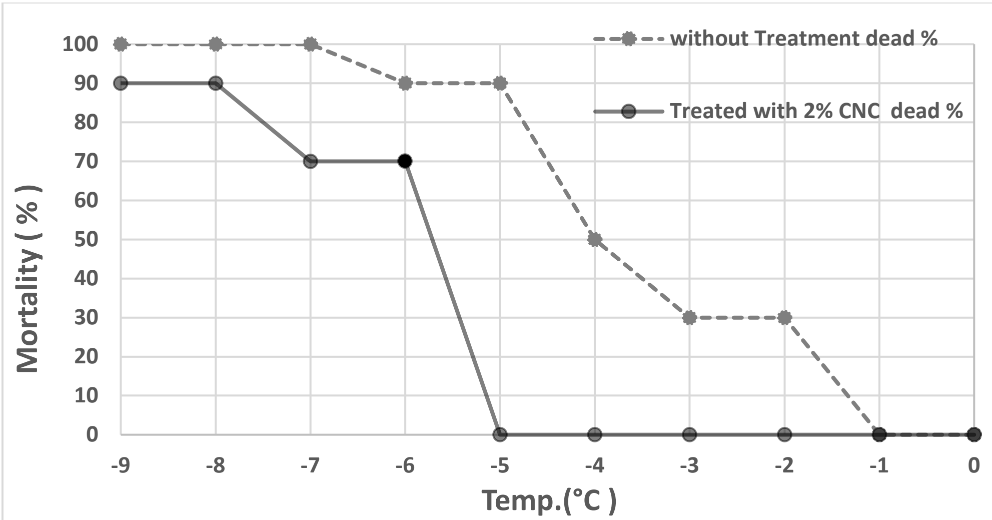


Fig. 1. Mortality of 'Benton' sweet cherry floral buds. Treatment made 19 March. Sampled on 20 March.

The effect of CNC spraying on the hardiness of the buds was determined again on March 26th following the same experimental procedure. Again, the CNC treatment significantly improved the hardiness of buds. Interestingly, untreated cherry floral buds collected in this week exhibited improved cold tolerance in the warmer temperatures evaluated (Fig.). On this sampling date, only 10% of the untreated buds were dead at -4 °C and 60% were dead at -5 °C. Similar to the previous treatment date, -7 °C was a critical temperature at which all reproductive buds were dead. In contrast, the CNC-treated buds exhibited similar sensitivity to cold damage on this date as the previous week. Tissue damage first occurred at -6 °C of CNC-sprayed buds and -9 °C was the critical temperature leading to mortality of all buds.

Based on these two sample dates, spray treatments of 2 wt.% CNC can prevent cold damage in sweet cherry. This is similar to our previous research (Alhamid et al., 2018). We found that all buds treated with CNC were free from damage until – 6 C. In contrast, untreated buds had either 90% or 60% bud death at – 5 C on 19 March and 26 March, respectively. This level of protection from cold damage is potentially significant for commercial growers during early spring frost.

Buds sampled 24 hr after treatment on 2 April exhibited significantly less hardiness, irrespective of treatment (Fig. 8). Untreated control buds exhibited 70% mortality at 0 ºC and complete mortality at -5C. In contrast, CNC-treated buds again were more tolerant to cold temperatures with 0% mortality at 0 C, 70% mortality at -5C, and complete bud death at – 6 C. The LT90 for the control and treated buds were determined at -4.5 ºC and -5.8 ºC. While still effective for improving cold tolerance, the efficacy of CNC treatment at this stage of bud development is less pronounced compared to the results from the previous two weeks.

