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Pilot Study to Evaluate Performance of Frost-Yuzu Fruit Trees under Protected Cultivation

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Abstract: This study was initiated to observe the performance of yuzu (*Citrus junos* Sieb. ex Tanaka) fruit trees when affected by a late freezing in 2018 and to evaluate the recovery of frost-damaged trees during post management under protected cultivation. A—4.9 °C of minimum daily temperature and 40-day drought occurred during dormancy, which then received heavy precipitation between early- and mid-March, with 15 m s^{-1} more than maximum instantaneous wind speeds frequently observed. This resulted in observed decreases in height, width and volume as well as in fruiting, fruit weight and yield, as well as yield index in 60–90% defoliated yuzu trees, in addition to higher rates of shoot dieback compared to trees that experienced only 0–30% defoliation. Lower performance and recovery rates of trees grown on flat land compared to trees on sloped land were also observed. Tree and net windbreaks did not significantly affect tree vegetative growth and fruit productivity but were found to have lowered shoot mortality in 2018 and 2019. Mulch with an irrigation after freezing or foliar urea application was shown to effectively increase vegetative tree growth and fruit productivity and reduce shoot mortality.

Keywords: freezing; mulch; slope; windbreak; yuzu

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1. Introduction

Yuzu (*Citrus junos* Sieb. ex Tanaka) is an upright evergreen tree 2–7 m tall and has been traditionally grown in South Korea, China and Japan [1]. The fruits were variously used for their refreshing fragrance, to make tea, seasoning, side dishes and for medicinal purposes, and contain a wide range of active ingredients, high amount of antioxidants and volatile oils [2–6]. Yuzu trees annually require higher amounts of sunshine (up to 2400 h) and water than other temperate fruit trees during the growing season [1,7]. The trees grow in a warm climate of between 13–38 °C annual average temperature, and are more cold tolerant than most other citrus species, being able to withstand daily minimum temperatures down to −9 °C [1,7,8], and are most extensively cultivated in Goheung-gun, Southern S. Korea, with 81.6% of the country's production [8].

Prolonged exposure to cold and drought conditions have frequently occurred across the country during the winter, notably in January and February, every 5 to 10 years for the last 50 years [7–11]. This increased water stress from dehydration and osmotic adjustments to cell walls as well as freezing injuries to de-acclimated perennial woody stems and bark tissues, significantly increases defoliation and shoot mortality in temperate fruit trees [11–14]. Most studies on the effects of freezing have focused on disease, melanose, gummosis, stem pitting and anthracnose occurrence in yuzu trees, which became the driving force for a considerable 66.8% decrease in yuzu cultivation area from 1996 to 2018 in S. Korea [7,8,15], with little scientific information available on tree recovery and strategies to prevent freezing damage.

Freezing damage was more often in yuzu trees and other temperate fruit tree species, such as apples, pears and peaches, cultivated in flat orchards rather than on hill orchards, mostly due to the frequent occurrence of nighttime radiant cooling and lower temperatures experienced by the exposed tissues [12,16–19]. The flat orchards were typically ground-covered using plastic mulches, hay or straw bales under windbreaks and wind machines to avoid cold dry air and to maintain heat energy balance [20–22]. Urea application increased tree T-N contents, a critical nutrient source for root growth and alleviation of xylem embolism during the winter, greater facilitating the onset of tree growth during de-acclimation in spring when compared to the application of plant growth regulators [12,23–25]. However, it is unclear whether the same strategy would effectively reduce stresses from freezing in yuzu trees.

This study was initiated to observe yuzu tree performance as affected by late-harvest freezing and to evaluate the recovery of damaged trees under protected cultivation and the effectiveness of post management practices.

2. Materials and Methods

2.1. Field Condition

This pilot study was conducted with 20-year old native variety of yuzu trees grafted with trifoliate orange trees (*Poncirus trifoliata* L.) on private orchard farms A, B, C, D and E in close proximity with each other in Goheung-gun, Southern S. Korea (34° N, 127° E) in 2018. Yuzu fruit trees in all orchards were planted with a density of 4 m between trees and 4 m between tree rows. The trees were trained with a vase shape type support with multiple pipes (more than five), a setup widely used in tree cultivation in S. Korea. Orchard soil texture was primarily a sandy loam soil with 0–30 cm depth rooting zone. Climate conditions are presented in Figure 1 for January and February in the last 20 years and in Figure 2 for late winter and spring, 2018 [26].

