Ecology and Epidemiology

A Relationship between Ice-Nucleation-Active Bacteria, Freeze Damage, and Genotype in Oats

D. Marshall

The Texas Agricultural Experiment Station, Texas A&M University Research and Extension Center, 17360 Coit Road, Dallas 75252.
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ABSTRACT

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Field and laboratory studies were conducted over a 2-yr period to determine if ice-nucleation-active (INA) bacteria played a role in the freeze damage of three oat cultivars and if cultivar:bacteria interactions were present. The winter-tender cultivar Florida 501 sustained greater freeze damage and supported higher populations of INA bacteria than did the moderately tender cultivar Coronado or the moderately hardy cultivar H-833. Populations of INA bacteria were 10^2–10^4 times greater on Florida 501 than on H-833. Field applications of Agristrep and Kocide 101 significantly reduced freeze damage and increased forage and grain yields of the three cultivars. Plots inoculated with an INA isolate of *Pseudomonas syringae* produced less forage and grain and had greater freeze damage than uninoculated plots. In a greenhouse and growth chamber study, the ability of individual plants to withstand freeze damage was tested at −4 C, for exposure times of 2–48 hr. Leaves of H-833 sustained freeze damage at a slower rate and supported fewer INA bacteria than Coronado or Florida 501. Although Coronado had the same detectable levels of INA bacteria as Florida 501, its leaves froze at a slightly slower rate than did leaves of Florida 501. When drenched in a mixture of Agristrep and Kocide 101, leaves of Florida 501 and Coronado had some freeze damage after 36 hr of exposure, while H-833 had no freeze damage up to 48 hr. The results suggest that INA bacteria as well as nonmicrobial factors were responsible for freeze damage in oats. Additionally, oat cultivars were found to differ in their resistance to INA bacteria, and in H-833, this resistance was expressed as a slow-freezing.

Additional keywords: disease resistance, winterhardiness.

Bacteria were first shown to act as ice nucleating agents in 1974 by Maki et al (14). They found that as the concentration of *Pseudomonas syringae* Van Hall cells increased, the temperature at which freezing occurred became warmer. Arny et al (2) showed that susceptibility to frost damage in corn was increased after application of *P. syringae* to leaves. Subsequently, *P. syringae* and other epiphytic bacteria have been found to be efficient initiators of ice crystal formation and to incite frost damage on numerous plant species and cultivated crops (1,10,13).

The presence and relative concentration of ice-nucleating-active (INA) bacteria varies in and between geographic locations and fluctuates over time (6,10,13). Additionally, leaf surfaces are not uniformly colonized by INA bacteria, and their populations can vary by more than 1,000-fold from leaf to leaf (8). The frequency distribution of INA bacteria on leaves has been found to approximate a lognormal distribution (8).

Besides the presence of INA bacteria, Ashworth et al (3) demonstrated that the length of exposure to subfreezing temperatures and leaf surface wetness were two other major factors in the ice nucleation temperatures of bean, corn, cotton, and soybean seedlings. However, research on snap bean leaflets (7) showed that the duration of time at subfreezing temperatures was less a determinant of nucleation frequency than was the test temperature.

In a study conducted on two oat cultivars in Wisconsin, the ice nucleation at higher temperatures was associated with larger bacterial populations, while nucleation at lower temperatures tended to be associated with smaller bacterial populations. No cultivar differences were noted in the study (7). In general, oats are susceptible to freeze damage and winterkilling. Because of this, oat production is limited to spring plantings in the northern United States. In central and southern portions of the country, oats can be fall planted, but there is often the risk of crop failure from freeze damage and winterkilling. Over the past 5 yr, oats have been planted annually on 486,000 ha in Texas (5). Most of the hectarage is in the south-central portions of the state. In Texas, oats are planted from September through October and are used primarily as a fall and winter forage for beef and dairy cattle. Oat hay and silage are also produced in the state. Only 25–30% of the oat crop is harvested for grain. Oats are preferred over other small grains for forage because of the abundant and rapid production of plant tissue. However, farmers and ranchers in northern Texas rely more on wheat as a forage because of its superior winterhardiness and increased value as a cash grain crop. It is possible that fall-planted oat hectarage could increase in more northern areas provided the risks of freeze damage and winterkilling were averted.

