

Effects of Chilling of *Bemisia argentifolii* (Homoptera: Aleyrodidae) Infesting Cabbage ¹

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ABSTRACT Cold temperatures affected the biology of *Bemisia argentifolii* Bellows and Perring on cabbage both directly and indirectly. Continuous chilling of 0°C and 4°C significantly increased mortality of adults and eggs. Variable daily chilling of 0°C for 6 h and then greenhouse conditions for the remainder of the day increased adult mortality but did not affect egg mortality for the 10 days of exposure. Prior plant exposure to 0°C resulted in reduced adult *B. argentifolii* preference and increased adult mortality. Previous 0°C and 4°C plant exposure also had an impact on within plant distribution of adults and eggs. On nonchilled and recently chilled plants, adults and eggs were found on the youngest (highest) leaves. On plants chilled at 0°C and 4°C three and five days before infestation, adults and eggs were found on lower leaves. Lower immature mortality was recorded on the nonchilled plants than on the chilled plants.

KEY WORDS Silverleaf whitefly, *Bemisia argentifolii*, *Bemisia tabaci* strain B, cabbage, *Brassica oleracea*, overwintering, cold temperatures, mortality

Bemisia argentifolii Bellows and Perring, which is also known as *Bemisia tabaci* strain B, has become a serious pest of both greenhouse and open field crops in many parts of the world, and causes an estimated \$500 million in damage annually (Perring et al. 1993, Bellow et al. 1994). This species or strain appears to have largely displaced strain A of *Bemisia tabaci* (Gennadius), which was indigenous to most of the tropical and sub-tropical regions of the world (Cock 1986) at least in the United States. Recently, *B. argentifolii* has expanded its range to areas with cooler winter temperatures (Bergh et al. 1995). Most studies of *B. tabaci* have been conducted on summer and fall crops or in greenhouses. However, the possibility of continuous development through the winter in many areas (Avidov 1956, Gerling 1984) has made an understanding of its biology at lower temperatures, and the succession and quality of overwintering hosts, important research goals. Some excellent studies have been conducted. Oviposition by *B. tabaci* was found to be impaired by rain and

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low temperature by Ohnesorge et al. (1981) and Avidov (1956) determined the lower threshold for oviposition to be 14°C. Husain and Trehan (1933) found no eggs laid under field conditions at temperatures below 22.8°C.

Overwintering hosts have been identified in many parts of the world. For example, in Israel, Gerling (1983, 1984) identified 19 species of weeds, ornamental, and cultivated plant hosts on which *B. tabaci* overwinters. Other studies have examined weed hosts as overwintering population reservoirs (e.g., Ozgur et al. 1989, Fattah et al. 1984, Coudriet et al. 1986). One cultivated crop often mentioned as an overwintering host is *Brassica oleracea* L. (e.g., Sharaf 1984, Coudriet et al. 1985, Husain and Trehan 1933, Watson et al. 1992). *B. oleracea* includes a number of varieties which includes cabbage (var. *capitata*), collards (var. *acephala*), broccoli (var. *italica*), cauliflower (var. *botrytis*) and brussels sprouts (var. *geminifera*). Gruenhagen et al. (1993) have found *B. argentifolii* to overwinter on broccoli in the San Joaquin Valley of California. Zalom et al. (1995) has shown cabbage to be a good host for *B. argentifolii* in the Marisme area of Catalunya, Spain.