Composted cattle manure [approx. 0.9% (*w*/*w*) N, 1.2% (*w*/*w*) P and 1.1% (*w*/*w*) K] was applied annually on all orchard floors in February of each year at 20,000 kg ha^{-1} as a basal fertilizer, with additional nutrient sources applied via a chemical soluble fertilizer in August according to the mineral nutrient requirements of 23.9 kg of N, 15.8 kg P_2O_5 and 18.4 kg K_2O per hectare for tree growth [27].

The yuzu trees in all orchards A–E received water from mobile sprinkler systems when rainfall was not reported over five consecutive days from March to November. Insects and disease were conventionally controlled during the growing season. Conventional pruning during the summer and winter was conducted with heading and thinning cuts on each tree. Perennial cover crops were naturally sown in the orchard floor and annually mown 3 to 4 times to minimize competition for water and mineral nutrients between the trees and the cover crops.

Flowering dates were recorded between 20–30 May in 2018 and 2019.

2.2. Observational Study

Yuzu fruit trees grown on flat land without protected cultivation were categorized into four groups, namely those experiencing 0%, 30%, 60% and 90% defoliation due to freezing, to evaluate tree performance and recovery on slightly sloping land—approximately 5–10° in orchard A in 2018 and 2019 (Figure 3A,B).

2.3. Protected Cultivation

Protected cultivation was employed to evaluate the performance and recovery of trees planted on flat land of approximately 0–5° rise, and sloping land of approximately 10–20° in orchard B, in 2018 and 2019, as well as to investigate the effects of tree windbreaks and net windbreaks, in orchard C. Five rows of Japanese spindle trees (*Euonymus japonica* Thunb.) 3–5 m in height, cold and wind tolerant windbreaks, were planted parallel in a north-south direction, in 2010.