In the subsequent week, 9 April sweet cherry buds had reached the first bloom stage. At this stage, buds become more susceptible to frost damage. For untreated control buds, we recorded 10% bud mortality at 0 C, with complete death at – 3 C. A temperature drop to -1°C was sufficient to cause 90% mortality of the untreated buds. The CNC treatment again improved bud hardiness, even at this vulnerable stage (Fig. 9). Bud mortality was not observed at 0 or -1 C in treated buds. There was a rapid increase in bud mortality as temperatures dropped, with 20%, 80%, and 100% death at -2, -3, and

-4 C. respectively. There was no rain on either April 9th (date of spraying) or April 10th (date of sample collection).

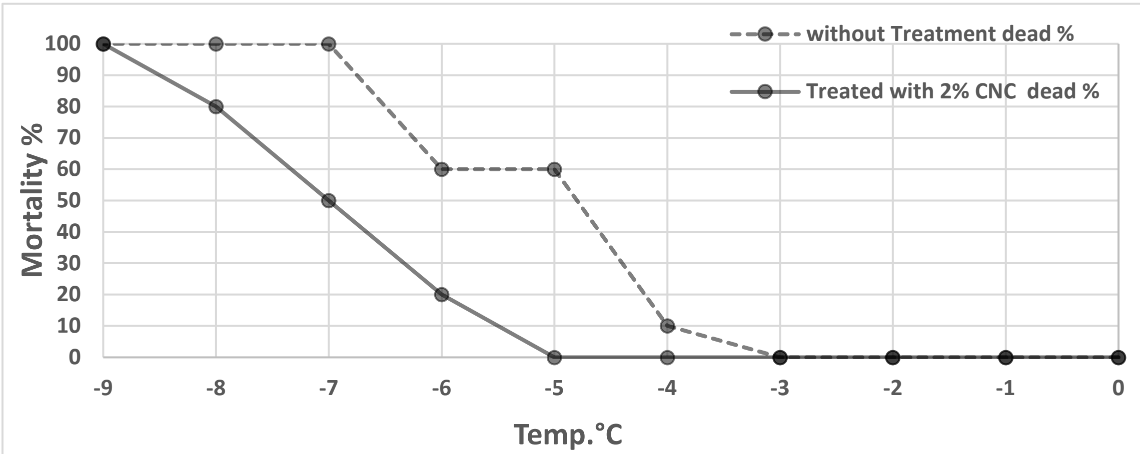


Fig. 2. Mortality of 'Benton' sweet cherry floral buds. Treatment made 26 March. Sampled on 27 March.

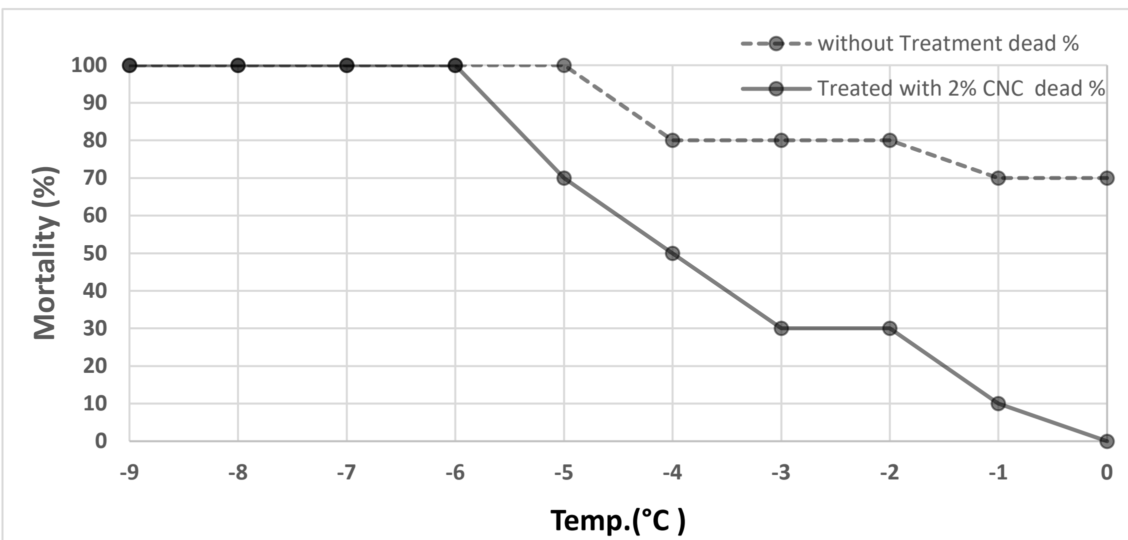


Fig. 3. Mortality of 'Benton' sweet cherry floral buds. Treatment made 2 April. Sampled on 3 April.

