SUPERCOOLING POINTS FOR RUSSIAN WHEAT APHID[1] AND WINTER WHEAT TISSUE AS INDICATORS OF COLD ACCLIMATION

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ABSTRACT

Supercooling experiments were conducted for Russian wheat aphid, *Diuraphis noxia* (Mordvilko), and wheat, *Triticum aestivum* (L), leaf tissue to determine critical low temperature thresholds and acclimation cues for both organisms. When supercooling points were compared for Russian wheat aphid life-stages (first instar through adult), there were no significant differences. However, when different life-stages of aphids from two rearing environments were compared, third-instar Russian wheat aphids reared at room temperature in a greenhouse supercooled significantly lower than those reared at sub-zero temperatures on plants in the field. This significance is due to variation in supercooling and not a result of acclimation. The average supercooling point for all Russian wheat aphid life-stages was -27.6^{0}C. There were no seasonal changes in supercooling points for fourth instar Russian wheat aphids reared at room temperature versus those reared under fluctuating subzero temperature in the field. Supercooling points of wheat leaf tissue averaged -2.8^{0}C in the fall and -7.3^{0}C in the winter. Variation in wheat leaf tissue supercooling increased from fall to winter as a result of cell damage caused by freeze-thaw cycles, indicating that host quality for Russian wheat aphids will decrease as the winter progresses. These findings contribute to a better knowledge of the understanding of the success or failure of Russian wheat aphid overwintering that are the prerequsite to management strategies.

INTRODUCTION

The implication of determining insect winter survival has been acknowledged only recently as an important consideration in integrated pest management (Leather et al.1993). The overwintering success or failure of an insect pest precedes any management strategy. It has been widely accepted through early work that three main components of insect winter survival are cold acclimation, freeze tolerance, and freeze intolerance (Salt 1962). Aphids have been classified as freeze intolerant, supercooling well below 0^{0}C to prevent freeze injury and death. Some aphid species possess the ability to acclimate as a response to cold exposure (Harrison and Barlow 1973), while others do not (Knight and Bale 1986). The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is the most economically threatening insect of winter wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., on the Central Great Plains. This aphid is anholocyclic, freeze intolerant and relies exclusively on supercooling to survive from fall to winter on winter wheat in northeastern Colorado (Armstrong and Peairs 1996).

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The supercooling point of an insect is defined as the temperature required for spontaneous ice nucleation of body water (Lee and Denlinger 1991). It can be measured by observing the lowest temperature previous to a sudden rise in temperature caused by the release of the latent heat of fusion. For freeze-intolerant insects such as aphids, the supercooling point is the absolute lower lethal temperature for survival.

Two climatic events responsible for causing 100% mortality in overwintering Russian wheat aphid populations in northeastern Colorado (Armstrong and Peairs 1996) in 1992 were a prolonged (>87 d) period under snow-cover and an air temperature drop to below -30.0°C for a minimum of one hour. In terms of using these two events to predict Russian wheat aphid mortality, the latter would be the simplest to use because temperature is the most widely available form of climatic data.

Supercooling points were measured for greenhouse and field-reared Russian wheat aphids to determine if there were differences among life stages or if they changed on a seasonal basis. If field-reared aphids collected in the fall and winter supercooled lower than aphids reared at a consistent warm temperature in a greenhouse, this would be an indication that cold temperature exposure is responsible for initiating cold acclimation. We also investigated seasonal changes in wheat leaf supercooling points for comparison with aphid supercooling points as an indicator of host quality.

MATERIALS AND METHODS

In the fall 1989, 1990, and 1991 a small plot (0.3 ha) of 'TAM 107' winter wheat was planted at the USDA-ARS Central Great Plains Research Station, Akron, CO, for the purpose of maintaining Russian wheat aphids for both greenhouse and field environments. Greenhouse wheat plants originated in the field, but were cored from there original soil into 12.5-cm deep x 15.2-cm diameter pots (Armstrong et al. 1993) and placed in the greenhouse without artificial lighting at Feekes growth stage two. Both greenhouse and field wheat were artificially infested with Russian wheat aphids collected from volunteer wheat on the research station. The aphids were allowed to acclimate to their rearing environment for at least three weeks before starting the experiments. Mean, maximum and minimum temperatures for both greenhouse and field environments were recorded with data loggers (Campbell Scientific, Logan, UT).