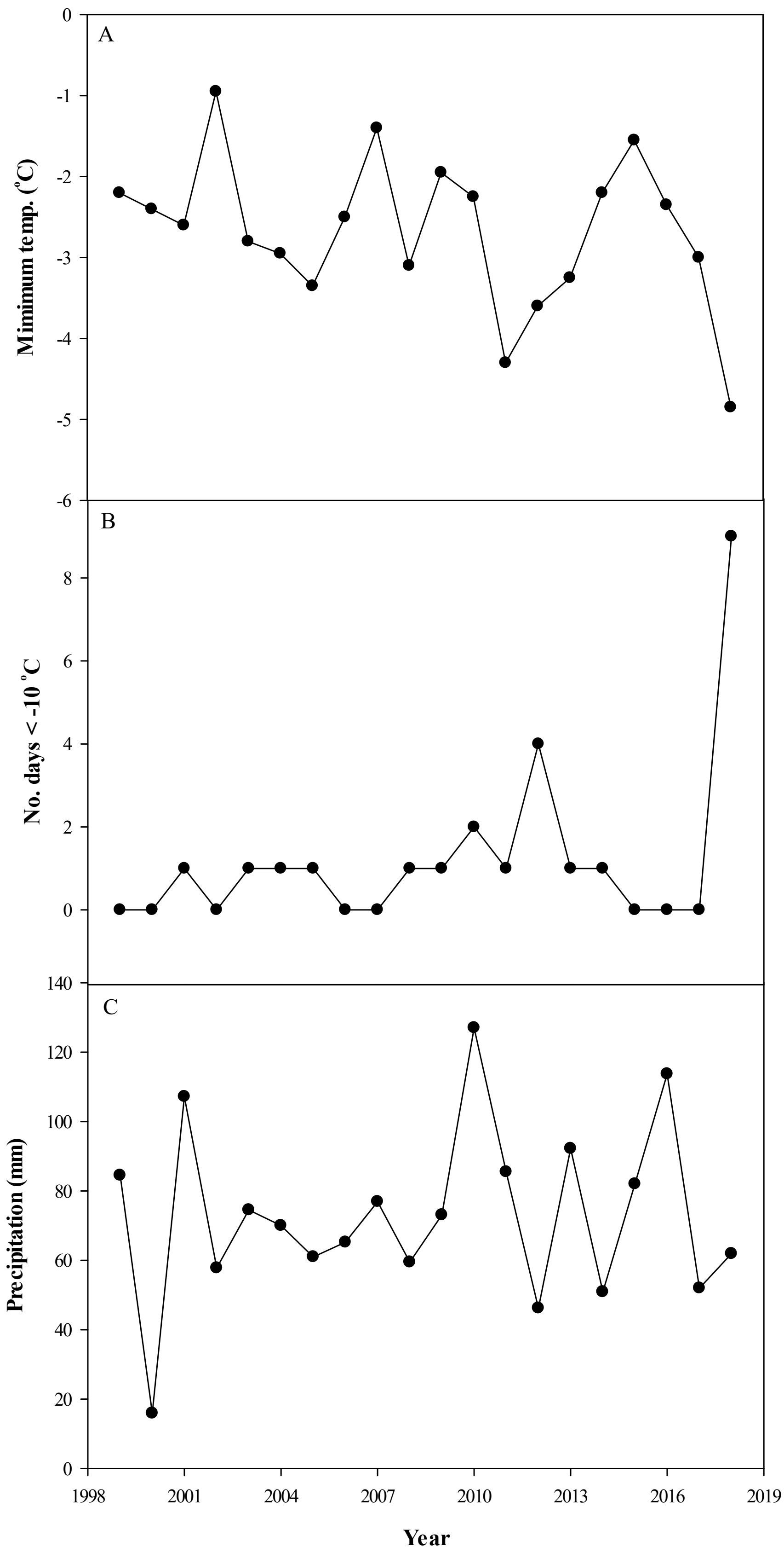

Figure 1. Average of minimum daily temperature (**A**), number of days of minimum daily temperature less than −10 °C (**B**), and daily precipitation (**C**) on January and February in the last 20 years in Goehung-gun, Korea.

