Effects of Temperature and Substrate on Survival and Lipid Consumption of Hibernating *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae)

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ABSTRACT The influence of temperature and substrate quality on the hibernation of the polyphagous predator *Coleomegilla maculata lengi* Timberlake was examined. Survival rate and lipid content were periodically evaluated at six temperatures (20, 10, 4, −0.5, −4, and −10°C) on two substrates (fallen leaves and wood chips). Rapid total mortality was observed at −10, 10, and 20°C. At −4 and −0.5°C, survival extended until May, but only at −0.5°C was the survival rate higher than that observed at natural hibernation sites. Fallen leaves were a better substrate than wood chips except in situations of sudden warming and subsequent snow melt. The rate of lipid consumption increased with temperature, and no significant variation was found between substrates.

KEY WORDS Insecta, Coccinellidae, hibernation, lipid

THE POLYPHAGOUS PREDATOR *Coleomegilla maculata lengi* Timberlake, is indigenous to agricultural crops in Quebec, Canada (Coderre 1982, 1984). *C. maculata lengi* feeds on corn and cereal aphids (Hodek 1973), certain lepidopterous eggs and larvae (Conrad 1959, Warren & Tadic 1967), and Chrysomelidae (Shade et al. 1970), as well as pollen from a variety of plants (Coderre et al. 1987, Hodek 1973).

Coleomegilla maculata adults hibernate in large aggregations in the grass and dead leaves accumulated at the bases of large isolated trees. This microhabitat provides insulation against fluctuating temperatures (Solbreck 1974), particularly in fall and spring. In winter, snow cover provides temperature sites further insulation, assuring a constant temperature of 0°C (Hagen 1962, Holmquist 1931 *in* Danks 1978) and protection from freeze (Gillot 1980, Hodson 1937, Lee 1980a). *C. maculata* also produces antifreeze proteins that lower the supercooling point (Duman et al. 1982) to between −17.8 ± 1°C (Lee 1980a) and −18.4 ± 0.4°C (Baust & Morrissey 1975). Despite this adaptation, mortality at hibernation sites is high and Wright & Laing (1982) found differing mortality rates between January (6.8%) and May (57.1%). In April and May, *C. maculata* loses cold resistance as increased daylight and increased spring temperatures stimulate the degradation of the antifreeze proteins (Duman & Horwath 1983). Loss of cold resistance and concurrent reductions in snow cover increases the vulnerability of *C. maculata* to late freezes (Harper & Lilly 1982).

The beginning of diapause, at the end of summer, is marked by an accumulation of lipid reserves (St-André 1988), the discontinuation of female reproductive development, and a decrease in respiration (Hagen 1962, Hodek 1973, Lee 1980b, Parker et al. 1977). Lipid reserves provide necessary energy for autumn migration to hibernation sites; they permit basal metabolism during hibernation, as well as spring dispersion and regeneration of the female reproductive system (El-Hariri 1970, Hagen 1962, Hodek 1973). Therefore, lipid reserves must be large enough to provide the energy necessary for migration and allow for survival until establishment when enough prey are available.

In 1985 N. St-André and D.C. (unpublished data) demonstrated that the lipid reserves of *C. maculata* on hibernation sites ranged from >1.28 to 0.88 mg between mid-April and mid-May, whereas at the end of November 1984 they were established at 1.68 mg.

In consideration of the high mortality rate at natural hibernation sites, we examined survival rate and lipid consumption of *C. maculata* at six temperatures on two substrates. A second objective was to determine whether *C. maculata* exhibited higher survival rates in an artificially controlled environment.

Materials and Methods

The specimens were hand collected from a hibernation site at La Présentation, Québec (72°56′ W, 45°39′ N) on 31 October and 1 and 2 November 1986. The 38,400 insects were separated at random into 48 experimental units (800 individuals/unit) and distributed evenly into incubators previously set to the controlled temperatures tested: 20, 10, 4, −0.5, −4, and −10 ± 1°C. At each temperature tested, two substrates were examined: fallen leaves

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of *Salix nigra* collected at the hibernation site, and wood chips (*Pinus* sp.). Four replicates were used for all treatment combinations.

