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Effect of surface wetness and duration of low temperature exposure on frost damage in sub-tropical fruit species

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ABSTRACT : Frost is the most devastating weather hazard for horticultural growth in the NW India. For mitigation of its impact the information on the process of freeze initiation a spread and the factors involved in aggravating the damage is of utmost importance. Studies were conducted at COHF Neri under controlled and radiation frost customized environment. REL was the measure adopted for accessing the damage. Two leaf surface wetness levels (wet and dry) along with three durations of low temperature (-2^{0}C) exposure 2 hours, 4 hours and 6 hours were taken under a Completely Randomized Factorial experiment conducted on container grown ten subtropical fruit species. Leaf surface wetness was more critical in case of mango and litchi whereas in case guava, jamun, karonda, galgal, lime extrinsic ice nucleation was not very important in inducing internal damage to the leaf tissues. Duration of low temperature exposure aggravated the damage level in almost all the species. In case of papaya and jackfruit higher susceptibility of these species to frost induced freeze damage the impact of frost duration was not found to be significant. The relative order off susceptibility of different species was observed to be as: Loquat, galgal, lime, guava, karonda, jamun, litchi, mango, jackfruit, papaya in the increasing order.

KEY WORDS : Frost damage, Mango, Litchi, Freezing susceptibility, Relative electrolyte leakage

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Frost and surface temperature inversion are phenomena of common occurrence during winter in the low hill and valley region of NW India. And, therefore, despite being subtropical (Thornwaite, 1948) this region is observed sub-optimal for growing of subtropical fruit crops. It occupies around 18 per cent of the total geographical area while supporting around 37 per cent of the total population of North-Western states of India. More than 80 per cent population of the region being agrarian is fighting tooth and nail for establishing horticulture based production system as more economic losses are caused by frost than by any other weather hazard during the recent past.It has been estimated that on an average, the direct losses due to frost in northern India exceed 130 million rupees per annum (Anonymous, 2010). The impact of frost on the farmers and local economy are so devastating that people are cutting an edge from horticulture and associated activities in the region.

Though frost is a phenomenon of natural occurrence but the extent of damage to a particular crop species entirely depends on the extent of freezing experienced by that species. Plant and plant parts usually freeze when they cannot avoid ice nucleation and growth of ice crystal with in their system during a frost induced freezing event. At freezing temperature the water molecules come together to form a stable ice nucleus either spontaneously or when catalyzed to do so in the presence of other substance with low specific heat (Ashworth *et al.*, 1985 and Franks, 1985). As per Levitt (1980) the extent of freeze induced damage largely depends upon the temperature drop rate rather than the duration of freezing event. Sharma (2012) have the opinion that the extent of damage observed and the persistence of freezing temperature during a radiation frost event are quite variable and have been found to be closely related to the temperature observed at the time of Sun set. During a natural frost freeze event as the frost point is achieved there occurs high level of condensation of water vapours onto the leaf surface from the saturated atmosphere prior to the occurring of the natural freezing event (Pescod, 1965). The speed of the growth of the ice crystal and its subsequent propagation is believed to have been facilitated by the amount of free water available at the leaf surface and the duration of the freezing temperatures persistence. Added to it various agencies have recommended surface water application through sprinklers as a reliable method of frost protection but the level of protection achieved under field conditions is quite variable. Therefore, keeping in view the above stated facts, the aim of present studies was to ascertain the role of leaf surface free water and duration of radiative cooling on frost induced freezing damage and to work out the relative susceptibility of subtropical fruit species for frost injury.