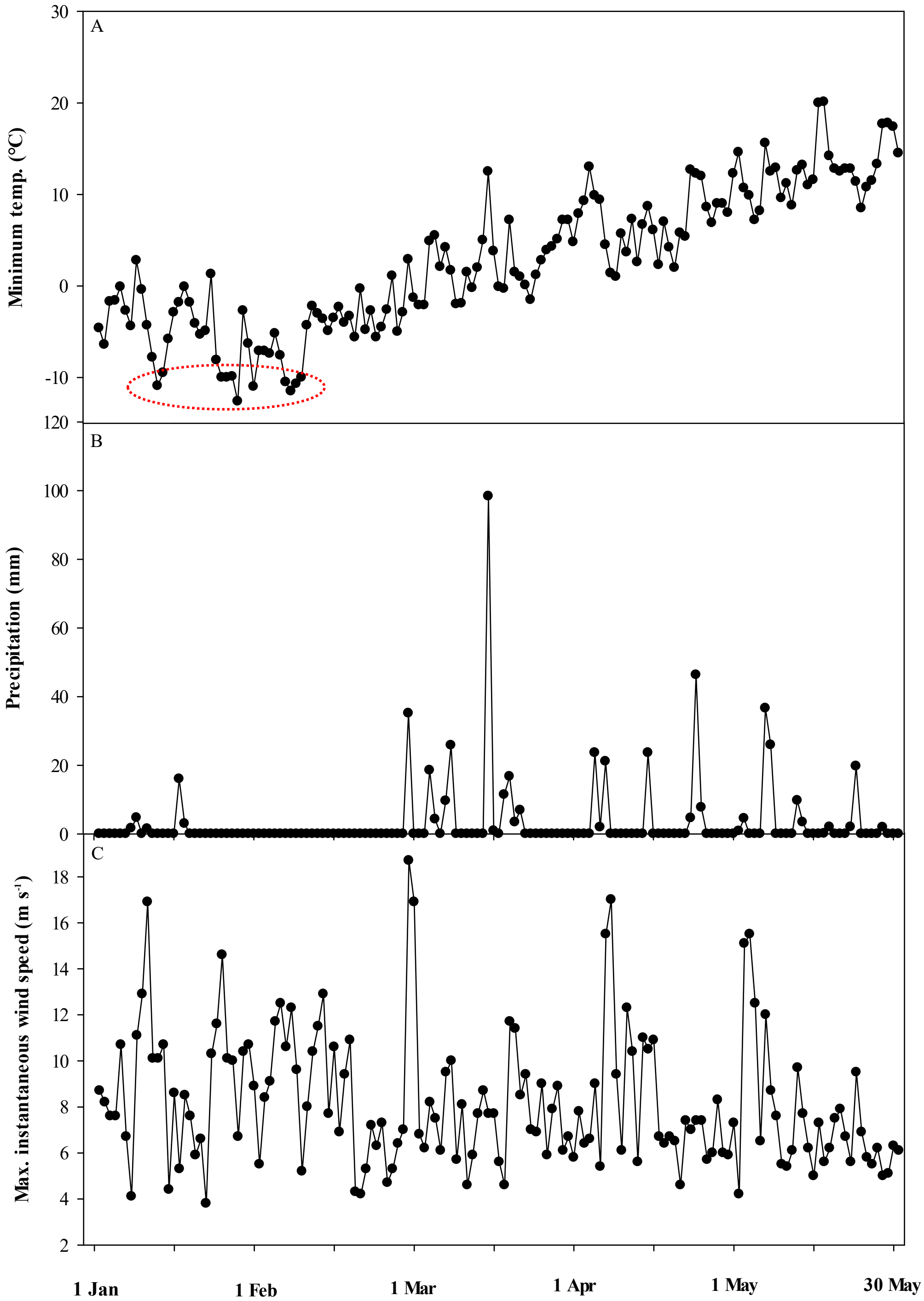

Figure 2. Daily minimum temperature (**A**), precipitation (**B**) and 15 m s^{-1} more than maximum instantaneous wind speed (**C**) in Goehung-gun, Korea from January to May, 2018.

2.4. Post Management

The post management treatment for freezing damaged yuzu trees included sod culture with irrigation (Sod + IR), rice straw mulch with irrigation (Mulch + IR), clean culture without irrigation (Clean-IR) on slightly sloped land in orchard D, in 2018 and 2019, to evaluate tree performance and recovery. Straw mulch was applied annually under the planted trees (1 m of mulch around each tree), in an approximately 5- to 10-cm-thick layer, which was then reapplied in April to maintain mulch depth in the following year. A 0.5% foliar urea application was made three times a week as another post management strategy to treat damaged trees planted on a slight slope in orchard E during the pre-harvest fruit drop in June 2018.

Figure 3. Yuzu orchard views of frost-damaged trees (**A**), planting sites (**B**), straw-mulched trees (**C**) and urea applied trees (**D**) in Goehung-gun, South Korea.

2.5. Tree Performance and Soil Nutrient Analysis

Growth parameters, such as tree height, tree width and tree volume were measured on all trees grown in orchards A–E, on 10 August 2018, when shoot growth ceased.

The 30-fruit per tree was harvested at the yellow color stage of fruit peel from early November to early December, counted and weighed to calculate the fruit yield and fruit yield index.

Three soil samples per tree were randomly taken with a 2 cm-diameter soil probe at a depth of 0–30 cm at 50% of the canopy drip-line radius, then mixed together in a PE bag and mesh sieve in orchard D for soil nutrient analysis according to RDA protocols [28]. Soil pH and electrical conductivity (EC) were measured with soil-distilled water, using a pH meter (FIVEEAST FE20, Mettler Tonedo CO., Jiangsu, China) and an EC meter (HI 2315 Conductivity Meter, Hanna CO., Seoul, Korea), respectively. Soil organic matter (OM) and available P_2O_5 were measured using the methods of Tyurin and Lancaster, respectively. The amounts of extractable soil cations, K, Ca and Mg were determined using the ammonium acetate method.

2.6. Statistical Analysis

Three single trees of similar size from the ten trees per treatment were randomly chosen for each treatment in each orchard A–E with a Completely Randomized Design.

Data were subjected to an one-way analysis of variance (ANOVA) to determine treatment differences with Duncan's multiple range test at $p < 0.05$. All statistical analyses were performed using Minitab Statistical Software v. 15.1 (Minitab, Inc., State College, PA, USA).