The objectives of this study were to determine whether INA bacteria were involved in freeze damage of oats and to ascertain if oat cultivars differed in their resistance to INA bacteria.

MATERIALS AND METHODS

Bacterial isolation. An epiphytic isolate (85D133) of *P. syringae* was collected from healthy leaves of cultivar Florida 501 in February 1985 at Dallas, TX. Leaves were washed in sterile distilled water and dilution-plated onto nutrient agar containing 2.5% glycerol. Any discrete colonies were transferred to modified Crosse's medium (4). Isolate 85D133 produced light-blue, domed colonies on modified Crosse's medium, and a diffusible fluorescent pigment on Pseudomonas Agar F (4,15). The arginine dihydrolase and oxidase reactions of 85D133 were negative. The isolate was determined to be ice-nucleation active at −4 C by the method of Lindow et al (13).

Field experiments. The oat (*Avena sativa* L.) cultivars Florida 501, Coronado, and H-833 were sown at a rate of 84 kg/ha in rows 17.3 cm apart at the Texas Agricultural Experiment Station in Dallas on 29 October 1985. Each plot was 1.2 m wide by 4.9 m long. The experiment was designed as a randomized complete block. Each block was a replication that consisted of the following treatments on all cultivars: 1) control, no treatment; 2) water, sterile distilled water applied at 46.7 L/ha; 3) streptomycin, applied at 5.6 kg/ha as Agristrep; 4) copper hydroxide, applied at 5.6 kg/ha as Kocide 101; and 5) *P. syringae* isolate 85D133, a concentration of 5.0×10^7 cells/ml applied at 46.7 L/ha. Bacterial inoculum concentrations were determined turbidimetrically. There were four blocks, giving a total of 60 plots. The treatments were applied every 2–3 wk beginning 16 November 1985 (two-leaf stage) and ending 2 April 1986 (heading stage).

In previous studies (Marshall, *unpublished data*), freeze damage in oats was evident in the field as necrosis of the leaf tips under a light freeze (0 to −2 C for 5–12 hr) and whole plant death under a heavy, prolonged freeze (below −2 C for longer than 12 hr). A scoring scale was developed to aid in the assessment of oat freeze damage in the field (Table 1).

Plots were harvested for forage on 12 January 1986; at that time, a sample of plant tissue was removed from the total forage per plot, weighed, dried at 65 C for 72 hr, and reweighed to obtain an estimate of percent moisture per plot. Plots were harvested for grain on 25 May 1986 and weighed for yield and test weight.

Temperature data were recorded at a standard National Oceanic and Atmospheric Administration weather station located approximately 120 m from the field experiments.

The field experiment was repeated during the 1986–1987 growing season with a planting date of 19 October 1986, forage harvest on 19 January 1987, grain harvest on 20 May 1987, and treatment applications beginning 9 November 1986 and ending 26 March 1987.

Laboratory assays. From each plot of the field experiment, 15 leaves were picked randomly, placed in sterile plastic bags, and put in a cooler for transportation to the laboratory. Collections were made in the first week of December, the third week of January, and the third week of March in both years. The tube nucleation test of Hirano et al (7) was used to assay leaf freezing because it provided a rapid means of obtaining estimates of the temperature at which ice nucleation occurred on each leaf. To begin the procedure, sterile test tubes were filled with 9 ml of sterile phosphate buffer and tested for the absence of ice nuclei in a controlled temperature bath at −10 C for 2–3 hr. The tubes were then shaken and those that did not freeze were allowed to warm to room temperature. Tubes that froze were discarded. Each leaf was placed in its own tube and chilled to 0 C in an ice bath. Leaves were first tested at −2 C for 90 min, at which time the tubes in which the buffer had frozen were recorded. The leaves were then sequentially tested at −4, −6, and −10 C (± 0.5 C) for 90 min each. The temperature at which freezing occurred was determined for each leaf. All leaves froze at −10 C.

After freezing, the leaves were allowed to reach room temperature. The 15 leaves and 135 ml of buffer from the samples of each plot were composited into a sterile 500-ml flask and shaken for 1 hr at 200 rpm. A series of 10-fold serial dilutions were plated onto Pseudomonas Agar F containing 100 mg/L of cyclohexamide to inhibit fungal growth. Bacterial colonies were counted after 5 days at 22 C. The replica freezing method of Lindow et al (13) was used to estimate the frequency of INA bacterial colonies.