Individual Russian wheat aphids were transferred by camel hair brush to the surface of a 30-gauge, copper-constantan thermocouple, coated with a thin layer of petroleum jelly. The thermocouple and aphid were placed in the center of a pyrex test tube (2.5 cm x 15 cm). The thermocouple wire was formed into a pig-tail configuration to allow the thermocouple and aphid vertical movement without touching the interior surface of the test tube. The test tube was held erect and partly submerged in a dry ice (0.5 kg) and 95% ethanol bath. The bath temperature ranged from -60 to -70°C. Temperature exposure to the thermocouple/aphid was controlled by gradual vertical movement of the thermocouple down the test tube. The thermocouple temperature was recorded every 0.2 second by the data logger. The temperature time series for each individual supercooling point determination was graphed over time to identify the temperature at the release of the latent heat of fusion (supercooling temperature).

The difference in the actual temperature (Ta) and the thermocouple temperature (T) was estimated to determine the error in Russian wheat aphid supercooling points. According to Fritschen and Gay (1979), $T - Ta = (-\beta\tau)$, where β is the slope of linear decrease in temperature, and τ is the time constant for a 30-gauge copper constantan-thermocouple. The time constant for 30-gauge thermocouples was determined to be 10.0 seconds for air and 0.1 second for water (Hinkle et al. 1991). The estimated error for Russian wheat aphid supercooling points using a time constant for air was $T - Ta = (-\beta\tau) = (-2.0^{\circ}C/60\ s)\ (10.0) = -0.33^{\circ}C$, and for water: $(-2.0^{\circ}C/60\ s)\ (0.1) = -0.0033^{\circ}C$. Time constants of this small magnitude allowed us to use the temperature just previous to the release of the latent heat of fusion as the supercooling point.

Twelve supercooling points were measured for first through fourth-instar and adult Russian wheat aphids reared in the field and greenhouse. The order of supercooling determinations were: first-instar field-collected, first-instar greenhouse-reared, second-instar field-collected, second-instar greenhouse-reared, third-instar field-collected, third-instar greenhouse-reared, fourth-instar field-collected, fourth-instar greenhouse-reared, adult field-collected, and
adult greenhouse-reared. Approximately 20 supercooling points could be measured in a single day. The experiments started on 6 March and were completed by 20 March 1990.

Fourth-instar Russian wheat aphids were used as a representative life-stage to determine if supercooling changed from spring to winter for field-collected aphids. Greenhouse aphids, reared under a more constant temperature (18.3 ± 4.6^{0}C), were used for comparison. A minimum of 10 supercooling points for aphids from each environment were conducted on nine different occasions from 5 May to 20 December 1990.

In order to further understand the relationship of overwintering Russian wheat aphid with the wheat plant and because of a lack of data on supercooling of the upper structures (above the crown) of winter wheat plants, supercooling points of 1-cm cross sections of 'TAM 107' wheat leaves were determined. The leaves were collected monthly on five different occasions starting 30 September 1992 to 8 January 1993. The third true-leaf was used as sample tissue regardless of plant growth stage.

Wheat leaf supercooling points were measured with the same methods used for Russian wheat aphid except that a 34-gauge, copper-constantan thermocouple was used. The thermocouple was inserted into the leaf tissue on one side of the mid-rib. Nine to fifteen supercooling points of wheat tissue were conducted each month.

The Russian wheat aphid life stage supercooling points were analyzed by *PROC ANOVA* (SAS Institute, PC SAS version 6.03, Cary, N. C. 1988) with source (greenhouse versus field) and level (instar) as variables. The *PROC t*-test was used to compare supercooling points by life stages for aphids reared in the two differing environments. The *PROC t*-test was also used to compare seasonal means of supercooling points for nine different dates between 11 May to 20 December 1990, and four different dates between 6 September 1991 to 3 January 1992. Supercooling point means and ranges for 10 to 12 replications of wheat leaf tissue were compared over the five sample dates by *PROC MEANS* (SAS Institute1988).