Low temperature responses of *B. oleraceae* have been studied by plant physiologists as a model to learn about cold-induced plant stress. At the cellular level, Lyons et al. (1979) suggested that low temperature invokes one or more primary events involving a change in the state of cell membranes leading to reduced permeability and changes in free or membrane-associated enzymes. These events occur immediately on exposure and are reversible. However, if the low temperature persists, the primary events are followed rapidly by secondary changes that are not reversible. These secondary changes include the inhibition of metabolic processes, the accumulation of toxic products, and various physiological dysfunctions. McWilliams et al. (1982) exposed *B. oleracea* seedlings to a constant temperature of 5°C and found that transpiration decreased immediately after exposure and stabilized at 40% of the control after about 6 hours. Net rates of photosynthesis and transpiration were inhibited, but not as rapidly or as extremely as in chilling sensitive plants. Despite the inhibition of photosynthesis, soluble carbohydrates and water soluble proteins accumulate in these conditions (e.g., Pollock 1986). This is probably a consequence of a metabolic shift brought about by cessation of growth (Kacperska-Palacz 1978), by cold-induced hydrolysis of polysaccharides (Levitt 1980), and by synthesis of amino acids such as alanine, malate, aspartate, and glutamate (Sosinska et al. 1977). Kulesza et al. (1986) found that by exposing young *B. napus* L. plants to continuous low temperatures above °C the first stage of hardening occurs in tissues capable of extension growth (such as young leaves) but not in tissues which had not yet entered that stage (such as apical buds or hypocotyls).

Gerling et al. (1986) reviewed the variables associated with survival and fecundity of *B. tabaci*, and noted that little attention has been paid to trophic influences and plant quality. They also suggested such investigations should include the behavior of adult whiteflies when selecting a leaf. In this study, we examined the direct effects of several chilling treatments on *B. argentifolii* adult and egg mortality on cabbage and the possible indirect effects of plant chilling on *B. argentifolii* behavior and biology.

Materials and Methods

Direct effects. Cabbage seedlings (cv. 'Savoy King') were grown in plastic pots in a greenhouse under agryl row covers which have been shown to exclude *B. tabaci* (Natwick et al. 1988, Perring et al. 1989). The plants were checked for the presence of whiteflies and other insects daily, and any insects found were removed. The experiment was initiated when the plants had three or four true leaves. Adult *B. argentifolii* were collected from the field with aspirators, and introduced into clip cages similar to those described by Zalom and Natwick (1987) measuring 1 cm in diam. The clip cages were placed on the highest or second highest fully expanded leaf. Ten adult whiteflies (five males and five females) were introduced into each of 120 cages, and the leaf at the base of the clip cage was marked with a felt tipped marking pen to indicate the area within the cage where the whiteflies were placed. The plants with the cages remained in the greenhouse under row covers for three days. After three days, the plants were divided at random into four groups and subjected to the following treatments: continuous $0^{\circ}\text{C} \pm 2^{\circ}\text{C}$, continuous $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $0^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 6 hours each day then returned to greenhouse under agryl, and a greenhouse-only control. Temperature in the greenhouse under the row cover was recorded daily during the experiment with a maximum-minimum thermometer, and temperatures averaged 27.6°C maximum and 9.1°C minimum (range 34°C to 7.5°C) for the 13-day period. An equal number of plants were removed from these treatments after 6 hours, 24 hours, 2 days, 4 days, 7 days and 10 days. After removing the plants, the number of adult whiteflies surviving each treatment including the nonchilled control was recorded. The number of eggs within the caged area was recorded, and the plants with the adults and cages removed were transferred to a walk-in environmental chamber maintained at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$, photoperiod 16:10 LD. The number of hatched and unhatched eggs was counted after 1 wk. Data for each date were analyzed using one-way ANOVA and Fisher's Protected LSD (SuperANOVA, Abacus Concepts 1989).

A separate group of 36 plants were infested with whiteflies in clip cages (one per plant) as described previously, but only after the plants had been exposed to the 0°C cold treatment for three days. Eighteen of the plants were returned to the 0°C treatment. Of these, six plants were removed after 1, 3 or 7 days of supplemental 0°C exposure. The remaining plants were kept in the greenhouse. Six nonchilled plants were infested with whiteflies in clip cages (two per plant), and these remained in the greenhouse as a nonchilled control. The number of dead and alive adults in each treatment was counted after the 1, 3 and 7 days of supplemental exposure. In addition, twelve plants which had been exposed to the 0°C treatment were infested with three female whiteflies in each clip cage. The whiteflies and cages were removed after three days, and the plants were moved to a walk-in environmental chamber maintained as above. The number of hatched and unhatched eggs were counted after 1 wk. Data were analyzed using one-way ANOVA and Fisher's protected LSD (SuperANOVA, Abacus Concepts 1989).

