Cold Hardiness within the Genus *Actinidia*

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Abstract. Two-year-old *Actinidia* vines, grown on their own roots, were subjected to artificial freezing tests in midwinter to determine their relative hardiness. Plant survival, growth recovery, and stem necrosis were used for estimating freezing injury. *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson var. *deliciosa* vines, which included 'Abbott', 'Bruno', 'Greensill', 'Hayward', and 'Jones' kiwifruit, were all severely damaged by exposure to a temperature of -18°C for 4 hours. *Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq., *A. kolomikta* (Maxim. et Rupr.) Maxim., and *A. polygama* (Sieb. et Zucc.) Maxim. appeared to be more tolerant to winter cold than *A. deliciosa*, indicating that potential germplasm exists for improvement of cold hardiness through interspecific hybridization.

Freezing temperatures may cause serious yield losses in kiwifruit, particularly in Europe. Previous observations have indicated that kiwifruit vines may incur serious freezing injury in spring on young shoots and flower buds, in autumn on fruits and leaves, and in winter on trunk, canes, and dormant buds, as observed in many European kiwifruit orchards in Jan. 1985 (Blanchet, 1985).

The threshold level of freezing tolerance of kiwifruit vines in winter under field conditions varies from -10°C to -18°C (Dozier et al., 1992; Kamota et al., 1989; Monet and Bastard, 1985). Damage to buds occurs on dormant vines at temperatures below -10°C (Hewett and Young, 1981), consisting of an increase in the proportion of dead buds and a subsequent decrease in the proportion of fruit-bearing shoots (Testolin and Messina, 1987). Exposure to temperatures below -18°C can cause more serious damage, as evidenced by bark splitting of the trunk (Monet and Bastard, 1985; Weet, 1979). The resumption of growth in the orchard in early spring predisposes the kiwifruit vine to the risk of cold damage. Dormant buds, deeply embedded in corky tissue during winter, require little chilling to overcome rest (Brundell, 1976) and often burst in March in France (Blanchet, 1985). On a mature vine, young shoots, arising from dormant buds, bear flower

buds in the lower leaf axils (Brundell, 1975). Young shoots can be damaged by exposure to -0.5°C (Pyke et al., 1986) and flower buds can be killed at -1.5°C (Hewett and Young, 1981). In autumn, kiwifruit maturity is reached later than for other deciduous fruit trees. Thus, the fruit may be damaged by early unexpected freezing before harvest (Ferguson et al., 1990).

Limited information is available on the cold hardiness of specific cultivars or species of *Actinidia*, although some botanical species are reported to be more cold hardy than *A. deliciosa* 'Hayward', the most commonly planted cultivar. Five species of *Actinidia* are reported by Avery (1991) to be hardier than *A. deliciosa* and are as follows: *A. arguta*, *A. kolomikta*, *A. melanandra*, *A. polygama*, and *A. purpurea*. Chat (1994) has previously confirmed the superior cold hardiness of three of these species. The objective of the present research was to determine the degree of freezing tolerance of container-grown *Actinidia* vines belonging to *A. deliciosa*, the cultivated species, as well as wild species, to identify suitable parental genotypes for a breeding program. Exposure to natural freezing conditions is the oldest and ultimate test of plant cold hardiness, with the major drawback that experimental results are not reproducible and therefore difficult to interpret (Li, 1984). The most reliable method for assessing freezing tolerance involves subjecting entire plants or plant parts to artificial cold treatment and evaluating the subsequent recovery and tissue damage incurred (Quamme, 1978).

Materials and Methods

Plant material. Seven *A. deliciosa* clones and six clones belonging to other *Actinidia* species were used in this study (Table 1). K7 and K8 are male clones selected at the Institut National de la Recherche Agronomique Fruit Research Station orchard of Bordeaux, France. Semi-hardwood cuttings treated in July 1990 with indole-3-butyric acid were rooted under

intermittent mist (Roche, 1983). Cuttings were transplanted and grown in a plastic greenhouse until May 1991. In winter, all cuttings were replanted into 5-liter pots containing 3 leaf-mold : 1 peat : 1 sand (by volume) and cut back to 20 cm above ground level. In May 1991, vines were transferred to an outdoor site for natural cold acclimation until they were tested. The pots were watered daily and fertilized from May to July 1991 with 15N–15P–15K for a total application rate of 30 g/pot.

