Cold Hardiness and Temperature Responses of Healthy and Mildew-Infected Terminal Buds of Apple During Dormancy

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ABSTRACT

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Cold hardiness of healthy and mildew-infected terminal buds of apple trees (*Malus domestica* 'Newtown') was determined monthly during the dormant period between 1 December 1981 and 1 March 1982. The lowest survival temperature (LST) of each bud was evaluated by the combination of visual observation, forcing, and grafting methods. The results obtained by using the three methods for the evaluation of LST agreed to within ± 2.9 C. The hardiness level of healthy buds was between −25.7 and −26.7 C during December to February and decreased to −18.9 C in March. The hardiness level of mildew-infected buds fluctuated between −16.4 and −21.6 C during the entire dormant period. Incubation of terminal buds for 1 wk at −12.2, −1.1, +10 C, and ambient (+3.3 and +7.7 C) temperature prior to freezing resulted in different survival patterns in January and February 1983.

Additional key words: freezing injury, freezing survival, *Podosphaera leucotricha*.

Severity of mildew infection of apple trees is associated with winter survival of mildew-infected terminal buds. When the minimum temperature of the preceding winter is −24 C or below, the mildew infection of Jonathan apple trees is very slight (9). The powdery mildew fungus, *Podosphaera leucotricha* (Ell. & Everh.) Salm., only survives when the mildew-infected buds are alive during the winter dormant period. Therefore, the measurement of survival temperatures of terminal apple buds during the dormant period would provide important information to apple growers for forecasting the degree of mildew infection the following season.

Unlike many dormant overwintering flower buds, such as deciduous azalea and stone fruits, apple flower buds do not survive by the mechanism of deep supercooling during a subfreezing period (1). Apple terminal shoots have both flower and vegetative buds, and the morphology of healthy and mildew-infected buds is quite different (9). A prolonged warm or cold interval during the dormant period has been reported to deacclimate or reacclimate the apple buds accordingly (8). When a visual method was used to evaluate bud viability, mildew-infected buds of three apple cultivars were more susceptible to freezing injury than were healthy buds (9). Neither additional methods for bud viability evaluation nor the effect of temperature prior to freezing on cold hardiness were studied.

The objectives of this study were: to determine the changes in cold hardiness through the dormant period of overwintering healthy and mildew-infected apple terminal buds using three methods to evaluate bud viability; and to determine the changes in cold hardiness of these terminal buds in response to a range of temperatures prior to freezing during the dormant period.

MATERIALS AND METHODS

Healthy and mildew-infected terminal buds were cut monthly from uniform, mature cultivar Newtown apple trees at the Mid-Columbia Experiment Station, Hood River, OR, between 1 December 1981 and 1 March 1982. Groups of each type of excised bud were divided into three subgroups. Buds from the first group were trimmed to ~5 mm of shoot while those from the second and

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third groups were trimmed to ~15 cm in length. Ten excised buds from each group were wrapped in aluminum foil and placed in a vacuum bottle containing an ice cube. Each vacuum bottle had a thermocouple inserted through the center of the lid and wrapped together with the excised buds in aluminum foil. The vacuum bottles with excised buds were incubated in a refrigerator at 3 C. When the bud temperatures equilibrated to ~2–3 C (overnight), the vacuum bottles were placed into a −34.4 C freezer. Freezing rate was about 5 C/hr or less depending upon the insulating effect of each vacuum bottle. Individual vacuum bottles with frozen buds were removed from the freezer when each of the following selected test temperatures was reached: −12.2, −15, −17.8, −20.6, −23.3, −26.1, −28.9, and −31.7 C. Each test temperature remained constant without further drop for at least 30 min in an incubator at 3 C and then increased to ~3 C after overnight incubation. The rate of warming was not recorded. Bud viability was evaluated by using three methods which included visual, forcing, and grafting. Buds from the first group were incubated in a moisture-saturated chamber at 20 C for 3 days, and the viability of sectioned buds was visually evaluated and rated by the previously described method which utilized a scale from 0 to 5 with 0 as no browning and 5 as all primordia and surrounding tissue browned (9). The mean score of 10 buds from either healthy or mildew-infected terminal buds was plotted against its corresponding freezing temperature. The lowest survival temperature (LST) of the terminal bud was arbitrarily defined as the temperature at which the mean score was rated at 2.5 as calculated from the linear regression curves.