Indirect effects. Twenty-five cabbage seedlings were grown in plastic pots in a greenhouse under agryl row covering as described previously to exclude insects. When the plants had four or five true leaves, they were

divided randomly into five groups and exposed to the following treatments: continuous $0^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 4 days then placed in greenhouse under agryl row cover for 5 wks, continuous $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 4 days then placed in greenhouse under agryl row cover for 5 wks, continuous $0^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 4 days then returned to greenhouse under agryl for 3 wks, and $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 4 days with no subsequent holding period. Five plants were maintained in the greenhouse during this time to serve as a greenhouse-only control. The leaves that had been exposed to chilling were marked so that they could be identified later. These plants were arranged in a 5×5 Latin square on a bench in a greenhouse that did not contain other plants. Each plant was 40 cm from the adjacent pot so that the leaves of the plants did not touch each other. The greenhouse was infested with *B. argentifolii* by placing potted poinsettia plants infested with *B. argentifolii* and plant cuttings from the field which were infested heavily with *B. argentifolii* under the benches. After 4, 7 and 10 days, the number of whitefly adults were counted on each leaf of all plants. The heating in the greenhouse was shut off the night before each sampling date and counts were made in the morning to minimize flight. The plants were taken from the greenhouse on the last sampling date, and the number of eggs on each leaf and leaf position on the plant recorded. The plants then were moved to an environmental chamber and maintained at 23°C for 1 month after which the number of empty pupal cases on 10 of the leaves was recorded. It was assumed that adults successfully emerged from these pupal cases if we could observe the T-shaped split associated with adult eclosion.

Mean number of *B. argentifolii* adults and eggs per plant were compared for the four chilling treatments and the nonchilled control using one-way ANOVA and Fisher's Protected LSD (SuperANOVA, Abacus Concepts 1989). In addition, the proportion of adults and eggs on chilled relative to nonchilled leaves, and the number of eggs per adult was calculated on a per plant and per leaf basis for all chilling treatments. The mean proportions of adults and eggs were compared by one-way ANOVA and Fisher's Protected LSD following arcsin transformation (SuperANOVA, Abacus Concepts 1989). Effect of treatment and leaf exposure to chilling on number of eggs per adult was compared by two-way ANOVA (SuperANOVA, Abacus Concepts 1989). Effect of chilling treatment on within-plant distribution was compared for proportion of whitefly adults and eggs per leaf by two-way ANOVA following arcsin transformation (SuperANOVA, Abacus Concepts 1989). Effect of the chilling treatments on whitefly survival from eggs to adult emergence was determined by one-way ANOVA and Fisher's Protected LSD following arcsin transformation (SuperANOVA, Abacus Concepts 1989).

Results

Direct effects. Exposure of both *B. argentifolii* adults and eggs to continuous chilling resulted in higher mortality than nonchilled controls, and significant differences between treatments were observed 2 days after treatments were initiated ($F = 9.549$, $P = 0.0004$, adults; $F = 5.023$, $P = 0.0377$, eggs).

Adult mortality resulting from the continuous 0°C treatment was significantly greater ($P < 0.05$) than that for all other treatments (Fig. 1). Adult mortality