Freezing tests. Two tests were carried out with a 3-day interval on 19 and 22 Feb. 1992. The first test involved *A. deliciosa*, the second involved the other species. All vines were subjected to the same temperature regime. One clone of a female *A. arguta* and 'Hayward' were used as reference genotypes in each test. In addition, two vines per genotype, not subjected to any artificial freezing, were used as controls. A $300 \times 345 \times 200\text{-cm}$ chamber, capable of precisely reproducing advective freezing conditions, was used. Air temperature could be controlled from 10 to -25°C . Pots were placed within the chamber in a $280 \times 210 \times 25\text{-cm}$ Styrofoam box and kept in darkness throughout the experiment. Two 8-cm-thick glass wool insulation layers, surrounded by polythene, covered the pots, thus reducing the incidence and duration of root system exposure to freezing injury. All the vines were first placed in the controlled-environment chamber at a constant 6°C before the freezing test. The air temperature program included five sequential 4-h steps at -10 , -2 , -18 , -2 , and -10°C . Alternating freezing temperatures were selected for the temperature regime since this simulated best the natural freezing conditions that occurred in France during Jan. 1985. Freezing and thawing were programmed at a rate of either 1 or $2^{\circ}\text{C}/\text{h}$. A temperature sensor, located in the air-conditioning machinery area of the chamber, measured the temperature of the return air flow. The air temperature measured in the chamber differed from that of the sensor by up to $+2^{\circ}\text{C}$, when temporal variation was restricted to 1°C . After the temperature had returned to 6°C , vines were removed from the chamber and transferred to the greenhouse.

Evaluating freezing injury. Vines were forced in the greenhouse for 6 weeks at a mean

Table 1. *Actinidia* species and clones tested for freezing tolerance in 1992.

Species	Sex ^z	Cultivar	Clone code
<i>A. deliciosa</i>	F	Abbott	W6
	F	Bruno	W7
	F	Greensill	W8
	F	Jones	W9
	F	Hayward	K5
	M	---	K7
	M	---	K8
<i>A. arguta</i>	F	---	W20
	F	---	W16
	M	---	W4
<i>A. kolomikta</i>	F	---	W12
	M	---	W10
<i>A. polygama</i>	F	---	W18

^zF and M indicate a female and a male plant, respectively.

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of 22C. The air ranged from a 35C daytime maximum to a 15C nighttime minimum. The date at which growth resumed was noted for each vine. Each vine was assessed for survival and freezing injury 6 weeks after the freezing test. Vines that showed no regrowth on this date were rated as dead. Freezing injury was estimated by the damage exhibited by the main stem. Undamaged stems remained green and firm, while damaged stems first darkened and ultimately dried out. Necrosis length (centimeters) was expressed as the length of the main stem showing necrotic lesions. Necrosis percentage was based on necrosis length relative to plant height (Lu and Rieger, 1990).

Statistical analysis. The design was a randomized complete block consisting of six single vines of each genotype for the 19 and 22 Feb. tests. Blocks were used to take into account a possible gradient due to the air flow between the two perforated opposite walls inside the controlled-environment chamber. All statistical analyses were performed considering the data of each freezing test separately. The data were examined by analysis of variance (ANOVA) for necrosis length and plant height. Means were separated by Newman-Keuls test ($\alpha = 0.05$). Friedman nonparametric statistical test (Sprent, 1992) was used for the analysis of necrosis percentage because the conditions for a parametric test were not all satisfied, even after transformation of the data. When significant differences were found in the latter test ($\alpha = 0.05$), separations were made by comparing the sum of ranks of each genotype with that of *A. deliciosa* 'Hayward'.