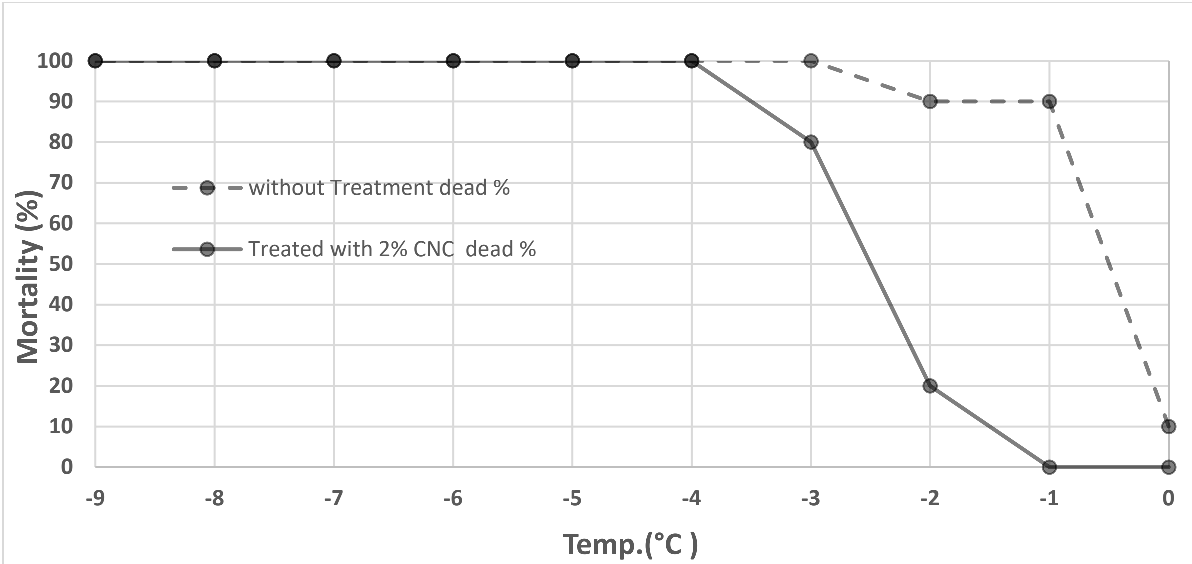
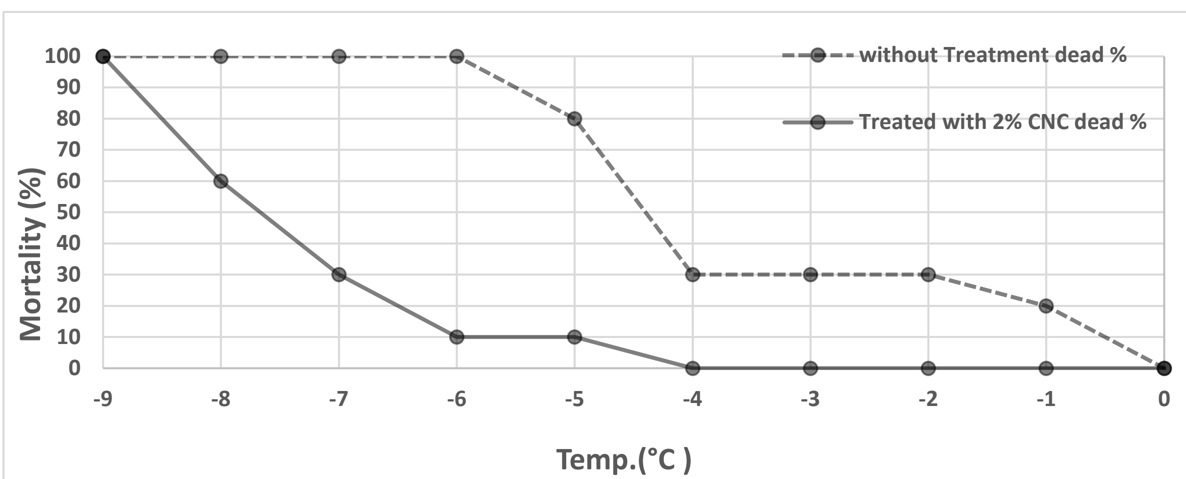