Effect of time on freeze damage. Seeds of the three cultivars were surface disinfected in 0.05% sodium hypochlorite for 2 min, soaked in sterile distilled water for 4 hr, and planted into sterile potting soil in 7-cm-square pots in the greenhouse. Three treatments were imposed on the plants over a 4-wk period. The treatments were: 1) control—no treatment; 2) drench—once a week, plants were dipped into a mixture of Agristrep plus Kocide, each at 0.2 kg/L; and 3) inoculated—once a week for the first 2 wk, plants were sprayed until runoff with a 5×10^7-ml concentration of *P. syringae* isolate 85D133. The *P. syringae*-inoculated plants were left untreated for the last 2 wk of the experiment. Care was taken during the course of this experiment to minimize cross-contamination between inoculated and uninoculated plants by using a separate building in which to inoculate with *P. syringae* and thereafter not allowing the plants to contact each other in low-temperature incubator and in the greenhouse.

At the end of 4 wk, all of the plants were placed in a controlled temperature incubator at 5 C. The temperature was then lowered to −4 C (± 0.5 C) over a 1-hr period. Five pots of each cultivar-treatment combination were removed from the incubator after 2, 5, 10, 24, 36, and 48 hr of exposure at −4 C. The plants were placed in the greenhouse at 24–28 C. After 24 hr in the greenhouse, plants were rated for freeze damage as the number of leaves per pot that exhibited water soaking. The number of INA bacteria was estimated by the same method used for the field-grown leaves.

RESULTS

Temperature data. During the 1985–1986 field experiment, the lowest temperature recorded before the 1 December sampling time

TABLE 1. Visual assessment scale for freeze damage in oats

0–9 scale	Amount of damage
0	No visible damage
1	0–10 of uppermost leaves necrotic
2	10–25% of uppermost leaves necrotic
3	25–50% of uppermost leaves necrotic
4	50–75% of uppermost leaves necrotic
5	75–100% of uppermost leaves necrotic
6	0–25% of whole plants dead
7	25–50% of whole plants dead
8	50–75% of whole plants dead
9	75–100% of whole plants dead

was −0.5 C on 28 November. Between the first and second sampling times, a low of −5.0 C was reached on 13 December and 8 January. Also, during that sampling time, lows of between 0 and −3.3 C were reached on 23 separate days. From 21 January to 23 March, there were 15 days when the temperatures reached between 0 and −3.3 C. The lowest temperature during that third sampling time was −7.2 C on 11 February.

During the 1986–1987 field experiment, no subfreezing temperatures were reached before the 3 December sampling date. From 3 December to 23 January, 22 days had lows of between 0 and −3.3 C. During that time, the lowest temperature was −4.4 C on 18 January. There were six subfreezing days of between 0 and −3.3 C between 23 January and 22 March.

The length of time the temperatures stayed at subfreezing levels was not not recorded. However, the daily high temperatures were never less than 1.1 C over both years of the experiment.

Field experiments. Because the cultivars differed in inherent forage and grain yield potential, only treatments within a cultivar were compared. Significant differences between years were found for forage yields but not for grain yields and test weights. Therefore, forage yields were reported for each year, while grain yields and test weights were averaged over 2 yr. Applications of Agristrep and Kocide 101 reduced freeze damage and significantly increased forage and grain yields over control plots of Florida 501 and Coronado (Table 2). There were nonsignificant increases in the forage and grain yields of H-833 sprayed with Agristrep and Kocide 101. Freeze damage on Florida 501 increased from a level of 10–50% leaf necrosis in bacteriocide-treated plots to 25–50% plant death in plots inoculated with isolate 85D133 of *P. syringae*. The freeze damage on Florida 501 inoculated with *P. syringae* was associated with a forage yield reduction of 40–44% and 43–50%, respectively, in 1986 and 1987, over the bacteriocide treatments. The decrease in grain yield in Florida 501 was approximately 20%. Plots sprayed with sterile distilled water tended to have increases in forage and grain yields; however, the increases were not significant. Grain test weight was unaffected by all treatments (Table 2).