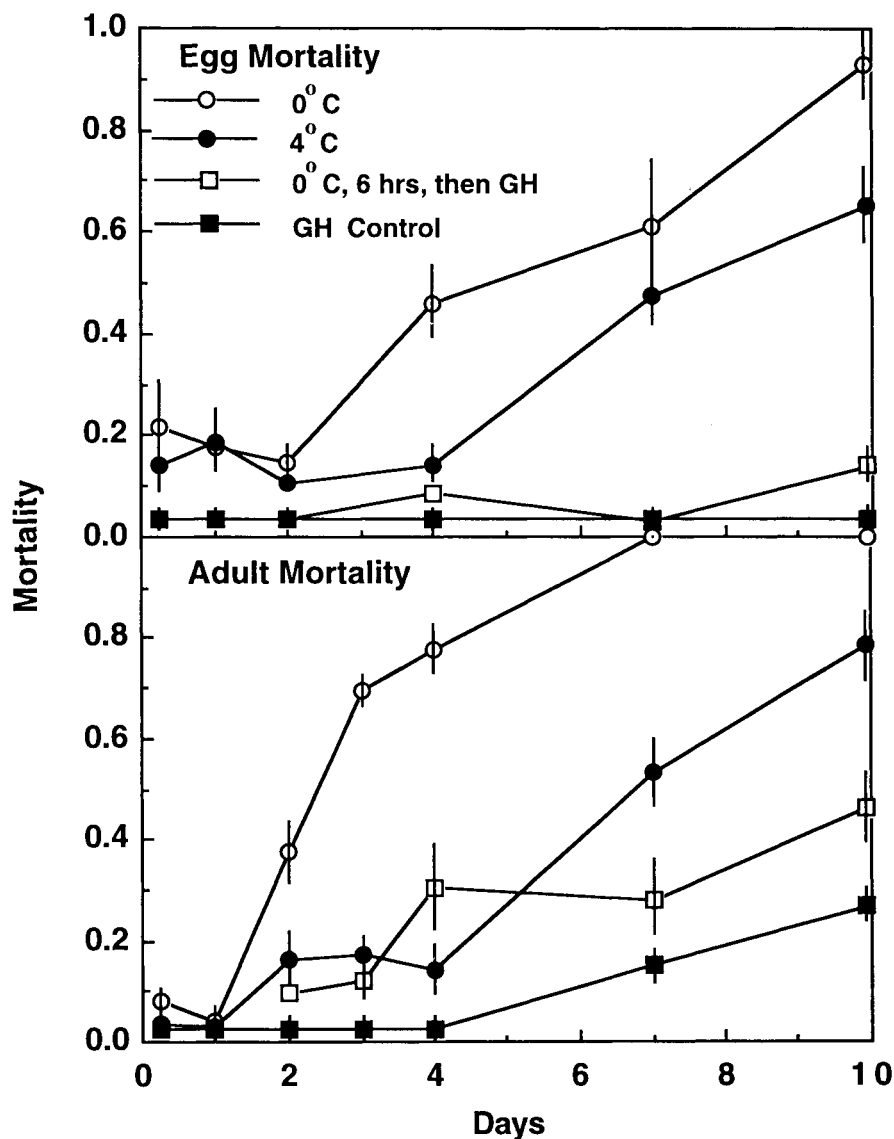


Fig. 1. Mortality ($\bar{x} \pm \text{SE}$) of *B. argentifolii* adults and eggs on cabbage leaves exposed to continuous 0°C or 4°C, or daily periods of 0°C for six hours, fluctuated with greenhouse ambient temperature, and on nonchilled greenhouse-only control plants.

resulting from the 4°C was significantly greater ($P < 0.05$) than that for the nonchilled control beginning the third day and for the variable chilling treatment beginning 7 days after initiation of chilling. Adult mortality in the variable 0°C/greenhouse treatment was significantly greater ($P < 0.05$) than that of the nonchilled control beginning 3 days after initiation of chilling, but did not increase following day 4.

Egg mortality resulting from the continuous 0°C treatment was significantly greater ($P < 0.05$) than that for all other treatments beginning the fourth day after initiation of chilling. Egg mortality resulting from the 4°C treatment was significantly greater ($P > 0.05$) than that for the other treatments beginning 7 days after initiation of chilling. Egg mortality in the variable 0°C/greenhouse treatment never differed significantly ($P > 0.05$) from that of the nonchilled control.