Fig. 4. Mortality of 'Benton' sweet cherry floral buds. Treatment made 9 April. Sampled on 10 April.

Multiple application testing results

All previous trials of CNC in fruit crops have assessed effects of single applications on cold damage. It is important, however, to understand whether repeated applications of CNC may further improve bud hardiness. In this trial we examined cold tolerance of buds that had received multiple applications of CNC. The initial CNC treatment on 19 March improved cold hardiness of buds to a similar degree to the other trial (Fig. 1), confirming the efficacy of single sprays. A second application was made to the same trees on 26 March, and buds collected after 24 hours following the 2nd second spray exhibited hardiness (Fig. 5) that was similar to the single treatment efficacy (Fig. 2). This suggests that a single application of CNC may be adequate for frost protection. Future research in our lab will assess this issue more thoroughly. The lethal temperatures obtained from control and treated buds are shown above, these data generally agree with the single applications testing results (Figs 1-4).



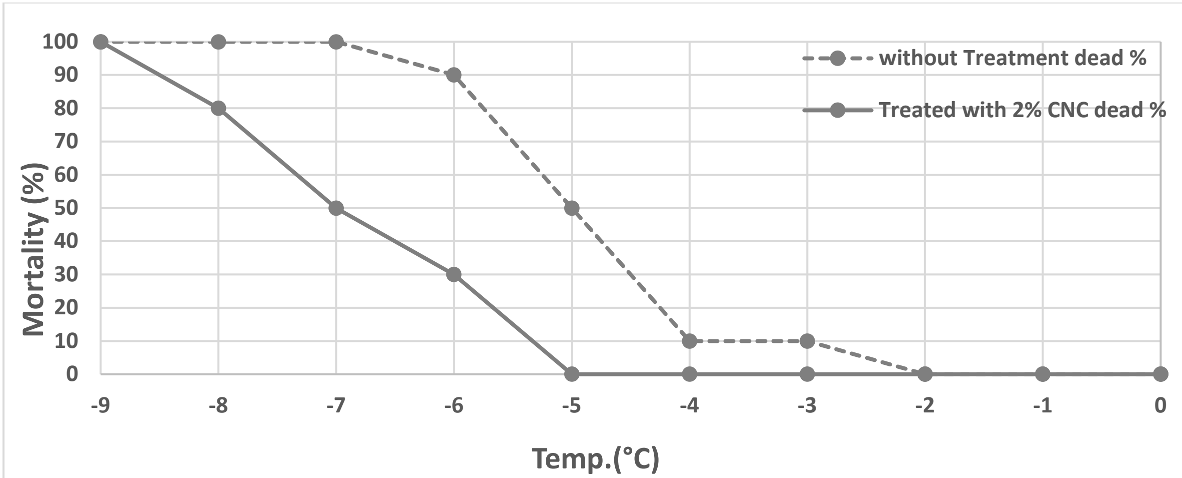


Fig. 5. Mortality of 'Benton' sweet cherry floral buds. Treatments made 19 April and 26 April to same trees. Buds were sampled on 20 April (above plot) and 27 April (lower plot).