Each experimental unit was made up of a 1-liter plastic container. Gravel spread with potting soil covered the bottom of the container to a depth of ≈10 cm. The insects were placed in a net bag with the substrate (fallen leaves or wood chips) and set onto the potting soil. The container was kept in complete darkness. For the units tested at temperatures >0°C, relative humidity was maintained between 70 and 90% by placing a wet sponge on the cover of the container. Snow when available, or crushed ice, covered the insects tested at temperatures <0°C.

Periodically, ≈80 insects were randomly collected from each experimental unit every 2 wk for the first month and every 4 wk subsequently. Sampling continued until week 28 (26 May 1987). All specimens were kept at room temperature for 4 h and those insects capable of moving after this period were considered to be viable.

Extraction of lipids from the surviving insects was carried out without regard for sex. Between 10 and 30 specimens were lyophilized and ground. Lipid extraction was accomplished through a soxhlet extractor (Model E6261, Corning EX5, Corning Glass Works, Corning, N.Y.) using petroleum ether solvent (Timmon-David 1930). Lipid extraction was complete after 4 h and extracts were weighed and expressed as milligrams of lipids per insect (dry weight). Daily lipid consumption was calculated for each temperature.

Statistical Analysis. Two-way analysis of variance and Student–Newman–Keuls multiple range tests were used to compare survival rate and lipid content at each sampling period. To compare survival between the two different substrates for each temperature tested at each sampling period, Mann–Whitney *U* tests were used. Lipid content was compared between substrates at each temperature by Student's *t* tests. The rate of lipid losses were compared by linear regression and comparison of slopes for each temperature and substrate. All statistics were carried out with the SAS statistical package (SAS Institute 1985).

Results

Survival. Extreme temperatures tested (20 and −10°C) did not permit survival beyond 8 wk in an artificial environment (Fig. 1A and F). Differences in survival of the insects between the two substrates were not significant. Survival at 10°C did not extend further than 12 wk (Fig. 1B). Survival extended to 20 wk at 4°C, however mortality was more rapid on fallen leaves than on wood chips (Fig. 1C). Survival rate was significantly higher on wood chips than on fallen leaves at weeks 8 and 12 (Mann-Whitney *U* Test: $U = 1$; $n_1 = 4$, $n_2 = 4$; df = 4, 4; $P = 0.03$). For the three temperatures tested >0°C, mold developed first on the substrates and subsequently on the insects. This mold developed more rapidly on the fallen leaves than on the wood chips.

At −4 and −0.5°C, the insects survived until the end of the experiment (26 May 1987) (Fig. 1D and E). At −4°C, survival was 42.1% on fallen leaves and 15.3% on the wood chips. Starting from week 20 the difference in survival rate between the two substrates became more marked, although not significant ($P > 0.05$). However, when we compared survival rates between the two temperatures (−0.5 and −4°C) at 16 and 20 wk, survival was significantly higher for the former (ANOVA, Student–Newman–Keuls: $F = 2.83$; df = 5, 18; $P = 0.05$) (Fig. 1D and E).

At −0.5°C, survival was significantly higher on fallen leaves than wood chips at 20 wk (Mann–Whitney *U* test: $U = 2$; $n_1 = 4$, $n_2 = 4$; df = 4, 4; $P = 0.05$). A warming in incubators at −0.5°C between weeks 20 and 24 resulted in the melting of the crushed ice in the units, and consequently a large proportion of the insects were lost. The values in Fig. 1D and E represented by the broken line were estimated from the significant regression established for survival rate at −0.5 and −4°C from the beginning of the experiment up to week 20. According to these estimates, survival on fallen leaves would have been 83 and 69% on wood chips (Fig. 1D and E).

However, when we examined data recovered after the unscheduled warming we noted only a slight decrease in survival of the insects on wood chips compared to the estimates (Fig. 1D). Alternatively, temperature increase caused the mortality of over 50% of the insects on fallen leaves (Fig. 1E).

