

Response Model for an Introduced Pest—
The Russian Wheat Aphid

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**Application of Ice-Nucleating Active
Bacteria Decreases the Supercooling
Capacity of the Russian Wheat Aphid
(Homoptera: Aphididae)**

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*Russian wheat aphids, Diuraphis noxia (Mordvilko), were exposed to a killed, freeze-dried preparation of ice nucleating active bacteria (Pseudomonas syringae) by ingestion in sucrose solution, and topically by misting them with the bacteria in distilled water. Untreated (dry) Russian wheat aphids consistently supercooled to -25.0°C before internal ice nucleation occurred. Ingestion of *P. syringae* suspensions of 10–150 mg/ml in 10% sucrose elevated the supercooling point an average of 16.3°C compared with those fed only 10% sucrose. Topical application of *P. syringae* in a water suspensions elevated the supercooling point an average of 9.0°C compared with aphids exposed to distilled water. Supercooling points from aphids that ingested *P. syringae* were more consistent and higher than for aphids exposed topically. This result would be expected because the gut provides the bacteria direct contact with body water. The soil surface temperature in winter wheat (winter 1993–1994) compared with the supercooling temperatures in these experiments shows that the Russian wheat aphid would be significantly more susceptible to winter temperatures and lethal internal ice formation after being exposed to *P. syringae*. *P. syringae* has the*

potential for use as a microbial insecticide that would decrease winter survival of the Russian wheat aphid by reducing its supercooling capacity.

The overwintering biology of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has been studied in Colorado since 1988. This aphid is freeze-intolerant, supercools to -26.0°C , and is prone to prefreeze mortality (mortality at temperatures above its supercooling point). Armstrong and Peairs (1996) found 2 climatic events that were responsible for causing 100% winter mortality in Russian wheat aphids infesting winter wheat, *Triticum aestivum* L. The 1st occurred when a rapidly moving cold front lowered the soil surface temperature to below -29.0°C for 24 h. The 2nd occurred when there were 40 d of continuous snowcover (>1 cm deep). Field research on winter wheat in northeastern Colorado and southeastern Kansas has demonstrated clearly that the Russian wheat aphid is the most cold-tolerant aphid infesting small grains in the Great Plains of the United States (Harvey and Martin 1988, Armstrong and Peairs 1996). The Russian wheat aphid is the most important, economically threatening insect pest of small grains in the Great Plains (Legg et al. 1993). Winter wheat and barley are infested by Russian wheat aphids in 2 ways. The 1st, and most economically threatening occurs when they successfully overwinter and infest the next year's crop. Alternatively, infestations occur later in the spring with winged migrations from the south. This infestation route may or may not develop into economically threatening densities.

Russian wheat aphid feeding on winter wheat causes significant reductions in the number of forming tillers, leaves, leaf area, root length, and dry weights of stem, leaf, root, and shoot (Burd and Burton 1992). These physiological losses in plant growth caused by Russian wheat aphid feeding are the result of plant water imbalances that reduce cell turgor and growth.

Because most overwintering insects are intolerant of internal freezing, it is critical that they either avoid exposure to low temperatures or resist freezing by increasing their capacity to supercool (Lee 1989, 1991). The limit of supercooling is defined as the lowest temperature to which an insect may be cooled before internal freezing begins and the heat of crystallization is detected. Various factors are known to influence the supercooling capacity of insects including body size, low-molecular-weight sugars and polyols, hemolymph antifreeze proteins and the efficacy of endogenous ice nucleators (Zachariassen and Hammel 1976, Storey and Storey 1988, Duman et al. 1991, Lee et al. 1993).

A small group of bacteria has the unusual capacity to catalyze ice nucleation at temperatures as high as -1°C (see reviews in Lee et al. 1995a). Most of these rod-shaped, gram-negative, asporogenous bacteria are epiphytic plant pathogens. By inducing ice nucleation in the plant tissues on which they reside, these bacteria are responsible for extensive frost-related crop losses (Lindow 1983). Bacterial representatives from this class

of highly efficient biological ice nucleators have been identified as normal flora in the insect gut where they play an important role in regulating the supercooling capacity of their host (Kaneko et al. 1991a, b, Lee et al. 1991, 1993; Tsumuki et al. 1992).