Large-scale commercial trial

The results obtained from the field trials conducted at the WSU experimental station have confirmed the earlier results from the laboratory studies (Alhamid et al., 2018). It is clear that CNC treatment is an innovative and effective technology to prevent frost damage to tree fruits. These trials have utilized small-scale application systems that may not represent well the actual efficacy of CNC treatments made on a large scale, with commercial application technologies. To further determine the commercial potential of this technology, large-scale trials were conducted in Spring 2019 at two commercial orchard locations, Mattawa, WA on 20 March 20, and Pasco on 21 March. One acre of sweet cherry and one acre of apple were selected at each location for the trials. During the trial, a commercial sprayer was used (On Target Spray Systems, Mt. Angel, OR, USA; Figure 12). The sprayer had 32 electrostatic nozzles and reservoir tank with a capacity of 100 gallons (378.5 L) (Figure 12a). Results are promising (Figs 6 &7), with protection lasting at least 6-7 days post application.

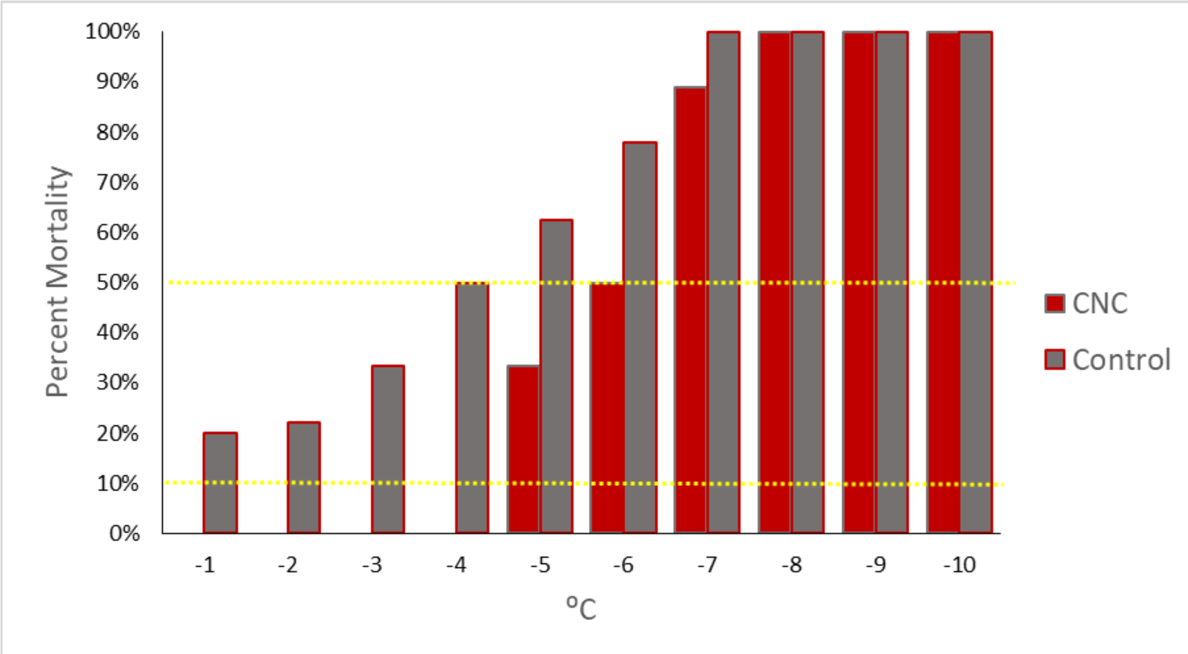


Fig 6. Mortality of 'Benton' sweet cherry buds 6 days following application of 2.5% CNC with commercial application equipment