RESEARCH METHODS

The studies were conducted during 2012 to 2015 at the institute of Biotechnology and Environmental Science Neri, which is now known as College of Horticulture and Forestry, Neri, Hamirpur (H.P.); one of the constituent college of Dr. Y.S. Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) India. For the studies ten subtropical fruit species namely galgal (*Citrus pseudolimon*), guava (*Psidium guajava*), jackfruit (*Artocarpus heterophyllus*), jamun (*Syzygium cumini*), karonda (*Carissa carandas*), lime (*Citrus aurantifolia*), litchi (*Litchi chinensis*), loquat (*Eriobotrya japonica*), mango (*Mangifera indica*) and papaya (*Carica papaya*) were container grown for two to three years and were subjected to natural acclimation process prior to experimentation. These studies were conducted in a growth chamber constructed and customized for radiation and convective frost conditions. The experiment was designed as Completely Randomized Factorial with surface wetness and duration of low temperature exposure as primary factors with two leaf surface wetness levels D_1=Dry leaf surface, D_2=Wet leaf surface, and three durations of low temperature exposure (-2^{o}C) *viz*., T_1= 2 hours, T_2= 4 hours and T_3 = 6 hours. The freeze induced damage caused to the different species was estimated as per REL (Relative Electrolyte Leakage) method as described by Soleimani *et al*. (2003). The upper exposed and damaged leaves of the plant were sampled and twenty five chips measuring 1cm^2 area were excised randomly from these damaged leaves. Three such samplings comprised one unit of replication and there were taken three replications per treatment combination. Immediately after excision these chips were transferred to the 50 ml of distilled water in a beaker which was sealed immediately with plastic film to avoid evaporation. These beakers were then put on the shaker for 6 hours and then the electrical conductivity of the solution was taken with the help of Eutech Low range digital conductivity meter. In a parallel experiment total electrolyte leakage was estimated by subjecting the beakers containing excised chips, in distilled water, to boiling for five minutes and after that putting these beakers on shaker and measuring electrical conductivity as narrated above. The REL per cent was calculated by dividing the EC recorded upon low temperature exposure by EC recorded upon total electrolyte leakage for that species. The relative susceptibility of different species was estimated on the basis of REL per cent obtained after pooling of data irrespective of surface wetting and duration of low temperature exposure for individual species. The species measuring higher per cent of REL were termed as more susceptible to frost induced freeze damage as it was defined by Dexter *et al.* (1932) and Laporta *et al.* (1994) and Soleimani *et al.* (2003). The pooling of data and statistical analyses were carried out as per the standard procedure described by Gupta and Gupta (1995) and Chandel (1998) for Completely Randomized Factorial experiments.

RESEARCH FINDINGS AND DISCUSSION

The data pertaining to effect of leaf surface wetness and duration of low temperature exposure on freeze induced damage as measured in terms of relative electrolyte leakage are presented in Table 1. It quite evident from the data that in case of galgal surface

Table 1 : Effect of surface wetness and duration of low temperature exposure on leaf REL (%) in different subtropical fruit species

Fruit species	Leaf wetness \ Time of low temp. exposure	T_1	T_2	T_3	Mean
Galgal	D_1	20	32	46	32.7
	D_2	20	42	77	46.3
	Mean	20	37	61.5	
	$CD_{(0.05)}(D_i)$ = NS (Non-significant)				
	$CD_{(0.05)}(T_i)$ = 17.4				
	$CD_{(0.05)}(D_i x T_i)$ = 14.4				
Guava	D_1	39	52	64	51.7
	D_2	42	64	79	61.7
	Mean	40.5	58	71.5	
	$CD_{(0.05)}(D_i)$ = NS				
	$CD_{(0.05)}(T_i)$ = 11.62				
	$CD_{(0.05)}(D_i x T_i)$ = 9.86				
Jackfruit	D_1	61	90	100	83.7
	D_2	65	91	100	85.3
	Mean	63.5	90.5	100	
	$CD_{(0.05)}(D_i)$ = NS				
	$CD_{(0.05)}(T_i)$ = NS				
	$CD_{(0.05)}(D_i x T_i)$ = 4.7				
Jamun	D_1	34	48	73	51.7
	D_2	49	65	94	69.2
	Mean	41.5	56.5	83.5	
	$CD_{(0.05)}(D_i)$ = 9.16				
	$CD_{(0.05)}(T_i)$ = 7.45				
	$CD_{(0.05)}(D_i x T_i)$ = 6.42				
Karonda	D_1	28	45	69	47.2
	D_2	47	64	94	68.3
	Mean	37.5	54.5	81.5	
	$CD_{(0.05)}(D_i)$ = 6.8				
	$CD_{(0.05)}(T_i)$ = 12.7				
	$CD_{(0.05)}(D_i x T_i)$ = 10.4				
Lime	D_1	20	42	56	39.3
	D_2	32	46	57	45
	Mean	26	44	57	
	$CD_{(0.05)}(D_i)$ = NS				
	$CD_{(0.05)}(T_i)$ = NS				
	$CD_{(0.05)}(D_i x T_i)$ = NS				
Litchi	D_1	47	57	60	54.7
	D_2	56	71	84	70.3
	Mean	51.5	64.0	72.0	
	$CD_{(0.05)}(D_i)$ = 11.4				
	$CD_{(0.05)}(T_i)$ = 12.0				
	$CD_{(0.05)}(D_i x T_i)$ = 10.4				
Loquat	D_1	5.0	20	32	19.0
	D_2	7.1	32	41	26.7
	Mean	6.01	26.0	36.5	
	$CD_{(0.05)}(D_i)$ = 5.2				
	$CD_{(0.05)}(T_i)$ = 12.4				
	$CD_{(0.05)}(D_i x T_i)$ = 8.4				
Mango	D_1	52	61	75	62.7
	D_2	64	86	92	77.3
	Mean	58	68.5	83.5	
	$CD_{(0.05)}(D_i)$ = 10.4				
	$CD_{(0.05)}(T_i)$ = 7.21				
	$CD_{(0.05)}(D_i x T_i)$ = 6.46				
Papaya	D_1	70	89	100	86.3
	D_2	100	100	100	100
	Mean	85	94.5	100	
	$CD_{(0.05)}(D_i)$ = 11.2				
	$CD_{(0.05)}(T_i)$ = NS				
	$CD_{(0.05)}(D_i x T_i)$ = 6.42				