Maintaining cabbage plants for 3 days at 0°C prior to infestation by *B. argentifolii* significantly affected adult mortality ($F = 6.871$, $P = 0.0076$, 1 day; $F = 145.379$, $P = 0.0001$, 3 days; $F = 158.482$, $P = 0.0001$, 7 days) (Fig. 2). Adult mortality on these chilled plants which then were exposed to 0°C was significantly greater ($P < 0.05$) than that on plants which were moved to a greenhouse

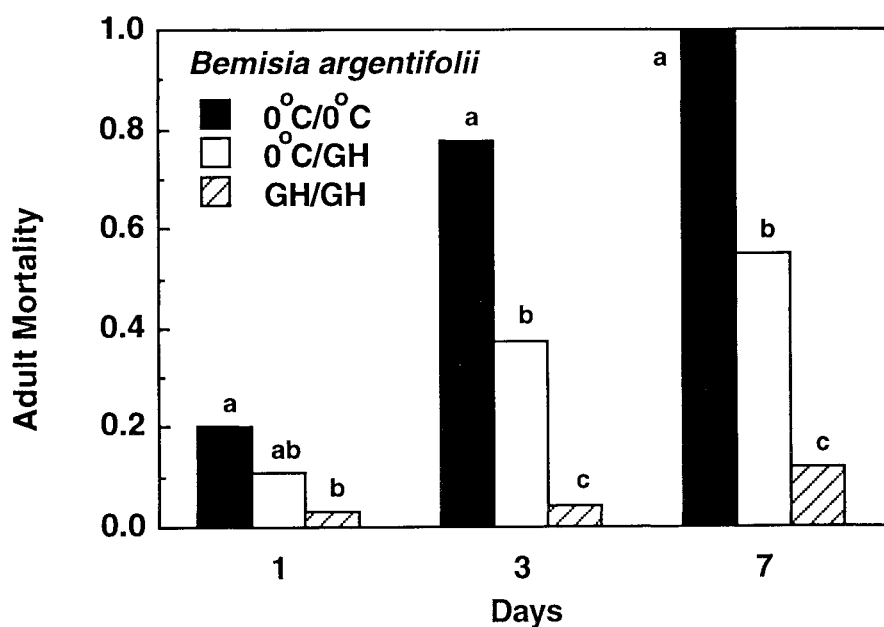


Fig. 2. Mortality of *B. argentifolii* adults on cabbage leaves exposed to continuous 0°C for 3 days prior to infestation, then returned to 0°C (0C/0C) or a greenhouse (0C/GH) after infestation compared to whiteflies on non-chilled leaves in a greenhouse (GH/GH). Bars accompanied by the same letter are not significantly different ($P < 0.05$) by Fisher's Protected LSD following arcsin transformation.

after infestation or never exposed to chilling after only 1 day of supplemental chilling (Fig. 2). Adult mortality on the chilled plants which were transferred to the greenhouse after infestation was significantly greater ($P < 0.05$) than on the nonchilled plants beginning 3 days after infestation. Egg mortality was similar on plants which were exposed to 0°C for 3 days prior to infestation and on non-chilled plants ($F = 2.073$, $df = 2$, $P = 0.1647$).