3. Results and Discussion

3.1. Climate Condition

Average minimum daily temperature during January and February in the last 20 years mostly declined to −4.9 °C in 2018 and to −4.3 °C in 2011 [26], when severe frost damage was reported in yuzu orchards in southern S. Korea, including other fruit tree species, apples, pears and peaches (Figure 1A). Days with a minimum daily temperature less than −10 °C, the cultivation limit of the yuzu fruit trees, were observed most frequently nine times in 2018 (Figure 1B) with 11 mm lower than-average precipitation (Figure 1C). Minimum daily temperatures lower than −10 °C continued over four days at the end of January and in early February (Figure 2A) when the frost resistance of temperate fruit trees was expected to decrease. Unusually high temperatures were observed in spring (approximately 13 °C), higher than the minimum temperatures for mid-March and early April, which then rapidly decreased to 0 °C within a few days [8]. Precipitation was not recorded for 40 days between 18 January and 27 February, but heavy precipitation was received between early- and mid-March (Figure 2B), which would increase freezing potential due to the prolonged drought in winter through embolism in xylem vessels and temperature fluctuations rather than freezing temperatures [12]. A wind speed of 15 m s^{-1} (greater than the maximum instantaneous wind speed) observed on 1 and 24 January, on 28 February, on 1 March and on 6 and 7 April (Figure 2C) would have caused additional potential for frost [8]. Even though the frost risk was little during the blossom onset of mid-May, with high temperatures and slow wind speeds observed, the blossom date could shift towards mid-April by the end of the 21st century due to global warming, resulting in trees experiencing more frequent freezing [18].

3.2. Observational Study

The 90% defoliated yuzu trees showed the smallest height and width, and tree volume, followed by the 60%, 30% and 0% defoliated trees in 2018 and 2019 (Table 1), as had previously been demonstrated in studies showing a positive correlation between freezing injury and the degree of avocado leaf senescence [25]. Shoot dieback of the yuzu trees was rarely observed due to freezing in the previous years, but was observed for all trees in 2018 when severe freezing frequently occurred during dormancy from January to February and during de-acclimation from March to April (Figure 2). Shoot dieback of yuzu trees due to freezing in 2018 was highly recorded to an average of 43.6% based on farm surveys at 18 randomly selected orchards in southern S. Korea [8]. 90%-defoliated yuzu trees considerably recovered from shoot dieback (from 72.3% in 2018 to 25.5% in 2019) compared to those values observed on other defoliated trees.

Table 1. Vegetative growth as affected by levels of freezing-induced defoliation in yuzu trees in 2018 and 2019.

Defoliation	Tree Height (cm)	Longest Width (cm)	Shortest Width (cm)	Tree Volume (cm^3)	Shoot Mortality (%)
			2018		
0%	421 a	356 a	325 a	34.1 a	4.5 d
30%	366 b	350 a	321 a	28.8 b	13.6 c
60%	321 c	335 a	316 a	23.8 c	31.8 b
90%	289 d	299 b	284 b	17.2 d	72.3 a
			2019		
0%	408 a	367 a	338 a	35.4 a	3.2 d
30%	382 b	362 a	336 a	32.5 b	10.6 c
60%	345 c	348 ab	337 a	28.3 c	15.4 b
90%	315 d	329 b	321 b	23.3 d	25.5 a

Means comparisons among treatments within a column by Duncan's new multiple range test; means followed by different letters are significantly different, 5% level.

Fruiting, fruit weight, fruit yield and yield index were higher on the 0% defoliated trees, followed by 30%, 60% and 90% defoliated trees in 2018 and 2019 (Table 2) likely influenced by the vegetative growth of each tree (Table 1). The 60% and 90% defoliated trees showed a fruit yield index less than 75% in 2019 as reviewed in the relevant article that a defoliation less than 60% prevented breaking paradormancy and inducing re-foliation and tree reserve depletion [12].

Table 2. Fruit productivity as affected by levels of freezing-induced defoliation in yuzu trees in 2018 and 2019.

Defoliation	Fruiting (No. tree^{-1})	Fruit wt. (g)	Fruit Yield (g tree^{-1})	Yield Index (%)	Fruiting (No. tree^{-1})	Fruit wt. (g)	Fruit Yield (g tree^{-1})	Yield Index (%)
		2018				2019		
0%	353 a	106 b	2357 a	100.0 a	360 a	113 c	2563 a	100.0 a
30%	315 b	105 b	2084 b	88.4 b	326 b	118 b	2425 b	94.6 b
60%	236 c	109 ab	1621 c	68.8 c	252 c	120 ab	1905 c	74.6 c
90%	149 d	112 a	1051 d	44.6 d	160 d	123 a	1240 d	48.4 d

Means comparisons among treatments within a column by Duncan's new multiple range test; means followed by different letters are significantly different, 5% level.

