

EFFECT OF COLD AND CO₂ ON THE SURVIVORSHIP
OF *LIRIOMYZA TRIFOLII* FEMALESPATRICK PARKMAN¹ AND R. L. PIENKOWSKIDepartment of Entomology, Virginia Polytechnic Institute
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Exposure to cold temperatures or CO₂ has been used often to immobilize insects and several studies have dealt with the short and long term effects of exposure to cold and CO₂ (Edwards & Patton 1965, Edwards 1968, Hooper 1970, Speirs & Zettler 1972, Berger & Zeyen 1987, Nicolas & Sillans 1989). Brief exposure to CO₂ was used to anesthetize adult leafminers, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), for toxicity studies with negligible effects on survival (Parkman & Pienkowski 1989); however, exposure to cold temperatures could have been used. This study was conducted to determine the short-term effects of cold temperatures or CO₂ at various exposure times on the survival of adult female *L. trifolii*.

Individuals used were from a *L. trifolii* colony maintained at Virginia Polytechnic Institute and State University for approximately 280 days (= 14 generations at 25°C). They were maintained on chrysanthemums, *Dendranthema morifolium* (Ramat) Tzvelev. cv. 'Manatee Iceberg', at 25 ± 3°C, 50 ± 10% RH, and a photoperiod of 14:10 (L:D).

To determine the effects of cold temperatures on adult survival, female *L. trifolii* (< 3 days old) held in 148 ml polystyrene vials with screen tops were placed in environmental chambers at 0, -5 and -10°C. Females were held for 0.25, 0.5, 0.75, 1, 2 and 4 h.

To assess the effects of CO₂, *L. trifolii* females (< 3 days old) held in similar vials were exposed to 100% CO₂ for 1, 1.5, 2 and 4 min. CO₂ was introduced directly into the vial holding the females with tubing through a screen hole in the top of the vial. Gas flow was controlled by a regulator mounted on the tank.

Four replications consisting of 12 individuals each were used for each exposure time at each temperature for the cold exposure study and for each exposure time for the CO₂ exposure study. Females were held at room temperature (24 ± 4°C) after exposure. A control group consisting of four replicates of 12 individuals each for each study was held at room temperature. Mortality for treated and control groups was assessed at 24 h. Honey smeared on the screen portion of the vial tops and a small water-soaked sponge placed in the vial were provided for treated and control groups.

Data were analyzed by analysis of variance, PROC ANOVA (SAS Institute 1985), and means separated with Duncan's multiple range test. Data from the cold exposure study were transformed by arcsine \sqrt{x} transformation before analysis.

No significant differences in mortality occurred at 0°C for all exposure periods (F = 2.13; df = 8,15; P > 0.05) (Table 1). Significantly more females died after the 4 h exposure at -5°C than after exposure periods of less than 1 h (F = 4.77; df = 8,15; P < 0.05) (Table 1). Mortalities of greater than 90% occurred after exposures of 2 h or longer at -10°C which were significantly greater than mortalities at exposure periods of up to and including 1 h (F = 12.16; df = 8,15; P < 0.01) (Table 1). Significantly more adults died when exposed to -10°C than at -5° and 0°C for all exposure periods, while there was no significant difference in mortality for adults exposed to -5° compared to those exposed to 0° for all exposure periods (Table 1). Mean percent mortality ± SD for the control group, 2.1 ± 4.2%, was significantly less than mortality occurring for all exposure periods at -10°C (F = 24.93; df = 9,18; P < 0.01) and for mortality occurring

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TABLE 1. MEAN PERCENT MORTALITY \pm SD OF ADULT FEMALE *L. TRIFOLII* WHEN EXPOSED TO COLD TEMPERATURES FOR VARYING PERIODS OF TIME.¹

Exposure period (h)	Temperature (°C) ^{2,3}		
	0	-5	-10
0.25	0.0 a	0.0 a	20.6 \pm 9.0 a
0.50	0.0 a	0.0 a	66.5 \pm 16.1 b
0.75	0.0 a	2.1 \pm 4.2 a	77.4 \pm 12.6 b
1.0	1.9 \pm 3.8 a	4.2 \pm 4.8 ab	76.5 \pm 17.7 b
2.0	3.9 \pm 4.4 a	10.4 \pm 10.5 ab	93.8 \pm 12.5 c
4.0	5.8 \pm 7.4 a	11.4 \pm 7.8 b	98.2 \pm 3.6 c

¹Four replications of 12 females each used for each exposure and temperature.