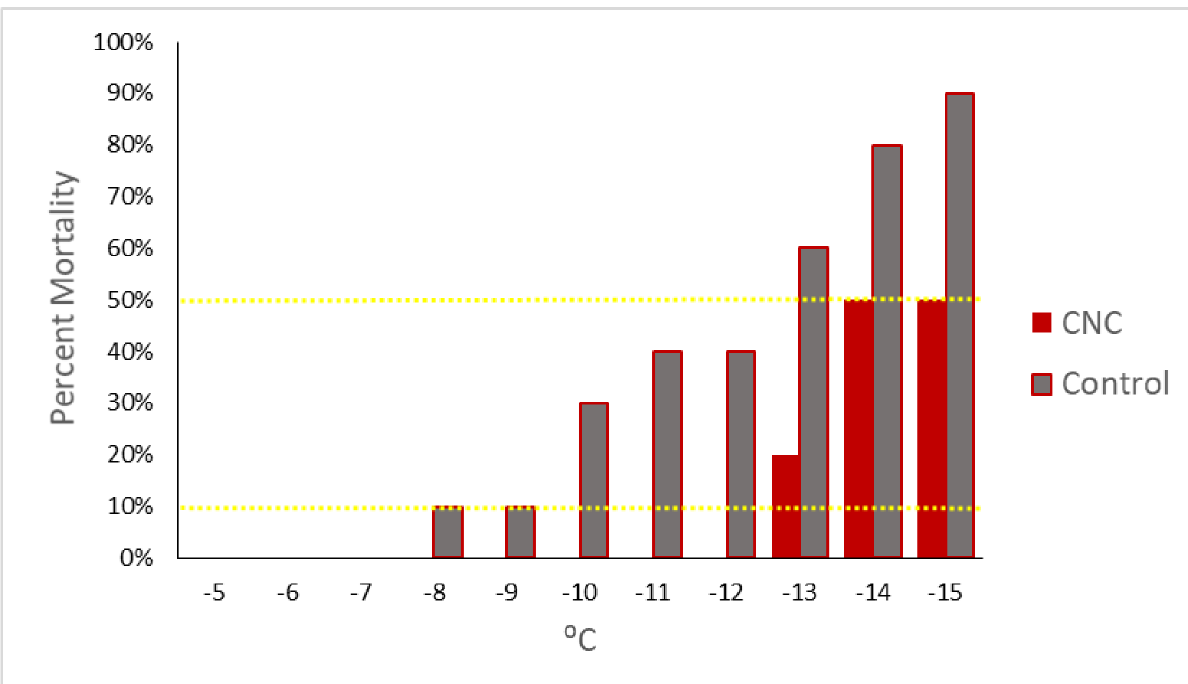


Fig 7. Mortality of 'Gala' apple buds 6 days following application of 2.5% CNC with commercial application equipment.

Scanning electron microscopy

CNC films were evaluated under scanning electron microscopy. Cross-sections reveal a coating thickness of ca. 29 micrometers and 40 micrometers for 2% and 3% CNC treatments, respectively (Fig 8.). The thickness of the CNC coating may relate to its efficacy.