Lipids. At the start of the experiment, lipid reserves were established as averaging 1.88 mg per insect (30.63% dry weight) (Fig. 2). In general, lipid content diminished rapidly as temperature increased. However, the rate of lipid loss (slope) was significantly higher (df = 1; $P < 0.05$) for coccinellids kept at 20, 10, 4, and −10°C than those kept at −0.5 and −4°C (Fig. 2). The rate of lipid loss (slope) did not differ significantly between the two substrates (Fig. 2).

Average lipid consumption rates of 0.049 mg lipids per insect per day were established for insects kept at 20°C, whereas lipid consumption rates were established at 0.009 mg lipids per insect per day for insects kept at 4°C. However, lipid consumption in insects kept below 0°C was substantially lower, ranging from 0.003 mg per insect per day at −0.5°C to 0.0001 mg per insect per day at −10°C (Fig. 3).

Discussion

Our data established that temperature plays a determining role in insect survival during winter hibernation in an artificially controlled environment. The rapid total mortality observed in the insects kept at −10°C demonstrates that this tem-

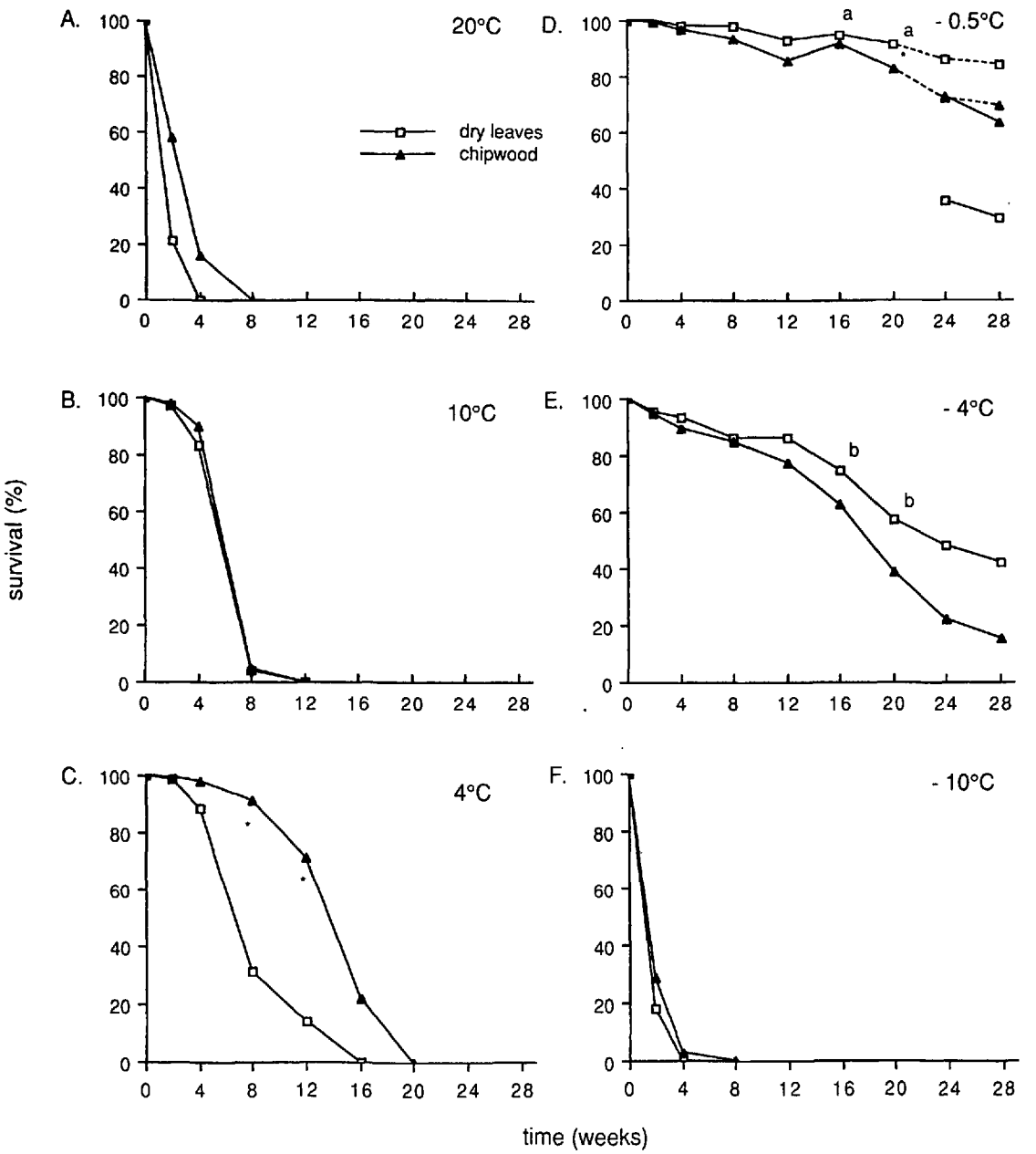

Fig. 1. Survival of *C. maculata lengi* in hibernation on two substrates at differing temperatures.