²Means in columns followed by the same letter are not significantly different by Duncan's multiple range test. Data transformed by arcsine \sqrt{x} transformation before analysis; nontransformed data presented.

³Mean percent mortality significantly greater at -10°C than at 0° and -5°C for all exposure periods. (At 0.25 h, F = 22.75; 0.50 h, F = 52.59; 0.75 h, F = 68.05; 1.0 h, F = 11.55; 2.0 h, F = 21.32; 4.0 h, F = 78.42; df = 5,6 and P < 0.01 for all exposure periods).

at 2 and 4 h at -5°C (F = 3.39; df = 9,18; P < 0.05). Control mortality did not differ significantly from mortality occurring at 0° for all exposure periods (F = 1.22; df = 9,18; P > 0.05).

Mean percent mortality \pm SD of adult *L. trifolii* exposed to CO₂ for 1, 1.5, 2 and 4 min was 2.1 \pm 4.2, 4.2 \pm 4.8, 4.2 \pm 4.8 and 8.3 \pm 6.8%, respectively. Mean percent mortality did not differ significantly for exposure periods (F = 2.76; df = 7,12; P > 0.05). Mean percent mortality for the control group (0%) was significantly less than that occurring for individuals exposed for 4 min, but not significantly different from mortality of individuals exposed for 1, 1.5, and 2 min.

Most terrestrial arthropods can endure prolonged exposure to freezing temperatures by producing an assortment of low molecular weight antifreeze/cryoprotective compounds, ice nuclei growth inhibitors, and/or proteins which augment ice nucleus formation (Baust & Rojas 1985). Although > 98% of *L. trifolii* adults exposed to -10°C for 4 h died, this species was found to overwinter in Maryland enduring temperatures as low as -26°C (Larew et al. 1986) indicating that at least one life stage of this leafminer, probably the pupa, can survive subfreezing temperatures for sustained periods of time.

Short exposures to CO₂ are known to affect behavior, reproduction, learning, and mortality of various insect species although the mechanism for CO₂ anesthesia of insects and how exposure to the gas affects various physiological and behavioral functions are not fully understood (Nicolas & Sillan 1989). For experiments with dipteran leafminer adults where exposure to CO₂ is minimal and treatment results are obtained within approximately 24 h, immobilization by CO₂ is an acceptable method.

Although our data indicate exposure of *L. trifolii* adults to CO₂ for up to 2 min does not significantly affect survival, exposure time should be kept as brief as possible to minimize mortality when anesthetizing individuals for short periods of time. Exposures of less than 1 min immobilized leafminer adults for approximately 30 s to 1 min with no apparent effects on short-term survival (Parkman & Pienkowski 1989). If individuals are to be immobilized for approximately 1 h or longer and temperature is not a crucial factor in experimental design, cold treatments of 0°C can be used to immobilize *L. trifolii* adults. However, consideration should be given to the rearing and maintenance temperature of the test insects. Our test insects were reared and maintained at approximately 25°C. The response to cold temperatures of *Liriomyza trifolii* or other species reared and maintained at cooler or warmer temperatures may differ from that of the individuals used in this study.

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**HELISCUS AND VERRES (COLEOPTERA: PASSALIDAE):
NEW SPECIES RECORDS FROM GUATEMALA AND PANAMA**

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Species of *Heliscus* are known from Mexico, Guatemala, Nicaragua and Costa Rica. Here I extend the range of this genus to Panama on the basis of a specimen of *H. rotundicornis* (Luederwaldt 1941) collected by E. Giesbert, 17-18 V 1987, 26km S. Rambala, Bocas del Toro, Panama, deposited in the Florida State Collection of Arthropods, Gainesville, FL. This species was previously cited from Costa Rica (Reyes-Cas-