3.3. Protected Cultivation

Trees grown on flat land showed significantly reduced height, width and volume, and higher rates of shoot dieback in 2018 and 2019, and higher defoliation in 2019, than those of trees grown on sloped land (Table 3). Severe frost damage in persimmon fruit trees was observed in low elevation orchards [17], possibly due to lower air temperatures compared to high elevations [19]. Radiant cooling more greatly occurred from late winter to early spring and effected cold air descending down the hill, which could decrease the temperature between 6 °C to 8 °C [12,29], resulting in lower fruit productivity, especially on flat land, in 2018 and 2019 (Table 4).

Table 3. Vegetative growth of yuzu trees planting on flat and slope as affected by freezing in 2018 and 2019.

Land	Tree Height (cm)	Longest Width (cm)	Shortest Width (cm)	Tree Volume (cm^3)	Shoot Mortality (%)	Defoliation (%)
			2018			
Flat	375	288	275	20.7	55.8	65.2
Slope	402	382	324	34.8	16.4	30.3
Significance	*	***	**	***	***	***
			2019			
Flat	382	325	312	27.1	16.5	12.8
Slope	388	381	336	34.7	5.8	13.6
Significance	ns	***	ns	**	***	ns

*, ** or *** Significantly different mean values (n = 3 trees) between treatment at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively. ns, not significantly different.

Table 4. Fruit productivity of yuzu trees planting on flat and slope as affected by freezing in 2018 and 2019.

Land	Fruiting (No. tree^{-1})	Fruit wt. (g)	Fruit Yield (g tree^{-1})	Yield Index (%)
			2018	
Flat	156	112	1101	100
Slope	301	116	2200	200
Significance	***	ns	***	***
			2019	
Flat	144	117	1061	100
Slope	296	123	2294	200
Significance	***	ns	***	***

*** Significantly different mean values (n = 3 trees) between treatment at $p \leq 0.001$, respectively. ns, not significantly different.

Vegetative growth and fruit productivity were not mostly different between trees protected by tree and net windbreaks in 2018 and 2019 (Tables 5 and 6). The windbreaks lowered shoot mortality less than 10.0% in 2018 as they significantly slowed down the wind speed, evaporation and temperature drop during the day [12,21,22]. However, freezing-induced defoliations increased to 25.4% in trees receiving tree windbreak protection and increased to 33.2% in those with net windbreaks from decreasing heat exchange between air layers at night, causing freezing on the leeside [21,22]. This high defoliation may advance bud burst in the subsequent spring, exposing sensitive tissues to freezing during the de-acclimation [12].

Table 5. Vegetative growth in yuzu trees protected by windbreaks using trees and nets as affected by freezing in 2018 and 2019.

Windbreak	Tree Height (cm)	Longest Width (cm)	Shortest Width (cm)	Tree Volume (cm^3)	Shoot Mortality (%)	Defoliation (%)
			2018			
Tree	368	376	355	34.4	4.6	25.4
Net	395	368	352	35.8	7.8	33.2
Significance	*	ns	ns	ns	*	*
			2019			
Tree	376	366	342	32.9	4.1	12.9
Net	382	352	358	33.7	5.2	12.5
Significance	ns	ns	ns	ns	**	ns

* or ** Significantly different mean values (n = 3 trees) between treatment at $p \leq 0.05$ or $p \leq 0.01$, respectively. ns, not significantly different.

Table 6. Fruit productivity of yuzu trees protected by windbreaks using trees and nets as affected by freezing in 2018 and 2019.

Windbreak	Fruiting (No. tree^{-1})	Fruit wt. (g)	Fruit Yield (g tree^{-1})	Yield Index (%)
		2018		
Tree	278	113	1979	102
Net	256	120	1935	100
Significance	*	ns	ns	ns
		2019		
Tree	289	116	2112	102
Net	272	121	2073	100
Significance	ns	ns	ns	ns

* Significantly different mean values (n = 3 trees) between treatment at $p \leq 0.05$. ns, not significantly different.