Results

Plant death or regrowth. Bud burst occurred 2 days after the artificial freezing test for *A. kolomikta* vines, but it took up to 36 days for some *A. deliciosa* vines (Table 2). Freezing injury developed during the weeks following the freezing test. Exudates leaked from certain nodes on the stems, indicating that some damage occurred within the buds; subsequently, such buds did not grow. When the shoot was injured, brown necrotic lesions developed along the stem from the upper to the lower part of the shoot. Regrowth arose from axillary buds on the previous-season shoots or from the basal part of the stem when the shoot was badly damaged. Four vines, all belonging to *A. deliciosa*, were killed entirely when frozen at -18C, whereas all *A. arguta*, *A. kolomikta*, and *A. polygama* vines survived at this temperature. Within *A. deliciosa* vines, no growth occurred above the height previously protected by glass wool during freezing, except for two vines (clone K7 and 'Jones'). Conversely, young growing shoots developed from a greater height for all *A. arguta*, *A. kolomikta*, and *A. polygama* vines tested relative to *A. deliciosa*.

Damage due to natural freezing. The most severe natural cold period before the artificial freezing test occurred on 17 Dec. 1991, with minima of -5.3 and -4.7C, at 10- and 50-cm heights above ground level, respectively. The arrival of autumn freezing induced leaf fall,

freezing injury on the terminal portion of the shoot, and cessation of growth. Terminal stem necrosis, due to natural freezing in Fall 1991 and Winter 1992, ranged from 5 to 32 cm for *A. deliciosa*, and from 2 to 34 cm for the other species (see controls in Tables 3 and 4). This experiment does not permit determining whether these differences are due to variation in temperature among the pots within the outdoor site or to variation in freezing susceptibility among genotypes.

Damage due to artificial freezing. All the vines subjected to the artificial freezing test exhibited more freezing injury than the controls when considering each genotype separately (Tables 3 and 4). Direct comparison between the two tests was permissible because

A. arguta (W16) and *A. deliciosa* 'Hayward' (K5) showed similar levels of cold hardiness in the 19 and 22 Feb. tests. Under our experimental conditions, the 3-day interval between the two freezing tests did not appear to modify the freezing tolerance of the *Actinidia* vines. For necrosis length, an ANOVA for data collected following the two freezing tests indicated no significant effect due to the blocks but a highly significant effect due to genotypes.

Within *A. deliciosa*, necrosis length appeared to vary greatly depending on the genotype, although it increased in direct ratio to plant height (Table 3). The *A. deliciosa* clone K8 exhibited the smallest amount of freezing injury in terms of necrosis length and also was the least vigorous genotype within *A. deliciosa*.

Table 2. Bud burst delay, death, or regrowth of *Actinidia* vines (n = 6, per clone) after exposure to freezing at -18C on 19 or 22 Feb. 1992.

Species	No. clones ^y	No. vines ^x	Bud burst delay ^z	Vines in each class (%)		
			(days)	Alive: regrowth		
			Min-Max	Dead	<20 cm	>20 cm
<i>A. deliciosa</i>	7	42	1-36	9.5	85.7	4.8
<i>A. arguta</i>	3	18	2-9	0	0	100
<i>A. kolomikta</i>	2	12	2-2	0	0	100
<i>A. polygama</i>	1	6	0-7	0	0	100

^zBud burst delay between the living vines subjected to the freezing test and the controls.

^yNumber of clones tested within each species.

^xTotal number of vines.

Table 3. Freezing injury of *Actinidia* vines subjected to an artificial freezing at -18C on 19 Feb. 1992, compared to the unfrozen controls.

Species	Clone	Artificial freezing			
		No	Yes		
		Necrosis length ^z (cm)	Necrosis length ^z (cm)	Plant ht (cm)	Necrosis ^y (%)
<i>A. deliciosa</i>	Abbott	17	199 a ^x	204 a ^x	98 S ^w
	Bruno	6	190 a	198 a	96 S
	Jones	14	174 ab	198 a	88 S
	Greensill	7	158 a-c	168 ab	94 S
	Hayward	5	148 a-c	154 ab	96 S
	K7	9	119 bc	147 ab	81 S
	K8	5	106 c	114 b	93 S
<i>A. arguta</i>	W16	25	25 d	167 ab	14 R

^zMean length of main stem showing necrotic lesion.