Laboratory analysis of field experiments. To determine if INA bacteria were responsible for the freeze damage found in the field, laboratory assays were conducted on the field-grown oat leaves. The percent of leaves that froze at −2, −4, −6, and −10 C was determined for each cultivar-treatment combination during each collection time. Frozen leaves ranged from 0% at 0 C to 100% at −10 C. Because of this range and because of unstable variances in the data, the arcsin-square root transformation of percent leaves frozen was used before statistical analysis. The arcsin-square root of leaves frozen was plotted against freezing temperature, and the area under the curve (AUC) was determined. AUC integrated the cumulative amount of leaves frozen over the range of temperatures tested. The AUC values for each cultivar-treatment combination did not differ significantly between years, and the values were combined (Table 3). The cultivar-treatment combinations whose leaves froze at cooler temperatures had smaller AUC than those that froze at warmer temperatures. It was found that the AUC values increased for all cultivar-treatment combinations as the seasons progressed. The control plots of H-833 had significantly smaller AUC values than Florida 501 and Coronado at all collection times. When *P. syringae* was applied to the leaves, the AUC values increased for all three cultivars; however, the AUC for H-833 with *P. syringae* was significantly less than that of Florida 501 and Coronado.

The temperature at which 50 (TP_{50}) and 90% (TP_{90}) of the leaves froze was calculated from the arcsin-square root of leaves frozen vs. freezing temperature curve for each cultivar-treatment combination at each collection time. The 2-yr combined data for TP_{50} and TP_{90} are shown in Table 3. Agristrep and Kocide 101 caused a colder TP_{50} and TP_{90} for the three cultivars over the controls. TP_{50} and TP_{90} increased in the control and sterile water treatments as the seasons progressed. In the March samplings, 90% of the leaves of the Florida 501 controls froze at −2.4 C, while those of Coronado froze at −4.3 C and H-833 at −7.8 C.

Figure 1 shows the populations of INA bacteria for each cultivar, treatment, and collection time. More INA bacteria were detected on control plots of Florida 501 and Coronado than on H-833, regardless of the collection time. Agristrep and Kocide 101 applications reduced the population levels on all cultivars. Generally, the populations increased as the seasons progressed. INA bacterial populations on control plots ranged from 10^2 to 10^4 times greater on Florida 501 than on H-833, depending on the sampling time.

Effect of time on freeze damage. In the greenhouse experiments, as the length of exposure at −4 C increased, the number of leaves that froze (exhibited water soaking) also increased (Fig. 2). This was true for all cultivars and treatments with the exception of the H-833 bacteriocide-drench and control treatment, which exhibited no signs of freeze damage after 48 hr. After 2 hr of exposure at −4 C, the *P. syringae*-treatment of Florida 501 had 60% of its leaves

TABLE 2. Effect of bacteriocides, *Pseudomonas syringae* isolate 85D133, and water on freeze damage, forage and grain yield, and grain test weight of three oat cultivars

		1986		1987			
Cultivar	Treatment[a]	Freeze damage[b]	Forage yield[c]	Freeze damage	Forage yield	Grain yield[c]	Test weight
Fla 501	Agristrep	3	4.32 a	3	4.62 a	7.76 a	48.7 a
	Kocide 101	3	4.57 a	2	5.22 a	7.79 a	48.3 a
	P. syringae	7	2.56 c	6	2.71 c	6.18 b	48.2 a
	Water	5	3.34 b	5	3.92 b	7.16 a	48.4 a
	Control	5	3.17 b	6	3.84 b	7.21 a	47.9 a
Coronado	Agristrep	3	6.01 a	3	6.53 a	6.04 a	47.3 a
	Kocide 101	2	6.15 a	3	6.31 a	6.21 a	47.1 a
	P. syringae	6	4.18 c	6	5.15 c	5.13 c	46.6 a
	Water	6	5.09 b	6	5.61 b	5.78 b	47.1 a
	Control	5	4.98 b	5	5.49 b	5.72 b	46.9 a
H-833	Agristrep	2	6.27 a	2	6.34 a	6.69 a	47.4 a
	Kocide 101	2	5.98 a	3	6.12 a	6.73 a	47.3 a
	P. syringae	6	5.13 b	6	5.29 b	6.00 b	47.4 a
	Water	4	5.85 a	3	6.09 a	6.50 a	47.0 a
	Control	4	5.76 a	3	5.98 a	6.58 a	47.1 a

[a] Treatments were Agristrep and Kocide 101 applied at 5.6 kg/ha, *P. syringae* isolate 85D133 in a concentration of 5.0×10^7 cells/ml, applied at 46.7 L/ha, and sterile distilled water applied at 46.7 L/ha. Treatments were applied every 2–3 wk from 16 November 1985 to 2 April 1986 and from 9 November 1986 to 26 March 1987.