wetness did not influenced REL (%) significantly whereas the duration of low temperature exposure was very much significant in increasing REL (%). But, when we go through the interaction effects of surface wetting and duration of low temperature exposure it is quite evident that non-significant effect of surface wetting was observed up to T_2 *i.e.*up to 4hours of low temperature exposure. Beyond that very high level of REL (77%) was observed when leaf surface was wet. Very similar results were obtained for guava also, but interaction of surface wetness and duration of low temperature exposure was observed non-significant only for initial two hours of low temperature exposure beyond which the REL was significantly higher when leaf surface was wet. In case of jackfruit the initial damage was so high that significant effect surface wetness or duration of low temperature exposure was not observed significant but their interaction was significant but only during initial hours of exposure. Surface wetness significantly enhanced the REL (%) during the first two hours of low temperature exposure; beyond that the REL (%) was so high that significant differences could not be recorded. In case of jamun and karonda the REL (%) increased as the duration of low temperature increased. Similarly the REL was observed significantly high in plants with wet leaf surface than on the plants with dry leaf surface. Interaction effects were additive and higher damage was observed with wet surface and higher level of low temperature exposure. In case of lime though there was observed increase in REL with surface wetness and with increase in duration of low temperature exposure was it was non-significant statistically. Interaction effect was also observed to be non-significant for this fruit species. In litchi the pattern of increase in REL with surface wetness and duration of low temperature exposure was very similar to that observed in case of guava but the damage level (REL%) observed was higher than guava. In loquat the effect of both wetness and low temperature was significant as it was observed in case of jamun and karonda, but damage level (REL %) observed was quite low in this case. In case of mango the REL (%) increased significantly with the increase in surface wetness or duration of low temperature exposure. With wet leaf surface the damage increase was significant with the increase in duration of low temperature exposure. In case of papaya the effect observed was very similar to that observed in case of jackfruit though the value of REL (%) recorded were higher in case of papaya than jackfruit. Incase of plants with dry leaf surface the 100 per cent REL was recorded upon 6 hours of low temperature exposure whereas in case of plants with wet leaf surface 100 per cent REL was observed within 2 hours of low temperature exposure.

In almost all the plants studied there was observed an increase in REL (%) due to wetness of the leaf surface. It implies that surface wetness significantly enhanced the freeze induced damage in almost all the species studied.These findings are in conformity with those of Wisniewski *et al.* (2001); Carter *et al.* (1999) and Workmaster *et al.* (1999) whose studies on herbaceous plants have shown that freezing may occur first on the leaf surface having free water which facilitate the ice formation and its further progression into leaf inner spaces through stomata. In some species like mango and papaya the effect of surface wetness was more pronounced even during the initial phase of low temperature exposure. It may be due to the reason that in these species there occur high stomata density on the upper surface (Rathore *et al.*, 2012) which might have provided more number of openings for inward movement of extrinsically nucleated ice favoured by the free water present on the phylloplane. Also, the findings of Wisniewski *et al.* (2001) and Pearce (2001) support the above findings with the explanation that there are species in which either cuticle is not an effective barrier or there are sufficient avenues of ingress that allow ice to readily propagate from outer surface of the leaf. Therefore, in case of such species allowing retention of condensation water on leaf surface during initiation of natural frost event or to achieve frost protection with the help of surface wetting techniques like sprinklers may prove disastrous.However, the use of hydrophobic compounds for abstaining free water onto leaf surface may prove better option for achieving better protection against frost induced freeze damage under natural conditions. Surface wetting can give effective protection against frost in guava and litchi in areas where critical duration of frost persists for two hours only. In case of galgal it can works for four hours. For jamun and karonda it may be achieved for longer hours with surface application of water. On loquat and lime there was observed lesser impact of surface wetness and duration of low temperature exposure. It might be the presence of cuticular waxes in these species which reduced the adhesion of water molecules on to the leaf surface thereby eliminating the free water on the leaf surface (Saulescu and Braun, 2015). Therefore, significant differences in the REL per cent were not observed. In case of jack fruit non-significant effect of surface wetness may be attributed to the faster intrinsic ice nucleation than the extrinsic one thereby the influence of available free surface water could not be seen in this specie. It may be due to the reason that in certain woody species the intrinsic ice nucleation is more common and faster than extrinsic one (Wisniewski *et al.*, 2001). Further, comparatively larger leaf area and lower leaf thickness of this species may be correlated to lower per unit solute concentration which might have favoured faster freezing and damage to jackfruit leaves. Similar were the observations recorded for papaya. In a series of field experimentation Sharma and Badiyala (2008) and Sharma *et al.* (2014) have reported higher damage in papaya even with the onset of frost events during winter. Anderson (1985) also reported similar type of findings that ice nucleation temperature of different species was influenced by length of sub-zero temperature. He further added that leaf surface wetness promoted the growth of ice nucleation bacteria at the leaf surface.