Indirect effects. The mean number of *B. argentifolii* adults and eggs per cabbage plant following different chilling regimes was lower ($F = 2.680$, $df = 4$, $P = 0.0540$, adults; $F = 2.786$, $df = 4$, $P = 0.0430$ eggs) on those plants chilled at 4°C five weeks before infestation than on nonchilled plants or those which were chilled at 0°C five weeks before infestation (Table 1). Number of eggs per adult was significantly different for treatment ($F = 5.120$, $df = 4$, $P = 0.0007$), chilled versus nonchilled leaves ($F = 20.258$, $df = 1$, $P < 0.0001$, and the interaction of treatment and leaf exposure to chilling ($F = 5.508$, $df = 3$, $P < 0.0013$). More eggs per adult were laid on nonchilled leaves ($x = 19.842 \pm 1.452$ SE, $n = 101$) than on chilled leaves ($x = 13.052 \pm 1.359$ SE, $n = 71$). On the plants receiving cold treatments, some leaves emerged after exposure. The mean ($\pm$ SE) number of nonchilled leaves per plant at the time of egg counts were made was 3.60 (± 0.25) for the 0°C followed by 5 wks of no chilling treatment, 4.00 (± 0.32) for the 4°C followed by 5 wks of no chilling treatment, 4.60 (± 0.25) for the 0°C followed by 3 wks of non chilling treatment, 1.00 (± 0.00) for the 4°C treatment infested immediately after exposure. Chilling had an impact on the within-plant distribution of adults (Fig. 3) and eggs (Fig. 4). Two way ANOVA for the interaction of chilling treatment and within plant distribution in terms of proportion of *B. argentifolii* on each leaf indicated highly significant differences ($P < 0.0001$) for

Table 1. Mean ($\pm$ SE) number of *B. argentifolii* adults and eggs on cabbage plants subjected to different chilling regimes before infestation.

Treatment	Weeks before infestation	Mean*/($\pm$ SE) adults per plant	Mean*/($\pm$ SE) eggs per plant
No Chill	na	70.9 (19.6) a**	917.4 (362.0) a†
0°C	5	57.9 (7.0) a	1049.8 (191.7) a
4°C	5	31.0 (9.0) b	335.4 (76.7) b
0°C	3	26.3 (7.2) b	447.6 (154.8) b
4°C	0	40.4 (10.0) ab	405.6 (79.8) b

* Means followed by the same letter are not significantly different ($P > 0.05$) by Fisher's Protected LSD following arcsin transformation.

** ANOVA - $F = 2.680$, $df = 4$, $P = 0.0540$.

† ANOVA - $F = 2.786$, $df = 4$, $P = 0.0430$.