Recently it was proposed that ice-nucleating microorganisms might be used to increase mortality of freezing intolerant insect pests that are attempting to overwinter (Strong-Gunderson et al. 1990, Lee 1991, Fields 1992, Lee et al. 1993). A number of studies have demonstrated that ingestion or topical application of these ice-nucleating active bacteria and fungi may be used to decrease the supercooling capacity of a wide range of insects (Diptera, Lepidoptera, Hemiptera, and Coleoptera), including stored grain pests and the Colorado potato beetle (M.R. Lee, et al. 1992, R.E. Lee, et al. 1992, 1994, 1995b, Strong-Gunderson et al. 1992, 1997, Fields 1993).

The purpose of this study was to determine if exposing the Russian wheat aphid to a killed, freeze-dried preparation of ice-nucleating active bacterium, *Pseudomonas syringae*, would significantly decrease its supercooling capacity and make it more susceptible to subzero temperatures. The soil surface temperature was measured in a wheat field to determine temperatures Russian wheat aphids are exposed to during the winter, and to compare them with the supercooling points of aphids exposed to *P. syringae*.

Materials and Methods

A colony of Russian wheat aphid was maintained through the 1993–1994 winter on potted 'TAM 107' winter wheat in the greenhouse. Russian wheat aphids used in starting the colony originated from volunteer winter wheat on the Central Great Plains Research Station, Akron, CO.

Supercooling points were determined by placing individual aphids (apterous, 4th instar) on the surface of a 24-gauge copper-constantan thermocouple with a camel hair brush and securing it with a thin layer of petroleum jelly. The thermocouple was placed inside a pyrex test tube (1.5 by 15 cm). This test tube was placed inside a larger test tube (3 by 16 cm) to stabilize the temperature differential. Both test tubes were held partially submerged in a dry ice 95% ethanol bath at a temperature between -60° and -70°C . The temperature was decreased $2^{\circ}\text{C}/\text{min}$ by lowering the thermocouple into the smaller test tube. The thermocouple was attached to a CR21X micrologger (Campbell Scientific, Logan UT) that was programmed to display and record the temperature every 0.20 s. These data were then transferred to a DOS personal computer via a cassette recorder, where they could be managed in a spreadsheet (Quattro Pro, version 3.0, Borland Int., Scotts Valley, CA) program. The data were plotted to show the lowest body temperature (e.g., supercooling point) before the release of the latent heat of crystallization. A killed, freeze-dried preparation of *P. syringae*, provided

by Genencor International, Rochester, N.Y., was mixed with distilled water at 10, 25, 50, 75, and 150 mg/ml (milligrams bacteria/milliliter water). This preparation had an ice-nucleating activity of 2.02×10^4 ice-nucleating sites/gram. The exterior of a single aphid was exposed to the solutions by spraying 1 time with a mist bottle. A control group was treated with distilled water using the same application method. Individual aphids were supercooled immediately following applications of bacteria and distilled water treatments. The same concentrations (10–150 milligrams *P. syringae* per milliliter water) were mixed in 10% sucrose solutions and fed to aphids 24 h before supercooling determinations. Aphids were fed the suspensions by placing small amounts in the top of a 1-ml plastic centrifuge vial covered by a tightly stretched piece of parafilm. Aphids were enclosed in the vials and allowed to feed through the parafilm. Only aphids that were actively feeding (had their stylets inserted through the parafilm) were used for determining supercooling points. A control group fed 10% sucrose alone for the same amount of time was included. Fifteen supercooling points were performed for all topical, ingested, and control groups. Significant differences in supercooling point values were detected by the Dunns multiple comparison test.