perature is too cold to permit prolonged survival. To increase cold resistance of *C. maculata* antifreeze proteins are produced in autumn (Baust & Morrissey 1975, Duman et al. 1982). The supercooling point represents a measure of the highest temperature at which a spontaneous freeze occurs within the body of the insect (Asahina 1969). Therefore, the supercooling point represents the temperature limit for the survival of a species (Asahina 1969, Duman et al. 1982, Salt 1961). However, the supercooling point is a punctual measure and cannot serve as an estimate of the capacity of the insect to survive a prolonged exposure to low temperature (Asahina 1969). Thus we conclude that *C. maculata* can survive only a few weeks at −10°C. Therefore, it is necessary to consider not only the temperature but the exposure time (Salt 1961, Asahina 1969, Somme 1982, Grafius & Collins 1986).

On the other hand, the extremely low rate of lipid consumption in insects kept at −10°C demonstrates that metabolic activity was very low. Furthermore, our results confirm that lipids are not

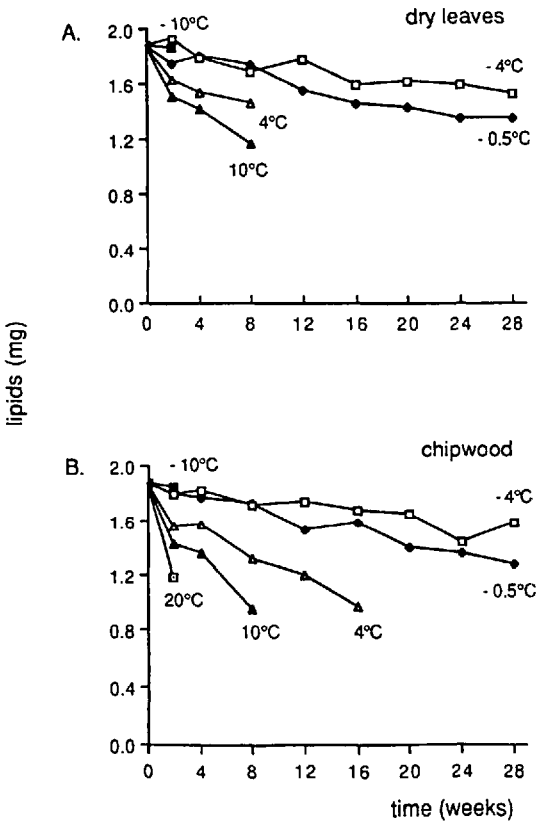

Fig. 2. Lipid content (mg/insect) of *C. maculata lengi* on two substrates at differing temperatures.

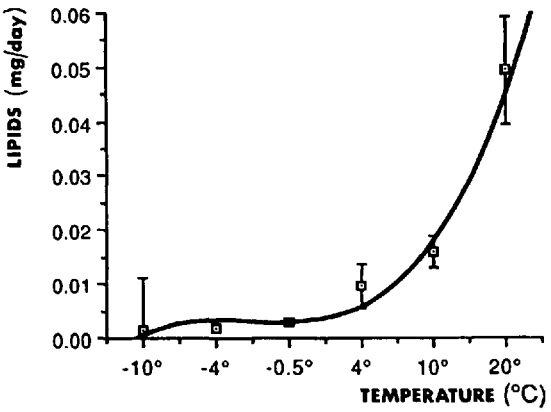

Fig. 3. Daily lipid consumption of *C. maculata lengi* at different temperatures.

involved in the mechanism of cold resistance in *C. maculata*, and that mortality due to temperatures is independent of lipid reserves, as has been shown for *Adalia bipunctata* (L.) (Mills 1981).