[b] Freeze damage was assessed by a visual rating on a 0–9 scale, where 0 was no sign of damage and 9 was complete plant death (see Table 1). Plots were rated for freeze damage just before forage harvest.

[c] Forage yields were in metric tons of dry forage per hectare. Grain yields were in metric tons per hectare. Grain test weights were in kilograms per hectoliter. Means followed by the same letter were not significantly different at $P = 0.05$ according to Duncan's multiple range test. Mean comparisons were valid between treatments within a cultivar.

TABLE 3. Freeze development on three oat cultivars in December, January, and March over 2 yr as affected by bacteriocides, *Pseudomonas syringae* isolate 85D133, and water

Cultivar	Treatment[b]	December[a]			January			March		
		AUC[c]	TP_{50}[d]	TP_{90}[d]	AUC	TP_{50}	TP_{90}	AUC	TP_{50}	TP_{90}
Fla 501	Agristrep	294 a	−6.9	−8.8	350 b	−6.8	−8.6	400 b	−6.6	−8.2
	Kocide 101	306 a	−6.9	−8.8	345 b	−6.5	−8.5	397 b	−6.2	−8.2
	P. syringae	779 e	−1.8	−4.9	865 f	−1.3	−2.3	887 f	−1.2	−1.8
	Water	671 d	−2.8	−5.7	757 de	−1.9	−4.7	816 e	−1.4	−4.0
	Control	719 e	−2.1	−5.3	798 e	−1.7	−3.6	854 f	−1.4	−2.4
Coronado	Agristrep	280 a	−7.2	−8.8	322 b	−7.0	−8.8	375 b	−6.6	−8.6
	Kocide 101	286 a	−7.0	−8.8	333 b	−6.8	−8.7	383 b	−6.8	−8.8
	P. syringae	734 e	−1.8	−5.6	862 f	−1.4	−2.9	879 f	−1.4	−2.0
	Water	576 cd	−4.2	−7.5	715 d	−2.3	−7.0	763 d	−1.8	−5.5
	Control	613 e	−3.4	−7.5	707 d	−2.0	−6.5	789 de	−1.7	−4.3
H-833	Agristrep	245 a	−7.4	−8.6	280 a	−7.2	−8.6	300 a	−7.2	−8.5
	Kocide 101	253 a	−7.2	−8.7	275 a	−7.0	−8.6	293 a	−6.8	−8.5
	P. syringae	660 d	−2.8	−7.7	780 e	−1.6	−4.8	805 e	−1.6	−3.6
	Water	382 b	−6.4	−8.4	453 c	−6.0	−8.2	526 c	−3.7	−7.8
	Control	442 b	−6.2	−8.4	483 c	−6.0	−8.2	562 c	−3.2	−7.8

[a] Samples were taken in the first week of December, the third week of January, and the third week of March in both years.

[b] Treatments were Agristrep and Kocide 101 applied at 5.6 kg/ha, *P. syringae* isolate 85D133 in a concentration of 5.0×107 cells/ml, applied at 46.7 L/ha, and sterile distilled water applied at 46.7 L/ha. Treatments were applied every 2–3 wk from 16 November 1985 to 2 April 86 and from 9 November 1986 to 26 March 1987.

[c] AUC is the Area Under the Curve of the arcsin-square root of leaves frozen plotted against freezing temperature from 0 to −10 C. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test. Mean comparisons are valid among all cultivar-treatment combinations within a sampling time.

[d] TP_{50} and TP_{90} denote, respectively, the temperature at which 50 and 90% of the leaves froze in each cultivar-treatment combination. TP_{50} and TP_{90} were calculated from the arcsin-square root of leaves frozen vs. freezing temperature curves.