The soil surface temperature was measured from 1 to 2 cm above the soil surface in a 4.3-ha field of 'TAM 107' winter wheat during the 1993–1994 winter. The thermocouples were positioned in close proximity to the wheat tillers where Russian wheat aphids overwinter. Temperatures were recorded in 4 separate locations with 30-gauge, copper-constantan thermocouples connected to a CR21X micrologger. Each location was an average of an array of 5 thermocouples covered with a 1-cm length of teflon rod (0.9 cm diameter) to prevent electrical interference in the presence of moisture. The temperature readings were recorded every 60 s and averaged to 1 h. Daily maximum and minimum temperatures were recorded by a CR21X from 18 September to 1 April 1994. Field temperature data were stored and managed in the same methods as supercooling temperatures.

Results and Discussion

Comparison of Exposure Methods. To determine if the exposure method affected supercooling in the absence of the bacteria, the control groups that differed only by exposure method were compared. The mean supercooling point (-18.7°C) of aphids topically exposed to distilled water was significantly higher ($P < 0.05$) than for dry aphids or those fed 10% sucrose (-23.3°C) (Table 1). The reason for this relative elevation in the supercooling point with external water exposure is probably caused by inoculative freezing, where the surface water on the cuticle freezes and initiates internal freezing (Salt 1963, Lee et al. 1993). Supercooling point

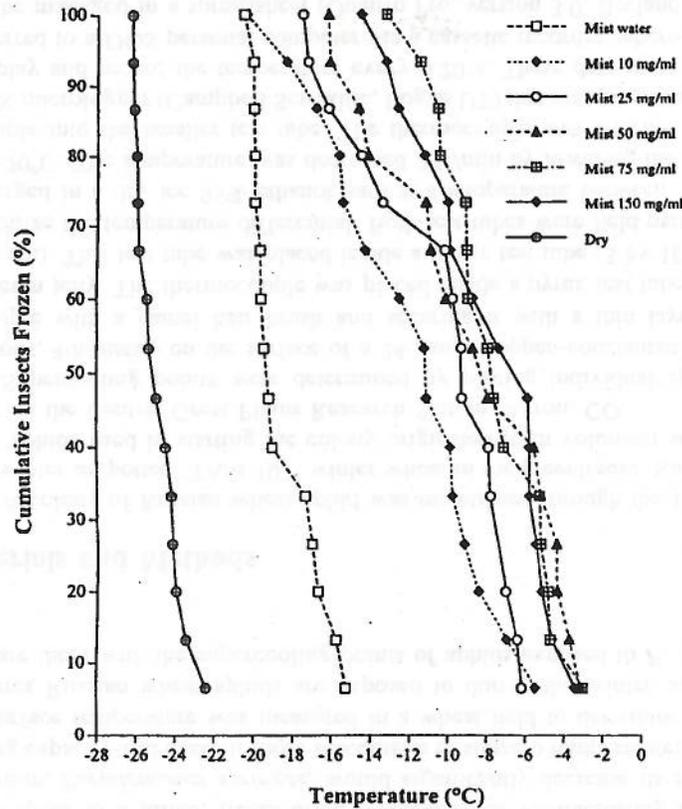


Fig. 1. Cumulative freezing profile of Russian wheat aphids ($n = 15$) after feeding for 24 h on a 10% sucrose solution containing 0–150 mg/ml of a killed, freeze-dried preparation of *Pseudomonas syringae*.

values of dry aphids closely matched those reported by Butts (1992) (-26.0°C), and Armstrong (1994) (-26.2°C).

Ingestion Studies. All supercooling point values for Russian wheat aphids fed *P. syringae* in 10% sucrose solution were significantly higher than those ingesting 10% sucrose alone (Table 1). Mean values increased 14.7 – 17.8°C . In the control group, no aphids began to freeze until temperatures decreased below -21.0°C (Fig. 1). In marked contrast, even at the lowest bacterial suspensions tested (10 mg/ml), aphids began to freeze above -4.0°C , with all aphids in this treatment group freezing by -13.0°C .