3.4. Post Management

Mulch + IR treatment plots resulted in higher soil pH, EC, OM and concentrations of P_2O_5, K, Ca and Mg than those of Sod + IR and Clean-IR plots (Table 7). All treatment plots satisfied desired levels of soil mineral nutrients to maintain annual tree growth. The sod cover crops, such as turfgrass, ryegrass and creeping red fescue, did not significantly increase nutrient availability or fruit yield compared to those of straw mulches over 6 years [22,30,31]. Mulch + IR treatment increased tree height, width and volume, and fruit productivity (Table 8).

Table 7. Soil chemical properties of yuzu trees in an experimental plot as affected by groundcover in 2018.

Treatment	pH (1:5)	EC (dS m^{-1})	OM (g kg^{-1})	P_2O_5 (mg kg^{-1})	ExCation (cmolc/kg)		
					K_2O	CaO	MgO
Clean-IR	6.2 b	0.28 b	2.7 b	506 b	0.69 b	7.46 b	1.72 a
Sod + IR	6.5 ab	0.32 ab	3.1 ab	651 ab	0.72 ab	7.65 ab	1.55 b
Mulch + IR	6.7 a	0.38 a	3.5 a	790 a	0.83 a	8.05 a	1.64 ab
Desired level	6.0–6.5	0.00–<0.20	2.5–3.5	200–300	0.30–0.60	5.0–6.0	1.5–2.0

Means comparisons among treatments within a column by Duncan's new multiple range test; means followed by different letters are significantly different, 5% level. -IR: without irrigation, + IR: with irrigation.

Table 8. Vegetative growth and fruit productivity in yuzu trees with different ground cover treatments as affected by freezing in 2018.

Treatment	Tree Height (cm)	Width (cm)	Tree Volume (cm^3)	Shoot Mortality (%)
Clean-IR	341 c	330 b	24.9 c	13.2 a
Sod + IR	365 b	372 a	32.6 b	8.4 b
Mulch + IR	386 a	380 a	36.1 a	5.6 c
Treatment	**Fruiting (No. tree^{-1})**	**Fruit wt. (g)**	**Fruit Yield (g tree^{-1})**	**Yield Index (%)**
Clean-IR	218 c	112 b	1538 c	100 c
Sod + IR	286 b	115 ab	2072 b	135 b
Mulch + IR	320 a	120 a	2419 a	157 a

Means comparisons among treatments within a column by Duncan's new multiple range test; means followed by different letters are significantly different, 5% level. -IR: without irrigation, + IR: with irrigation.

Foliar urea application increased tree height, tree volume and fruit productivity, and reduced shoot mortality of the tree freezing (Table 9) by providing a rapid T-N source to individual leaves on shoots [25].

Table 9. Vegetative growth and fruit productivity of yuzu trees foliar-applied with urea as affected by freezing in 2018.

Treatment	Tree Height (cm)	Width (cm)	Tree Volume (cm^3)	Shoot Mortality (%)
Control	352	204	19.5	12.9
Urea	376	238	21.8	8.7
Significance	*	ns	*	***
Treatment	**Fruiting (No. tree^{-1})**	**Fruit wt. (g)**	**Fruit Yield (g tree^{-1})**	**Yield Index (%)**
Control	255	112	1779	100
Urea	286	120	2162	122
Significance	**	*	**	**

*, ** or *** Significantly different mean values (n = 3 trees) between treatment at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively. ns, not significantly different.

4. Conclusions

A higher risk of yuzu trees experiencing freezing was likely caused by extremely low minimum daily temperatures, prolonged drought and temperature fluctuations from January to April in 2018, which was significantly minimized and shoot mortality reduced to less than 10% by protection cultivation and post management strategies. However, farmers struggled, with planting locations and protected cultivations facing economic difficulties in most urbanized countries. Supplement urea applications applied right after fruit harvest and a week before freezing would be recommended to enhance freezing tolerance during thawing and spring ecological hibernation in temperate region as well as to retard the effects of leaf senescence although it was difficult to judge with different locations of individual experiments. However, the statistical power of the pilot study were not sound due to the low sample size. Additional research is needed to further understand the long-term physiological responses of yuzu trees protected with various methods and their potential interactions to provide best management practice to tolerate freezing occurring in temperate areas, especially during de-hardening periods, and the eco-dormancy phase in spring.

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