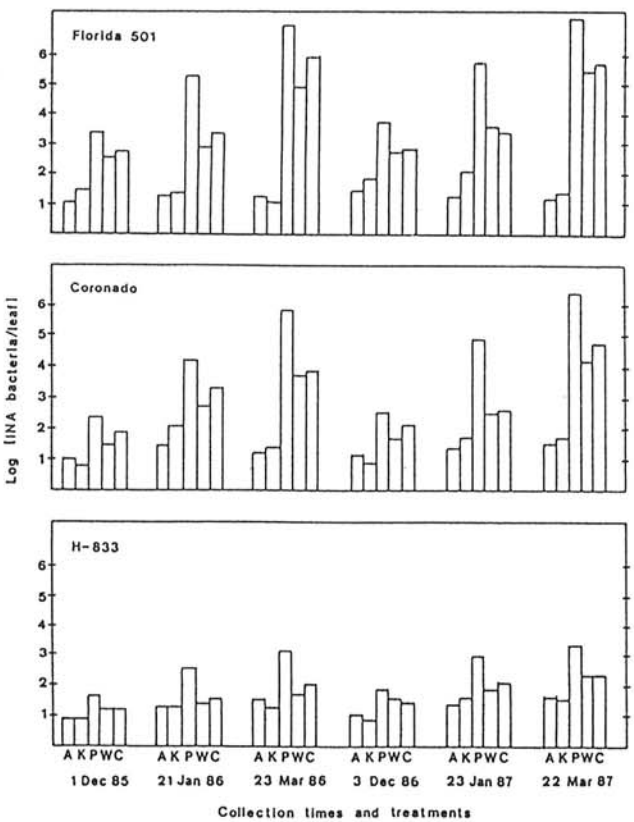

Fig. 1. Leaf populations of INA-bacteria on oat cultivars Florida 501, Coronado, and H-833 determined at six collection times. Treatments were A = Agristrep, K = Kocide 101, P = *Pseudomonas syringae* isolate 85D133, W = sterile distilled water, and C = control.

frozen, while Coronado had 51% and H-833 had only 8% frozen. The leaves of H-833 froze at a slower rate than leaves of Coronado and Florida 501. Florida 501 froze the fastest of the three cultivars, with 100% of its leaves frozen at 10 hr of exposure to −4 C. The rate at which Coronado froze was intermediate between H-833 and Florida 501 (Fig. 2C). The Agristrep-Kocide 101 drench treatment effectively increased the time that leaves could be held at −4 C before exhibiting freeze damage (Fig. 2B). The H-833-bacteriocide and control treatments showed no signs of freeze damage after 48 hr (Fig. 2A and B). However, leaves of Florida 501 and Coronado had increasing amounts of freeze damage in both the control and bacteriocide drench treatments (Fig. 2A and B). It is shown in Figure 3 that INA bacteria were present on control leaves of Florida 501 and Coronado, but not H-833. No INA bacteria were detected on bacteriocide-drenched leaves of any of the cultivars.

DISCUSSION

Before the tissue water in leaves freeze, the water generally supercools (9,16). Ice forms in supercooled water when an ice nucleus is present to initiate formation (17). Lindow et al (11,12) have shown that some epiphytic bacteria may act as ice nuclei and prevent the supercooling of plant tissue water, thereby inducing frost damage. Certain species of plants have been found to harbor more INA than other species (13). Sampling time, maturity stage, and geographic location are major factors that determine the concentrations of epiphytic INA bacteria (1,7,8,10). In the present work, associations were found between the freezing resistance of oat cultivars and INA bacteria. Additionally, the moderately winter-hardy cultivar H-833 was found to possess the ability to suppress INA bacterial populations and subsequently to reduce freeze damage.

In this study, forage production was increased by reducing the population levels of INA bacteria, which allowed plants to survive subzero temperatures. Because of their cost, the two bacteriocides, Agristrep and Kocide 101, could not be applied commercially at the rates and frequencies used. It is unlikely that bacteriocides could be economically used to prevent freeze damage in oats.

Crop yield loss from freeze damage was assessed in the field over 2 yr. The winter-sensitive cultivar Florida 501 showed a reduction of more than 40% in forage yield and 20% of grain yield when Agristrep-treated plots were compared with *P. syringae*-inoculated plots. If the same treatment comparison was made for the winter-hardy cultivar H-833, it showed that an 18% loss in forage and a 12% loss in grain yield occurred. Furthermore, the ice nucleation temperature of the individual leaves and the concentration of INA bacteria on those leaves indicated that H-833 suppressed INA bacterial populations and that this suppression resulted in less freeze damage and less yield reduction.