After the freezing test, buds from the second and third groups were stratified at 5 C until 25 January 1982 or later. The stratified buds from the second group were inserted into flasks with water and incubated at room temperature to determine the number of buds that could be forced to break and grow. The LSTs of buds at each sampling date were determined as the temperatures at which 50% of buds were capable of growth.

The stratified buds from the third group were grafted individually onto 1-yr-old apple seedlings that had been potted and grown in the greenhouse. The grafted plants were kept in a growth chamber for at least 1 mo. Chamber conditions were 26 C, 75 ± 5% relative humidity, and a 12-hr photoperiod. The LSTs of buds at each sampling date were, therefore, determined as the temperatures at which 50% of buds were capable of continued growth. For all three methods, LST was calculated from the linear regression equation of percent survival or injury rating versus temperature.

In 1983, healthy and mildew-infected buds with 15-cm shoot attached were cut from the cultivar Newtown apple trees located at the same orchard as the previous year's study. Sampling dates were 17 January and 21 February. Healthy and infected excised buds were each divided into four groups. Buds from each group were wrapped in plastic bags and incubated at −12.2 ± 2, −1.1 ± 1, +10 ± 1 C, and ambient (+3.3 average for 17 January and +7.7 C average for 21 February) for 7 days. After 7 days of incubation, buds were subjected to the freezing test and then forced at room temperature to determine the percent of survival at each test temperature. LST was calculated from the linear regression equation of percent survival versus temperature. When percent survival at the two or three coldest freezing temperatures was 0, only the highest of these temperatures was included in calculating the regression equation.

RESULTS

The three methods of viability evaluation for LST determination were in agreement to within ± 2.9 C and the best agreement was within ± 1.1 C (Table 1). There was no significant difference ($P = 0.05$) between the three methods of evaluation for healthy or infected buds.

The hardiness level of healthy buds was −25.7 to −26.7 C from 1 December 1981 to 2 February 1982, and deacclimated to −18.9 C on 1 March. However, the hardiness level of mildew-infected buds fluctuated between −16.4 and −21.6 C during the entire winter period (Table 1).

Between 1 December 1981 and 1 March 1982 the maximum and minimum air temperature was 16.7 and −13.3 C, respectively. The relationship between average LST at each of the four sampling dates and average daily maximum and minimum air temperature at 1, 3, 7, 10, and 14 days prior to sampling was examined with linear regression analysis. Correlation coefficients (r) between temperature and LST of healthy buds ranged from −0.48 to +0.56 and were not significant. All correlation coefficients between temperature and LST of infected buds were negative values, and average daily minimum temperature 3 and 7 days prior to sampling correlated significantly ($P = 0.95$) with LST ($r = -0.968$ and −0.972, respectively). Average daily minimum orchard temperature during the 7 days before sampling ranged from −2.7 to 2.2 C with corresponding LSTs of −16.9 to −21.6, respectively.

The survival patterns of healthy and mildew-infected buds held at selected temperatures for 7 days prior to freezing are shown in Fig. 1. Both healthy and infected buds subjected to −12.2 C for 1 wk had very irregular survival patterns at each test temperature. However, the survival never reached 50%, indicating that a prolonged 1-wk exposure of buds at −12.2 C might have injured the bud tissues, and further freezing enhanced the degree of injury.

The LSTs of healthy buds incubated at −1.1 C and ambient temperatures were ~−20.5 C for the sampling on 17 January and −17 C for 21 February 1983. The LSTs of healthy buds exposed to +10 C for 1 wk were −19.5 and −14.8 C for the samples of 17 January and 21 February, respectively. Buds sampled on 17 January were hardier than those sampled on 21 February, indicating that cold hardiness of healthy buds is closely related to the stage of bud dormancy.