RESULTS

There were no differences (F = 1.15, df = 59 and 59, $P > F$ = 0.59) in supercooling points of greenhouse-reared versus field-collected Russian wheat aphid when life-stages were pooled. Supercooling means by rearing environment were -26.94 ± 0.71^{0}C for greenhouse aphids and -27.07 ± 0.65^0 C for field aphids.

When Russian wheat aphid supercooling points were compared for life stage x rearing environment, third-instar, field-reared aphids supercooled 0.5^{0}C higher than greenhouse-reared, which was statistically significant (df = 11, $P >$ t = 0.025) as indicted by no overlap of the standard error bars (Fig. 1). The average daily rearing temperature starting 1 February to 6 March 1990 for field-collected aphids was -0.22^{0}C (max = 17.8^{0}C, min = -19.8^{0}C) compared to 19.5 ± 5.0^{0}C (max = 24.3^{0}C, min = 15.1^{0}C) for greenhouse-reared. A 0.5^{0}C difference in supercooling between third-instar aphids reared from the two environments would not be biologically significant. Outdoor ambient temperatures can change several degrees within minutes. There were no significant differences for the remaining instar or adults (Fig. 1).There were no seasonal differences in supercooling for greenhouse or field-collected Russian wheat

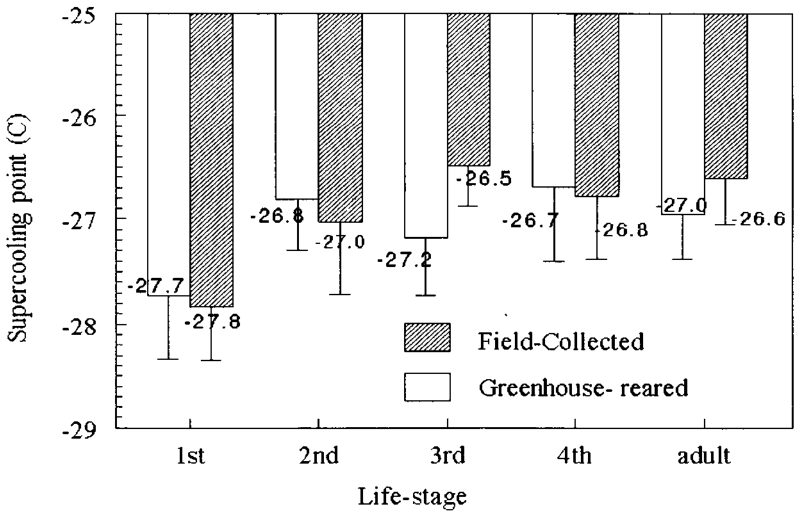

FIG. 1. Mean supercooling points (n=12) and standard errors for life-stages of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), March 1990.

TABLE 1. Exposure Temperatures and Mean ± S.E. Seasonal Supercooling Points Recorded for Fourth-instar Russian Wheat Aphids Collected from 'TAM 107' Wheat Grown In the Field and Greenhouse, May through December, 1990.

		Supercooling point		
Date	Exposure temperature[a]	Field-collected	Greenhouse-reared[b]	$(P > t)^c$
11 May	16.4, 11.2	-26.5 ± .8	-26.4 ± .6	0.46
11 June	25.3, 9.4	-26.6 ± .4	-26.7 ± .7	0.31
11 July	33.2, 15.4	-27.2 ± .8	-26.6 ± .6	0.48
17 Aug	28.8, 13.3	-26.7 ± .4	-26.8 ± .5	0.29
21 Sep	31.2, 13.7	-26.5 ± .6	-26.7 ± .5	0.42
10 Oct	21.2, 5.4	-26.8 ± .5	-26.5 ± .5	0.34
26 Oct	18.5, -0.3	-26.7 ± .9	-26.6 ± .8	0.41
14 Nov	15.64, 2.7	-27.2 ± .8	-27.4 ± .6	0.31
20 Dec	10.08, -6.4	-27.3 ± .6	-27.0 ± .4	0.33

[a] Mean daily maximum and minimum ambient temperatures (^{0}C) between sample dates for field- collected Russian wheat aphids.
[b] Greenhouse Russian wheat aphids were reared at 18.3 ± 4.6^0 C.
[c] Degrees of freedom = 11 and 11, P = 0.05.