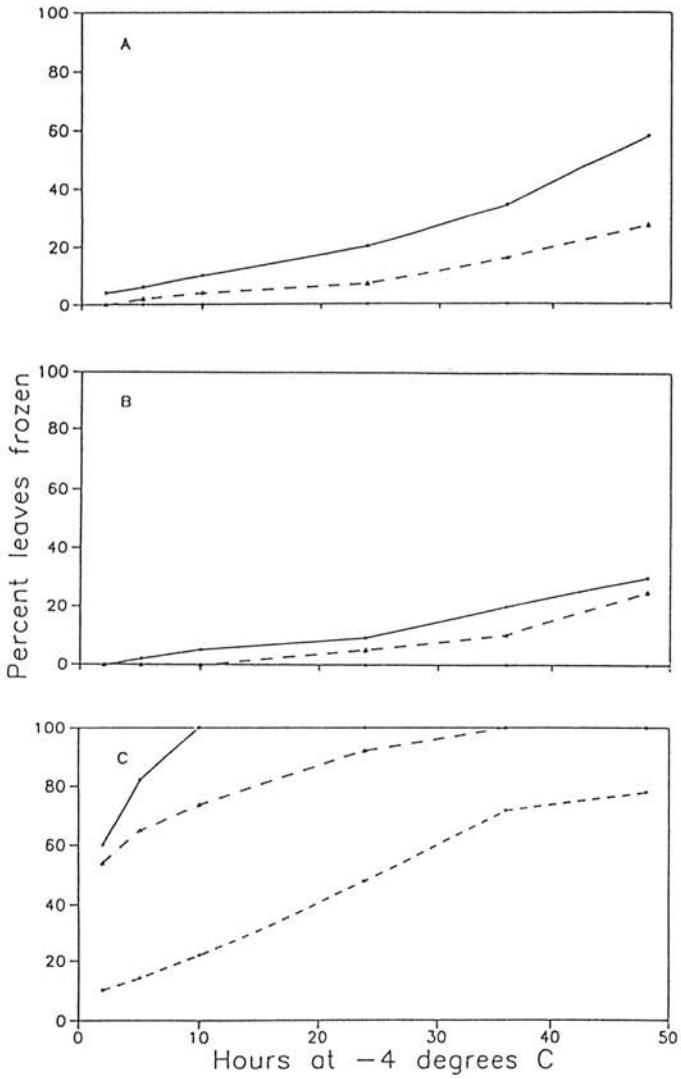

Fig. 2. The percent frozen leaves of three oat cultivars as a function of exposure time at −4 C. **A,** Control treatment; **B,** bacteriocide drench; **C,** inoculation with *Pseudomonas syringae* isolate 85D133. * = Florida 501; △ = Coronado; and ■ = H-833.

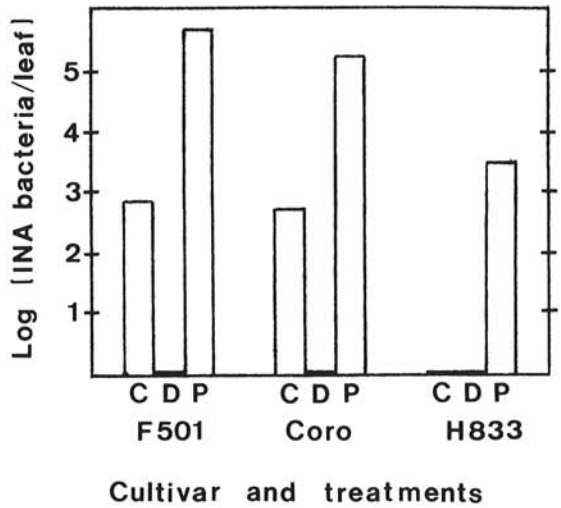

Fig. 3. Leaf populations of INA-bacteria on oat cultivars Florida 501, Coronado, and H-833 after exposure to −4 C for up to 48 hr. Treatments were C = control; D = drench in bacteriocides, and P = inoculation with *Pseudomonas syringae* isolate 85D133.