For mildew-infected buds, 1 wk of incubation at −1.1 C injured most buds sampled on 17 January as evidenced after freezing. However, those sampled on 21 February had 50% bud survival at −17.9 C. Low survival of infected buds incubated at ambient temperature or +10 C for 1 wk was observed when the test temperature was at −20.6 C or below. The survival patterns after freezing were generally independent of sampling dates and also quite different from the healthy buds. The LSTs of infected buds incubated at the ambient temperature or +10 C were between −12 and −16 C irrespective of the sampling dates.

DISCUSSION

Each of the three methods to evaluate bud viability possessed both inherent advantages and weaknesses. The visual method allows evaluation of bud viability within 5 days after each freezing

TABLE 1. The lowest survival temperatures (LST) of healthy and mildew-infected terminal buds of cultivar Newtown apple during winter months of 1981–1982 as evaluated by three different methods

	LST (C)							
	Healthy bud				Infected bud			
Date	Visual	Forcing	Grafting	$\bar{X}$ + SD	Visual	Forcing	Grafting	$\bar{X}$ + SD
1981								
1 December	−27.3	−28.1	−22.8	−26.1 ± 2.3	−19.3	−21.8	−16.0	−19.0 ± 2.4
1982								
6 January	−29.2	−25.9	−25.0	−26.7 ± 1.8	−16.7	−18.3	−15.8	−16.9 ± 1.0
2 February	−23.2	−26.4	−27.6	−25.7 ± 1.9	−20.7	−23.0	−21.0	−21.6 + 1.0
1 March	−19.1	−20.4	−17.2	−18.9 ± 1.3	−15.6	−18.4	−15.2	−16.4 ± 1.4