The data on comparative REL (%) as measure of relative susceptibility of different fruit species to frost induced freeze damage are presented in Fig. 1. The ascending order of relative susceptibility of different species and on the basis of critical difference observed it can be said that among the species studied papaya followed by jackfruit were the most frost sensitive. Mango, litchi and jamun were at the second level of frost sensitivity, may be termed as frost sensitive species. Karonda, guava may be termed as the plants with lower frost sensitivity whereas galgal and lime as least frost sensitive subtropical fruit crops. Loquat may be termed as frost tolerant subtropical fruit crop depending upon the REL (%) observed. In a field study Sharma *et al.* (2014) observed almost similar type of relative susceptibility of subtropical fruit crops to frost under natural condition but the contrast observed was not as clear as under the present studies.

Conclusion:

From the above discussed results it can be concluded that relative electrolyte leakage (REL%) was influenced significantly by leaf surface wetness in almost all the species studied. Duration of low temperature exposure further added to the REL(%) increase. But, significant impact of these two factors was quite variable in different species and this give a way for thinking about different methods of frost protection which can be employed under field conditions. In case of litchi and guava surface wetting can give frost protection up to two hours of critical low temperature exposure whereas this can be achieved for four hours in case of galgal. In fruit species like papaya, jackfruit, mango, jamun and karonda the surface wetting cause significant damage at low level of critical low temperature exposure hydrophobic compounds may be considered essential for

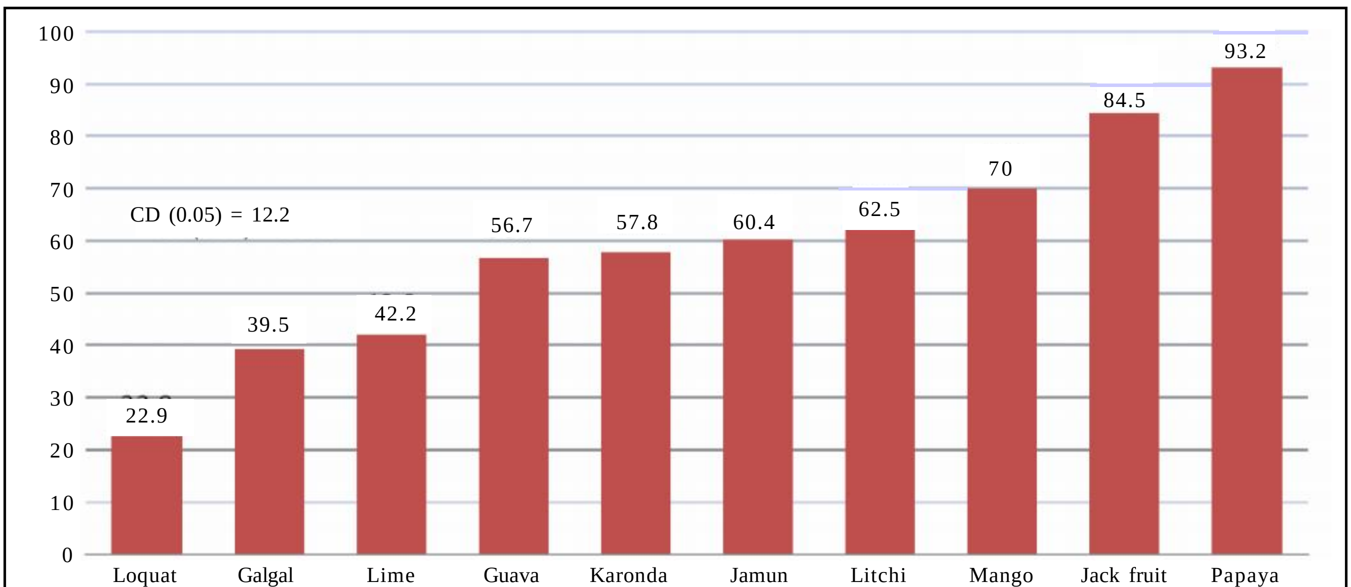

Fig. 1 : Ascending order of frost induced freezing succeptibility of different fruit species as estimated interms of REL (%)

achieving effective frost protection. The relative order of frost susceptibility of different fruit species was observed as: Loquat >lime >galgal >guava >karonda >jamun >litchi >mango >jackfruit >papaya. This order may be taken into consideration while selecting fruit species for a particular frost prone environment.

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