Conflicting results have been reported on the effect of exposure time at subzero temperatures on freeze damage. Hirano et al (7) found that 95% or more of ice nucleation events occurred in the first 30 min of a tube nucleation test with snap bean leaves. However, Ashworth et al (3) concluded that ice nucleation depended on the time spent at subzero temperatures. Bean, corn, cotton, and soybean seedlings as well as sterilized suspensions of kaolin exhibited more freezing as the length of exposure time increased (3). In the present study, it was found that oat cultivars vary in the rate at which they freeze when they are held at −4 C for up to 48 hr. The rate of freezing depended on the concentration of introduced INA bacteria present and the cold hardiness of the cultivars. Florida 501 supported higher levels of INA bacteria than Coronado or H-833 and also suffered the greatest freeze damage at a more rapid rate. When leaves of Florida 501 were drenched once a week in the bacteriocide mixture, then tested, a proportion of the leaves still froze as exposure time increased. No INA bacteria could be detected on those leaves. Explanations for this could be that the bacteria were present, but at low, undetectable levels or that the lack of cold hardiness of the leaves simply rendered Florida 501 more susceptible to damage by subzero temperatures. Because INA bacteria were detected on the control leaves of Florida 501 and Coronado, it is possible that the INA bacteria were present in the ambient air of the greenhouse. Whether the populations on the control leaves of Florida 501 and Coronado were actually isolate 85D133 is not known, because the isolate was not labeled in any way. However, this is insignificant compared to the fact that INA bacteria grew on Florida 501 and Coronado, but not on H-833 under ambient greenhouse conditions. This again suggests that H-833 possessed the ability to suppress increases in INA bacterial populations.

Figures 3 and 2B show that when no INA bacteria were detectable on leaves, plants of Florida 501 and Coronado had increased amounts of freeze damage over time, while plants of H-833 exhibited no freeze damage. Thus, H-833 had a higher level of cold hardiness that made it less susceptible to ice formation and subsequent damage. The addition of *P. syringae* served to initiate freeze damage, but the damage increased at a slower rate on H-833 than on *P. syringae*-initiated damage on Florida 501. Therefore, H-833 can be said to exhibit a slow-freezing resistance. Slow-freezing in H-833 is expressed as a delay in freeze damage as compared with the fast-freezing oat, Florida 501. The freeze resistance of H-833 is possibly a reflection of its inability to act as a host for INA bacteria coupled with a resistance to cold temperatures. The mechanism of freeze resistance in H-833 is unknown. The resistance could act directly on the INA bacteria or it could favor the growth and reproduction of non-INA bacteria, thereby keeping the number of ice nuclei below damaging levels.

A hypothesis can be offered on how some oat cultivars withstand freeze damage. Freeze damage in oats appears to be a function of inherent cold hardiness coupled with an ability of the plant to resist increases in population levels of INA bacteria. An oat cultivar will freeze at a warmer temperature if it is more conducive to colonization by INA bacteria. Thus, the amount of freeze damage that occcurs depends on the the plant's inherent ability to withstand cold temperatures, the concentration of INA baceria on its leaves, and the length of exposure to subfreezing temperatures.

The experiments in this study were conducted at a temperature fluctuation of ±0.5 C. This could have caused some shifts from bacterial ice nucleation to intrinsic, oat plant nucleation. However, the data unequivocally demonstrate that oat cultivars differ in their levels of freeze resistance, and that a component of freeze resistance is lower INA bacterial populations on leaves.

Because oat cultivars differ in resistance to freeze damage and because this is due, at least in part, to INA bacteria, then there must be genetic factors that cause cultivars to be less conducive to INA bacteria. It should be possible, therefore, to conduct genetic studies on oat cultivars that vary in their receptivity to INA bacteria and to determine the mode of inheritance of the genetic factors.

It is apparent that freeze damage in oats occurs when the host and bacteria are together in the presence of a conducive climate. It is perhaps unique in this system that damage occurs only when temperatures drop below freezing. Thus, the plant and the bacteria

can be in intimate association for a long period of time, but it is not until the temperature is conducive that damage occurs. The temperatures do not have to be below freezing for a very long period of time in order for Florida 501 to suffer freeze damage. However, below-freezing temperatures must remain so for longer periods of time in order for H-833 to sustain appreciable freeze damage.

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