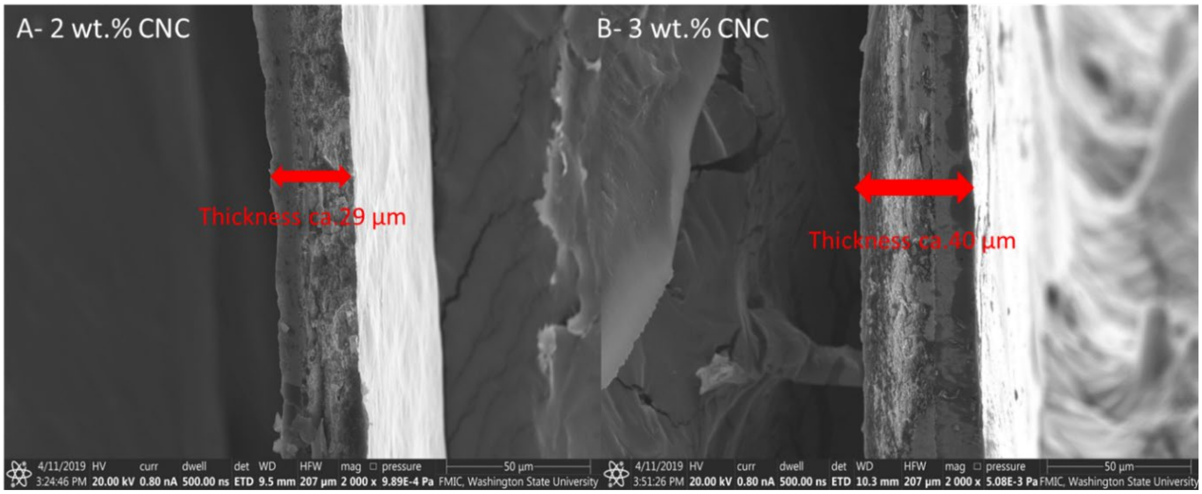


Fig. 8. Scanning electron microscopy micrographs of (a) 2 wt. % CNC film (magnitude 2000×); (b) 3 wt. % CNC film (magnitude 2000×)

EXECUTIVE SUMMARY

Project title: Reducing cold damage with cellulose nanocrystals

Key words: Frost, freezing, CNC, abiotic stress, apple, sweet cherry

Abstract: Applications of cellulose nanocrystal dispersions can reduce cold damage in apple (*Malus domestica* Borkh.) and sweet cherry (*Prunus avium* L.). Reductions in cold damage are related positively to CNC concentration. Protection can last at least 1 week. CNC treatment does not affect flowering time nor fruit set.

Every year, tree fruit growers lose money from cold damage to reproductive buds or flowers. The U.S. Food and Agriculture Organization reported that cold damage has caused more economic losses to crops in the U.S. than any other weather hazard. The potential losses from cold damage are devastating, and predicted to become more commonplace with increasingly variable spring weather. Cellulose nanocrystals (CNC) represents a new generation of renewable nano-biomaterials with unique physical, and chemical properties, including their low thermal conductivity (i.e., they provide excellent insulation). In this work our team has synthesized a CNC dispersion that can be sprayed onto trees, forming a thin, uniform and insulating film on the surface of the buds. The CNC film is transparent, allowing sunlight to penetrate during the day, while its insulating property prevents heat loss during cold conditions.

Single applications of CNC, using electrostatic application technology, can provide significant protection to dormant and post-dormant reproductive buds in apple and sweet cherry. Benefits of CNC treatment are related positively to rate of CNC – 1% CNC provided only slight reductions in cold damage whereas 2% was consistently effective, and 2.5 – 3% provided even greater reductions in cold damage. In 'Scifresh' apple, for example, the LT10 was 30.2 F for untreated flowers and 26.6 for flowers treated with 2% CNC (an improvement of 3.6 F). In this trial the greatest improvement in flower hardiness was observed at 21.2 F, a temperature at which 80% pistil death was recorded for control, 90% for 1% CNC, and only 30% for 2% CNC. Based on these results, untreated trees would have complete crop loss at about 19 – 20 F, and trees treated with 2% CNC would have ca. 50% crop remaining, not losing the entire crop until ca.16 F. Importantly, we discovered that CNC efficacy is not lost in large-scale applications using commercial sprayers (OnTarget Spray Systems). We found that a 2.5% CNC treatment improved hardiness of sweet cherry and apple by

Interestingly, multiple applications of CNC dispersions to trees did not improve tolerance to cold temperatures any more than single applications. This was evaluated however in only a single trial, and this is an area for further research. We have shown that the protection of CNC coatings is lost during rain, but that a single treatment can provide significant improvement in hardiness for up to one week in the absence of precipitation. It is unclear how much precipitation is sufficient to reduce the CNC film's efficacy – this too is an area for further research.