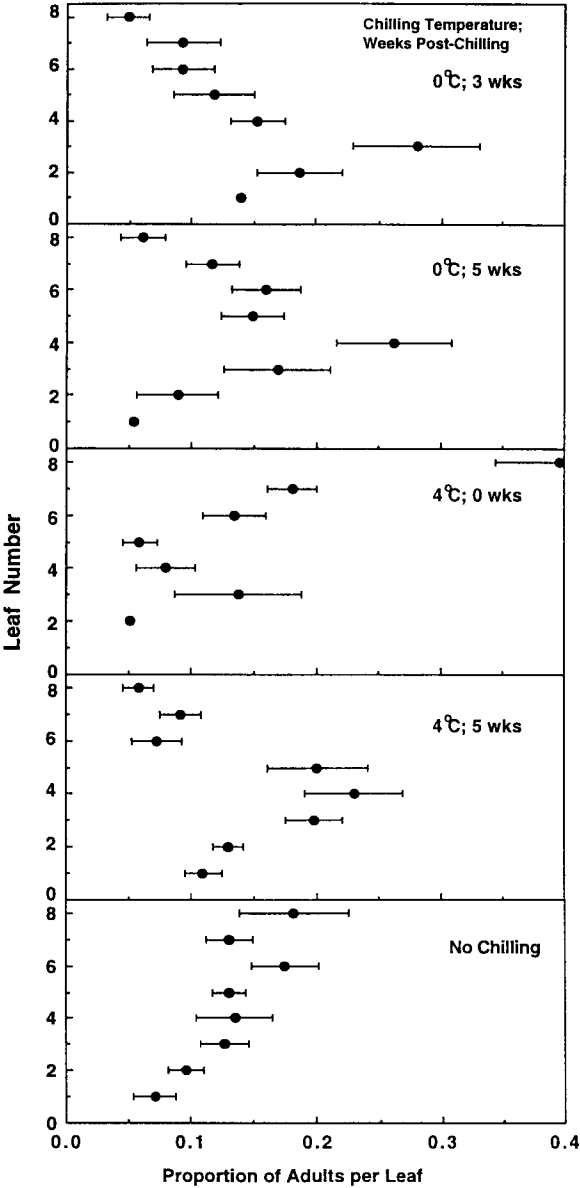


Fig. 3. Proportional distribution ($\bar{x} \pm SE$) of *B. argentifolii* adults on each leaf (leaf 8 = newest) on cabbage plants receiving four days of continuous 0°C or 4°C five weeks before infestation (old), 0°C three weeks before infestation (medium), 4°C immediately before infestation or no chilling (new) (n = 5).

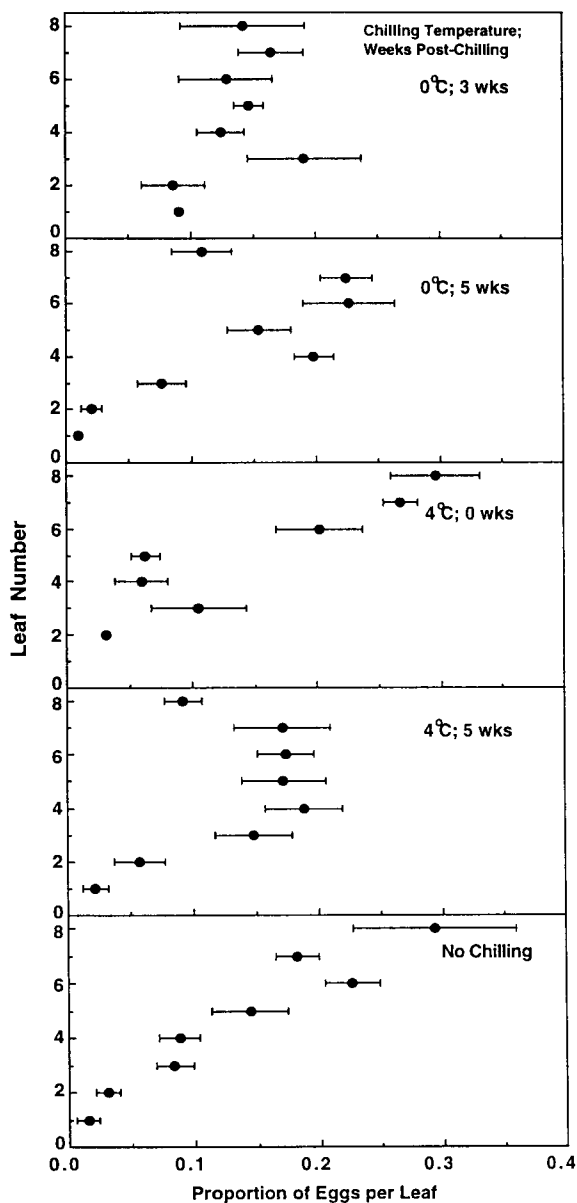


Fig. 4. Proportional distribution ($\bar{x} \pm SE$) of *B. argentifolii* eggs on each leaf (leaf 8 = newest) on cabbage plants receiving four days of continuous 0°C or 4°C five weeks before infestation (old), 0°C three weeks before infestation (medium), 4°C immediately before infestation or no chilling (new) ($n = 5$).

Table 2. Mean ($\pm$ SE) percent *B. argentifolii* adult emergence from eggs laid on leaves of plants exposed to different chilling treatments.

Treatment			
Temp.	Weeks before infestation	n	Mean ($\pm$ SE)* % emergence
No Chill	0	9	70.7 (4.4) ab
0°C	5	8	77.6 (3.7) a
4°C	5	8	62.9 (6.6) bc
0°C	3	8	55.3 (3.7) c
4°C	0	9	53.8 (4.2) c

*Means followed by the same letter are not significantly different ($P > 0.05$) by Fisher's Protected LSD following arcsin transformation.

both adults ($F = 6.482$, $df = 28$) and eggs ($F = 3.513$, $df = 28$). No significant difference in number of eggs per adult was found for the interaction of chilling treatment and within-plant distribution ($F = 6.482$, $df = 28$, $P = 0.1310$).