Topical Exposure. Although Russian wheat aphids exposed to a solution of 10 mg/ml *P. syringae* had a mean supercooling point 6.3°C greater than those misted with distilled water, this difference was not statistically

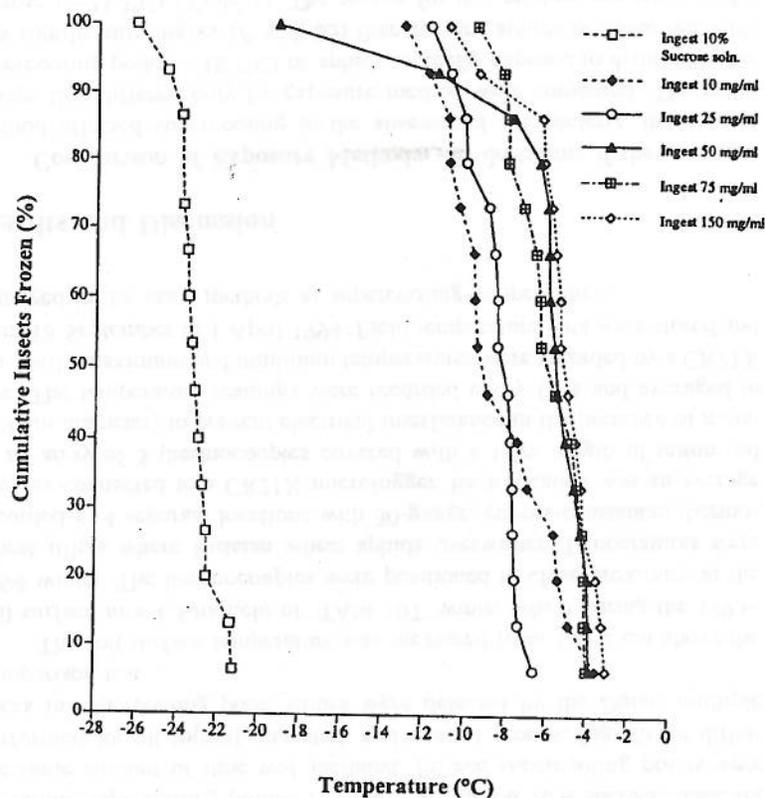


Fig. 2. Cumulative freezing profile of Russian wheat aphids ($n = 15$) after being misted with a killed, freeze-dried preparation of 0–150 mg/ml of *Pseudomonas syringae* in distilled water.

significant (Table 1). Increasing concentrations of *P. syringae* significantly reduced the supercooling capacity of the Russian wheat aphid by 8.0°C (25 mg/ml) to 10.4°C (150 mg/ml) (Table 1). In the control group of dry aphids, no individual began to freeze until temperatures decreased to below -22.0°C , whereas those misted with water had supercooling points in the range of -15.0 – -20.0°C (Fig. 2). Aphids misted with *P. syringae* suspensions began to freeze at temperatures as high as -3.0°C . For the group treated with 50 mg/ml, half of the aphids froze at temperatures above -9.0°C .

Comparison of Field Temperatures with Supercooling Points. Soil surface temperatures were mild during the 1993–1994 winter compared with the 1989–1992 winters when they were measured using the same methods (Armstrong and Peairs 1996). The lowest hourly soil surface temperature recorded for the 1993–1994 winter was -18.4°C on day 40 or 9 February

Table 1. Mean \pm SEM supercooling temperatures of Russian wheat aphid after ingestion or topical application ice nucleating active (INA) bacterium *Pseudomonas syringae*, March 1994