TABLE 2. Exposure Temperatures and Mean ± S.E. Seasonal Supercooling Points Recorded for Fourth- instar Russian Wheat Aphids Collected From Field and Greenhouse Environments, September 1991 to January 1992.

			Supercooling point		
Date	*n*	Exposure temperature[a]	Field-reared	Greenhouse-reared[b]	$(P > t)^c$
6 Sep 91	10	30.7, 12.3	-26.8 ± .6	-26.5 ± .7	0.26
5 Oct 91	12	24.1, 6.9	-26.9 ± .5	-26.3 ± .5	0.37
25 Nov 91	10	12.4, -3.5	-26.7 ± .8	-26.8 ± .3	0.55
3 Jan 92	12	5.0, -11.9	-26.7 ± .5	-26.4 ± .4	0.41

[a] Mean daily maximum and minimum ambient temperatures (^{0}C) between sample dates for field-collected Russian wheat aphids.
[b] Greenhouse Russian wheat aphids were reared at 20.76 ± 5.65 ^{0}C.
[c] Degrees of freedom = 11 and 11, $P = 0.05$.

aphids from May to December 1990 or from September 1991 to January 1992 (Tables 1 and 2 respectively). There was no evidence for Russian wheat aphid acclimation to low temperatures in the field. As mean minimum daily temperatures declined, field-collected Russian wheat aphid supercooling remained consistent (Table 1).

'TAM 107' wheat leaf sections collected from the field supercooled at -2.8 ^{0}C on 30 September 1992 and decreased to -8.6 ^{0}C on 24 October 1992 (Table 3). The highly variable nature of the wheat leaf supercooling points resulted in no significance when means were compared by collection date (Table 3). The range of means increased over time due to damage caused by freeze-thaw cycles in the field. Observations of 1-cm leaf sections under magnification, before supercooling, indicated that as seasonal temperatures progressively decreased, plant cell damage increased in the form of ruptured cell walls and leaking solutes. After wheat plant solutes leak from freeze injury, irreversible damage and death results (Palta et al. 1978). As the amount of plant cell damage increased, the variation in supercooling capacity increased because the latent heat of fusion was interrupted by cells that were less than capacity with solute, leading to increases in the standard errors and range of means (Table 3).

TABLE 3. Exposure Temperatures and Mean ± S.E. Seasonal Supercooling Points for 1-cm Sections of 'TAM 107' Wheat Leaves Grown In the Field, Central Great Plains Research Station, Akron, Colorado, September 1992 to January 1993.

Date	*n*	Exposure temperature[a]	Supercooling point[b] ± S.E.
30 Sep 92	9	26.98, 6.29	-2.78 ± -1.14
7 Oct 92	10	25.95, 7.40	-5.23 ± -1.39
24 Oct 92	10	19.18, 0.03	-8.64 ± -2.63
10 Dec 92	15	5.31, - 5.24	-7.44 ± -6.38
8 Jan 93	11	1.92, -13.92	-7.25 ± -6.31

[a] Mean daily maximum and minimum ambient temperatures between sample dates of collecting 1-cm sections of wheat tissue.
[b] Supercooling points were not significantly ($P = 0.05$) different across all dates.

DISCUSSION

The mean supercooling points for Russian wheat aphid life-stages were similar to those reported from Alberta, Canada, although significantly lower supercooling points were found for Canadian first-instar compared to second or third, and second-instar supercooling points were significantly lower than fourth-instar (Butts 1992). Canadian first-instar supercooling points had the greatest variation. Lower Russian wheat aphid fresh-weights were positively correlated with lower supercooling points in the Canada study.