On nonchilled plants, both *B. argentifolii* adults and eggs were found on the youngest (highest) leaves. A similar distribution was seen on plants exposed to 4°C for 4 days, then infested. Adults were found on lower leaves of plants chilled 3 and 5 wks before infestation, although eggs tended to be laid higher on those plants. This relationship appears to be associated with previous leaf exposure to chilling.

Adult emergence was significantly different between chilling treatments ($F = 3.921$, $df = 4$, $P = 0.0094$, with a higher percentage emergence recorded on the no chill and 0°C followed by 5 wks before infestation chilling treatments (Table 2).

Discussion

Cold temperatures affect the mortality and biology of *B. argentifolii* both directly and indirectly. Continuous chilling was especially significant in increasing mortality of both adults and eggs. Variable chilling during which time daily temperatures included 6 hours at 0°C and the remainder at ambient greenhouse temperatures increased adult mortality, but did not affect eggs for the 10 days of exposure. Continuous chilling of 0°C to 4°C in the open field is possible within the expanding range of *B. argentifolii*. Such conditions may not occur frequently, but they could impact *B. argentifolii* populations in some years.

Chilling cabbage plants resulted in reduced adult *B. argentifolii* preference and increased adult mortality, but this effect was diminished 5 wks after the exposure to chilling. The plant response also was reflected by the different

adult and egg abundance on chilled and nonchilled leaves within plants. Although overall abundance was lower on plants exposed to chilling, the adults preferred the older chilled leaves rather than the new leaves which are higher on the plant. This distribution is contrary to what we observed with nonchilled or very recently chilled cabbage plants where adults are typically more abundant on younger leaves, and in studies on other plant species with *B. tabaci*. (e.g., Ohnesorge et al. 1980, Herakly and El Ezz 1970). Change in distribution due to chilling could result in higher winter mortality as nymphs hatching from eggs laid on older leaves may not complete development before leaves senesce when temperatures are low. Butler et al. (1986) state that incompatibility between whitefly development and leaf survival at low temperatures results in higher winter mortality on some crops. Alternatively, the preference of adults for older, lower leaves may reflect the location which, in a field setting, would be less exposed to cold temperatures as the lower leaves would be closer to the soil surface which could moderate temperature, especially in an irrigated setting. When plants had only chilled leaves in the 4°C and immediately exposed to *B. argentifolii* treatment, the higher leaves tended to host more adults. In this case it is possible that if nonchilled leaves are not available the adults select higher leaves preferentially, or that changes in leaf chemistry resulting from chilling occurs only after a period of exposure to warmer conditions.

Other studies have shown that *B. tabaci* are affected by changes occurring in plants. For example, Byrne and Draeger (1989) demonstrated that lettuce declines in quality as a host as plants mature, and Van Arx et al. (1983) showed fecundity was influenced by leaf age. Although our study was not designed to define mechanisms responsible for our observations, it is possible that changes in the availability or distribution of carbohydrates or proteins in the plants could be a factor. Increased fertilize applications have been shown to increase the abundance of *B. tabaci* in beans (Sardana and Verma 1987). The role of sugars as oviposition stimulants has been shown in another aleyrodid, *Aleurocanthus wogumi* Ashby (Dowell and Steinberg 1990). Cohen et al. (1989) related *B. tabaci* leaf preference to leaf pH levels.

As discussed in our introductory comments, low temperatures are known to evoke metabolic shifts in plants that can result in accumulation of soluble carbohydrates and certain proteins. It seems possible that these shifts would influence host quality for *B. argentifolii*. Further studies of direct and indirect impacts of cold temperatures on *B. argentifolii* might help us better predict its potential as a pest in regions where moderately cold temperatures occur, and could help to identify factors influencing its success.

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