^yLength of main stem showing necrotic lesion relative to plant height.

^xMeans (n = 6) followed by the same letter are not significantly different within each column based on Newman-Keuls test, $\alpha = 0.05$.

^wMeans followed by R indicate a genotype significantly more cold hardy than *A. deliciosa* 'Hayward' and S a genotype as susceptible as *A. deliciosa* 'Hayward', according to Friedman's nonparametric statistical test ($\alpha = 0.05$).

Table 4. Freezing injury of *Actinidia* vines subjected to an artificial freezing at -18C on 22 Feb. 1992, compared to the unfrozen controls.

Species	Clone	Artificial freezing			
		No	Yes		
		Necrosis length ^z (cm)	Necrosis length ^z (cm)	Plant ht (cm)	Necrosis ^y (%)
<i>A. deliciosa</i>	Hayward	32	156 a ^x	166 ab ^x	93 S ^w
<i>A. arguta</i>	W4	2	69 b	188 a	36 R
<i>A. kolomikta</i>	W12	2	58 bc	175 ab	36 R
<i>A. arguta</i>	W20	33	56 bc	150 ab	35 R
<i>A. polygama</i>	W18	34	40 bc	168 ab	26 R
<i>A. kolomikta</i>	W10	3	31 bc	178 ab	17 R
<i>A. arguta</i>	W16	12	14 c	126 b	9 R

^zMean length of main stem showing necrotic lesion.

^yLength of main stem showing necrotic lesion relative to plant height.

^xMeans (n = 6) followed by the same letter are not significantly different within each column based on Newman-Keuls test, $\alpha = 0.05$.

^wMeans followed by R indicate a genotype significantly more cold hardy than *A. deliciosa* 'Hayward' and S a genotype as susceptible as *A. deliciosa* 'Hayward', according to Friedman's nonparametric statistical test ($\alpha = 0.05$).

To take into account differences in genotype growth habit, the necrosis was expressed as a percentage. The necrosis percentage exhibited little variation, ranging from 81% to 98% when vines were subjected to the artificial freezing test. Using either parametric tests on the necrosis length or nonparametric tests on the necrosis percentage, no significant difference appeared between *A. deliciosa* 'Hayward' and the other *A. deliciosa* genotypes.

Freezing injury differed between *Actinidia* species: the necrosis length of *A. deliciosa* ranged from 106 to 199 cm, whereas that of the other species tested ranged from 14 to 69 cm (Table 4). This difference was significant, as established by parametric and nonparametric statistical analysis. For *A. kolomikta*, *A. arguta*, and *A. deliciosa*, several clones were tested, thus giving information on variation within species. Clones W4 and W16, damaged by freezing on 36% and 9% of their main shoot, respectively, do not belong to the same freezing-susceptible class established by the Newman-Keuls test ($\alpha = 0.05$). Variations appeared to exist within *A. arguta*, although the geographic or genetic origin of the clones tested was not known. All the other clones belonging to *A. arguta*, *A. kolomikta*, or *A. polygama* reached the same level of cold hardiness at the date of the test, based on necrosis length.

Discussion

The minimum temperature tolerated by plants depends mainly on their stage of development and previous environmental conditions. The importance of environmental factors, such as temperature and light, on the development of freezing tolerance in plants has been established for a long time (Levitt, 1980). As found in many plant species, short photoperiods and low nonfreezing temperatures induce acclimation of kiwifruit vine (Lu and Rieger, 1990). Artificial freezing tests throughout the winter demonstrated that the freezing tolerance of *Actinidia* vines was maximal in midwinter (Kim and Kim, 1986a, 1986b; Pyke et al., 1986). Nevertheless, short-term fluctuations in hardiness caused by changing temperatures are often superimposed on seasonal fluctuations (Howell and Weiser, 1970). *Actinidia* vines dehardened early in the spring so that bud burst in France occurs from the end of February for *A. kolomikta* (Blanchet, 1989) until mid-March for *A. deliciosa* 'Hayward'. Consequently, our *Actinidia* vines were probably not at their maximum level of cold hardiness when they were subjected to artificial freezing tests. Neither potential for maximum cold hardiness nor death threshold temperatures were evaluated in this experiment.