Treatment	n	Temperature, °C
Ingested INA		
10% sucrose (control)	15	-23.3 \pm 0.31
10 mg/ml	15	-8.6 \pm 0.72**
25 mg/ml	15	-8.6 \pm 0.38*
50 mg/ml	15	-6.6 \pm 1.02***
75 mg/ml	15	-6.1 \pm 0.46***
150 mg/ml	15	-5.5 \pm 0.56***
Misted INA		
Distilled water (control)	15	-18.7 \pm 0.45
10 mg/ml	15	-12.4 \pm 1.12
25 mg/ml	15	-10.7 \pm 1.03**
50 mg/ml	15	-9.1 \pm 1.20***
75 mg/ml	15	-7.9 \pm 0.75***
150 mg/ml	15	-8.3 \pm 0.90***

Within a treatment, means followed by asterisks are significantly different from the control values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; Dunns multiple comparisons test).

(Fig. 3). Overall, the average soil surface temperature was below -10.0°C for 136 h, and below -15.0°C for 71 h during the winter. Eighty four percent of all Russian wheat aphids ingesting *P. syringae*, and 59% of those topically exposed in our experiments, froze above -10.0°C . Those respective percentages for both *P. syringae* exposure methods increase to 100 and 93% when the temperature was lowered to -15.0°C .

Many insects, including the Russian wheat aphid, experience pre-freeze mortality at temperatures above their supercooling point (Butts 1992). The significance of exposing Russian wheat aphids to *P. syringae* is that even short exposure to low temperature causes internal ice formation and death, compared with prefreeze mortality that usually requires hours or days of exposure to low temperature.

The results of this research demonstrate that the supercooling, (thus the cold tolerance) of Russian wheat aphids is reduced markedly following exposure to the ice-nucleating active bacterium *P. syringae*. These findings justify further research on the use of ice-nucleating microorganisms as a control agent for cereal aphids in the field. Spraying the foliage of Russian wheat aphid-infested winter wheat with diluted suspensions *P. syringae* in distilled water would be a simple application method. However, it is not clear if aphids would be exposed to the bacterium because their overwintering habitat is inside a curled wheat leaf. Further research would be needed

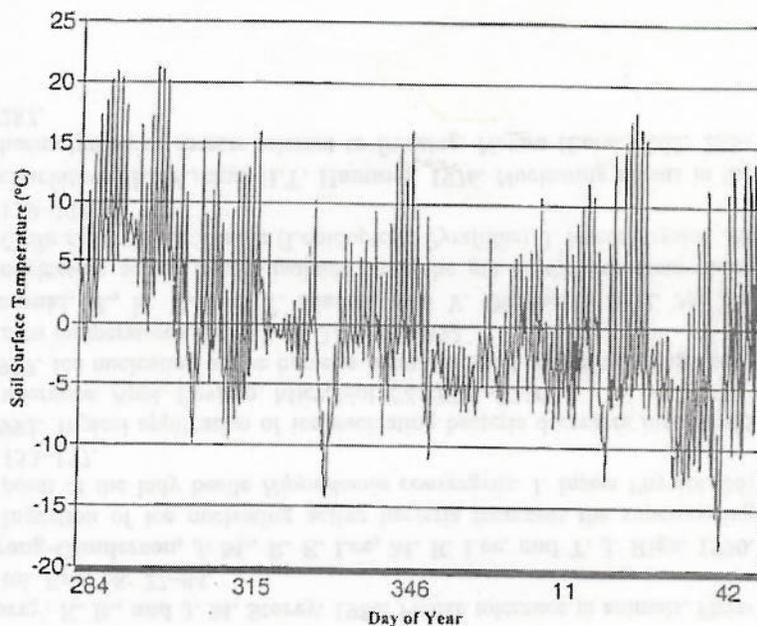


Fig. 3. Average hourly soil surface temperature in a 'TAM 107' winter wheat field on the Central Great Plains Research Station, Akron, CO. Day 284 is 11 October 1993, day 42 is 11 February 1994.

to assess freeze damage to wheat foliage (tillers and leaves) following the application of *P. syringae*. Previous research (Fowler et al. 1981) of freeze injury to wheat plants indicates that survival is closely related to freeze injury to the crown, rather than the upper growth.

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