Although aphids were not weighed before supercooling in these experiments; younger instar, and especially first instar supercooled at lower temperatures than later instars (Fig. 1). This relationship is common in insects and is attributed to smaller size rather than a biochemical component that resists freezing (Angell 1982). Butts (1992) data also support the fact that smaller aphids supercool at lower temperatures than larger ones.

Russian wheat aphids used in these experiments turned a distinctive brownish-red color after freezing. This color change has also been observed in the field after extremely cold (-30^{0}C) temperatures and is indicative of high mortality. The cause for the color change is most likely denaturing of proteins from ice crystal formation.

Comparison of the supercooling points of Russian wheat aphid and 'TAM 107' wheat leaf tissue indicates that wheat leaves have a higher probability of freezing before Russian wheat aphids under field conditions. Although variation of supercooling points increased for wheat leaf tissue as the winter progressed, supercooling points averaged 18 to 23 ^{0}C higher than that of Russian wheat aphid. The variation increased because winter wheat freeze injury increased following freeze-thaw cycles in the field (Pontis 1989).

Supercooling points for winter wheat plants are usually recorded for the crown tissue because crown survival is highly related with wheat plant survival. High molecular weight fructans are responsible for freeze resistance in winter wheat crowns (Pontis 1989, Suzuki and Nass 1987). Supercooling lethal temperatures (LT_{50}) for bare winter wheat crowns ranged from -11.9 to -19.6 ^{0}C for 41 winter wheat cultivars in Canada (Fowler et al.1981). From field observations in this experiment, wheat tillers, culms and leaves froze before crown tissue. The crown is a below ground and the depth at which the crown establishes after planting, along with the amount of fructan in crown tissue, are genotypic traits. The crown is insulated by the surrounding soil which results in temperature stabilization. The greater the distance wheat tissue is above the soil surface, the greater the probability of freezing when exposed to sub-zero temperatures.

Physical adaption that contributes to Russian wheat aphid overwintering ability are its small size and the lack of ice nucleators in its feeding source. Supercooling points of aphids reared outdoors and in a greenhouse did not differ on a seasonal basis. Some aphid species can acclimate to cold temperatures. This increase in acclimation may be related to overwintering mortality but is not related to supercooling (Adams 1962, McLeod 1987, Bale et al. 1988, Harrison and Barlow, 1973).

The results of these studies support the concept that aphids as a group are consistent in being void of ice nucleators in all life stages, (Duman et al. 1991, O'Doherty and Ring 1986). The absence of nucleates is a result of the feeding process. Feeding directly into phloem tissue reduces the chance of nucleate contamination. Plant fluids are void of nucleators, and the gut of an insect is the most efficient site for nucleator activation under the influence of cold temperature.

Wheat plant freezing and subsequent cell damage would have at least three significant implications for overwintering Russian wheat aphid. The first would be the length of time the plant tissue is frozen, related to the inability of the Russian wheat aphid to insert stylets into frozen phloem tissue. The greatest probability of having environmental conditions that keep wheat plant tissue frozen are when low temperatures occur in the absence of insulating snow. The second important factor would be the physical relationship of the aphids stylets inserted in plant tissue when the plant tissue freezes. Powell (1974) found that when the green spruce aphid, *Elatobium abientium* (Walker), was feeding on spruce needles, it supercooled at a higher temperature compared to those with mouthparts not engaged in host tissue. Since these experiments determined the wheat plant has a higher probability of supercooling at higher temperatures than the Russian wheat aphid, and when aphids with stylets inserted into host plant tissue supercool at a higher temperature than those that do not, it is possible that feeding Russian wheat aphid will freeze above their normal supercooling point. Finally, if phloem tissue were completely destroyed by freezing, the wheat plant would die, resulting in Russian wheat aphid starvation, even if temperatures were not low enough to freeze the Russian wheat aphid. Thomas and Butts (1990) found LT_{50} values for wheat crowns and tillers damaged by the Russian wheat aphid in the fall to increase by 2.2 to 2.5^{0}C and 2.6 to 4.3^{0}C, respectively, in Canada. Fall-infested wheat plants therefore have a higher chance for winterkill and thus a higher probability for death of above-ground tissues from both aphid feeding and freeze injury.

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