Temperatures >0°C (20, 10, and 4°C) did not provide the conditions necessary for survival of *C. maculata* during winter hibernation in an artificially controlled environment and survival rate declined with increasing temperatures. Long-term survival of *C. maculata* during winter hibernation is not possible at temperatures of 20 and 10°C because these elevated temperatures stimulate activity in the insects. Our results with *C. maculata* kept at 4°C support those obtained by Hämäläinen (1977) in an experiment with *Coccinella septempunctata* L. Total mortality resulted within 4 mo when the insects were kept in the laboratory at 6°C. Further, a survival rate of 50% was noted with *A. bipunctata* kept under the same conditions for 8 mo.

Decomposition of the dead leaves was stimulated by high temperatures and an abundant growth of mold was observed. Because of their capacity to absorb water, wood chips provided more favorable conditions for insect survival at temperatures >0°C.

Lipid consumption is influenced by metabolic activity which is directly related to temperature (El-Hariri 1966) and increases with increasing temperature. Our results (Fig. 3) demonstrated that the relationship between temperature and daily lipid consumption was stable between −0.5 and −4°C and exponential between 0 and 20°C. In artificially controlled conditions there appears to be a threshold level of lipid reserves that falls at 0.95 mg per insect.

No surviving specimen had lipid reserves below this level. However, El-Hariri (1966) measured lipid reserves between 0.5 and 0.9 mg per individual in *A. bipunctata* and *Propylea quatuordecimpunctata* (L.), and both are comparable in size to *C. maculata*.

Survival extended until May for insects kept at −4°C. However, survival rate was too low to consider this temperature as providing favorable winter hibernation conditions for *C. maculata*. Benton & Crump (1979) proposed that the size of the aggregation of insects at hibernation sites could influence heat storage and thus survival rate; Wright & Laing (1982) examined this hypothesis in a study of survival at natural hibernation sites. Despite a lower survival rate in smaller aggregations, differences in survival rate were not significant (Wright & Laing 1982).

In our study, repeated sampling considerably reduced the initial population of 900 insects to 800. This reduction potentially increased the mortality rate by an intensified heat loss due to decreased aggregation size toward the end of the experiment. Furthermore, a living organism can survive for only a limited period of time in a state of supercooling. Therefore, potentially those insects hibernating in a state of supercooling may at any point during hibernation suffer a spontaneous lethal freeze (Salt 1966, Zachariassen 1985). The increased mortality of insects kept at −4°C in March may be due in part to this phenomenon.

The highest survival rate was observed among insects kept at −0.5°C on a substrate of fallen leaves. Thus, the controlled conditions that resulted in the highest survival rate, corresponded to those found

at natural hibernation sites under winter snow cover. However, the warming and subsequent melting of the ice cover in the laboratory allowed us to verify the importance of the water absorptive properties of wood chips.

Although lipid reserve levels at the end of the experiment were higher for insects kept at −4°C than those kept at −0.5°C, the levels of lipids found in the latter (1.35 mg/insect) appear to be sufficient. These levels are higher than levels measured at natural sites at the end of hibernation before spring dispersion and reproductive development (St-André 1988). Furthermore, lipid levels in *A. bipunctata* at the end of hibernation were 1.2 mg/insect. Over-consumption of lipids did not occur in an artificially controlled environment and lipid reserves were high enough to allow for use of these insects.

Our experiment clearly demonstrates that optimal conditions for survival and lipid consumption in hibernating *C. maculata* are present at natural hibernation sites with snow cover. Maintenance of insect populations under these conditions at the end of winter by the future management of natural hibernation sites could considerably increase survival rate. Furthermore, survival of *C. maculata* was higher in an artificially controlled environment than at natural hibernation sites. This represents an alternative for the increase of populations and their future use as biological control agents.

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