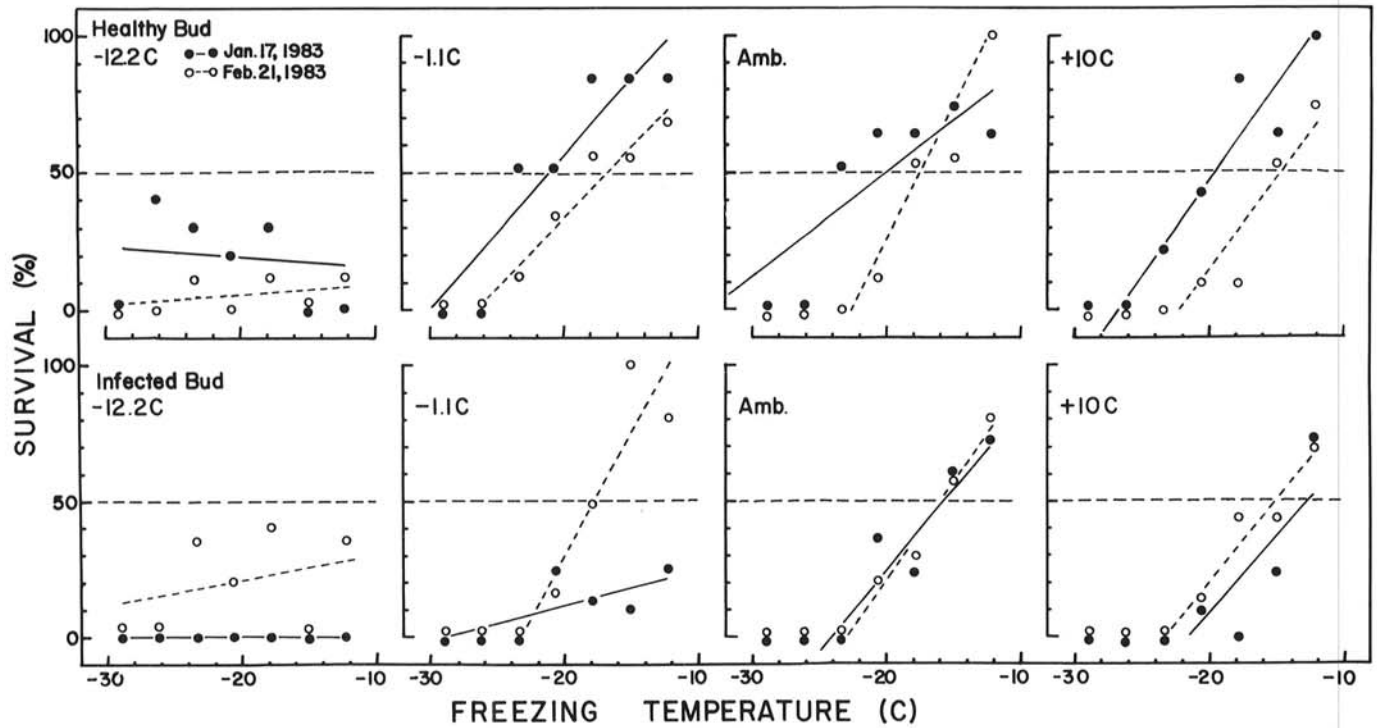

Fig. 1. Percent survival of healthy and mildew-infected terminal buds of cultivar Newtown apple trees after 1 wk of incubation at different temperatures followed by various freezing temperatures. Buds were sampled on 17 January (●) and 21 February (O) 1983.

test, but is somewhat subjective. The forcing method is the simplest for objective evaluation. However, it does not determine whether the opening buds are capable of continued growth. The grafting method evaluates the true growth of the buds after freezing, but is tedious. Furthermore, it is not known whether the death of a grafted bud is due to freezing injury or grafting failure. Although all three methods were agreeable to within ± 2.9 C, we prefer the forcing method for viability testing of apple buds after the freezing process.

Cold hardiness of healthy buds was not significantly affected by ambient temperature prior to freezing, but it was associated closely with the stage of dormancy and rest. Although the relationship between dormancy and cold acclimation found in the literature was confusing, Irving and Lanphear (3) observed that dormant plants deacclimated less than nondormant ones. Litzow and Pellett (5) also showed that warm temperatures did not deacclimate *Cornus serica* during rest. Kobayashi et al (4) demonstrated that acclimation of dogwood stem increased with deepening rest.

Cold hardiness of mildew-infected buds decreased as daily average minimum air temperature 3 and 7 days prior to freezing decreased. This relationship also appeared valid for infected buds held at constant −12.2, −1.1, and +10 C for 7 days prior to freezing on 17 January but not on 21 February when deacclimation may have begun. Infected buds held at ambient temperature (3.3 C average for January samples and 7.7 C average for February samples) for 1 wk prior to freezing were generally hardier than those held at any of the constant temperatures. The negative relationship between minimum air temperature and freezing survival of infected buds indicated that freezing survival of infected buds was not always associated with the state of bud dormancy and rest as in the case of healthy buds.

Avoidance of freezing by deep supercooling of water inside the bud primordium is one of the survival mechanisms for bud tissues during freezing (6). However, apple flower buds did not survive by deep supercooling (1). The alternate survival mechanism is tolerance of extracellular freezing (1). Morphologically, all the bud scales of mildew-infected buds dried prior to entering dormancy while those of healthy buds were sound and intact (9). One function of bud scales was proposed by Dorsey (2) as the accommodation of ice which freezes out of the flower primordium extracellularly. If the survival of apple buds is via tolerance of extracellular freezing, then bud scales might play an important role for the different freezing patterns between healthy and infected buds.

Anatomically, most healthy terminal buds of cultivar Newtown apple trees are reproductive while most mildew-infected buds are vegetative (field observation). The mechanisms of freezing survival of apple vegetative buds has not been studied. A further study of water freezing patterns in healthy and mildew-infected buds by the technologies of nuclear magnetic resonance (NMR) and differential thermal analysis (DTA) (7) would be necessary to elucidate the actual mechanisms of freezing survival of both types of apple terminal buds.

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