As assessed by the controlled freezing test of 22 Feb., the *A. arguta*, *A. kolomikta*, and *A. polygama* vines used are significantly harder than those of *A. deliciosa*. These results are consistent with those obtained previously by Blanchet (1989), following the natural freeze of 1985 where the temperature dropped below -18°C . From all the results obtained in both natural and artificial freezing conditions, *A.*

arguta, *A. kolomikta*, and *A. polygama* exhibited less damage than *A. deliciosa*. These results are also in accordance with the geographic distribution and natural habitat. The genus *Actinidia* is widespread, but the Section *Leiocarpae* (Dunn) Li, including *A. arguta*, *A. kolomikta*, and *A. polygama*, extends farther north than the others (Ferguson, 1990). All *A. arguta*, *A. kolomikta*, and *A. polygama* species originated from China to Japan and Korea, and the first two can be found in Siberia, although the cultivated species, *A. deliciosa*, is restricted to China (Li, 1952).

In the present experiment, *A. arguta*, *A. kolomikta*, and *A. polygama* were not significantly different in their ability to withstand subzero temperatures. The differences observed reflect as much variation within as between species. Conversely, in the literature, varying degrees of freezing susceptibility have been reported among these three *Actinidia* species. According to Avery (1991), *A. arguta* and *A. polygama* can withstand -31°C without freezing injury, whereas *A. kolomikta* can resist down to -40°C . The method in which the plant material is subjected to a series of temperatures is generally considered more suitable for differentiating genotypes that are close in their degree of cold hardiness (Levitt, 1980). However the application of this method to the present study was not feasible due to the very large number of plants that would be required.

Tolerance of *A. deliciosa* vines to freezing shows little variation between cultivars as based on necrosis percentage. This result is in accordance with previous ones involving natural and artificial cold experiments. Under orchard conditions, the male vines of 'Matua' and 'Tomuri' have been more tolerant to freezing injury than 'Hayward', although these differences were small (Dozier et al., 1992; Testolin and Messina, 1987). Using a controlled-environment chamber, Pyke et al. (1986) found only small differences in freezing tolerance between the two female cultivars Hayward and Bruno. The cultivars used in this study may not reflect the potential of *A. deliciosa*, as only a very small part of the genetic variability has so far been exploited (Ferguson, 1991). The first seeds of *A. deliciosa* introduced to New Zealand were collected in China at the beginning of this century from a restricted geographic area. Presumably, all the cultivars developed further in New Zealand and now distributed throughout the world derive from a few vines descended from these seeds (Ferguson and Bollard, 1990). Due to their common origin, only a small variation in their environmental requirements are expected from the cultivars tested here. The screening of introduced vines originating from the coldest area of China may be the best way to select cold-hardy clones within *A. deliciosa*. Moreover, in so far as genotype rank may change with year and season (Quamme, 1978), it would appear advisable to examine the cold hardiness more than once. Evaluating cold hardiness in autumn, winter, and spring would give a better representation of the overall cold hardiness than would a single midwinter determination.

Conclusion

This study has shown that genotypes vary in their response to freezing stress, indicating the potential for genetic enhancement of cold hardiness within the *Actinidia* genus. *Actinidia arguta*, *A. kolomikta*, and *A. polygama* show less freezing injury than *A. deliciosa*, and these results agree with observations made under natural freezing conditions. Further studies should include other *Actinidia* species or clones: *A. melanandra* has so far not been tested, neither has *A. purpurea*, now considered a variety of *A. arguta* (Ferguson, 1990).

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