Development and Mortality of Greedy Scale (Homoptera: Diaspididae) at Constant Temperatures

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ABSTRACT Developmental times and mortality were determined for four stages of greedy scale Hemiberlesia rapax (Comstock), a key pest of kiwifruit, Actinidia deliciosa (A. Chevalier) C. F. Liang et A. R. Ferguson variety deliciosa, in New Zealand. Scale were reared on potato tubers at seven constant temperatures and developmental stage and mortality were assessed at regular intervals. This required the removal of the scale cover to enable stage and mortality to be accurately determined. High numbers of scale (>1,400) were required at each temperature to sustain a frequent destructive sampling regime. Scale survival to the reproductive stage ranged from 0% at <12.5°C to 45% at 24.6°C. The time for 50% of the live scale population to reach each developmental stage was determined using a Bayesian smoothing program. A linear model of scale development was developed for each stage based on a single, temperature-independent distribution of normalized developmental time. A linear regression of development rate against temperature was used to estimate developmental thresholds that were 9.6, 9.7, 10.3, and 10.6°C for the second, third instar, mature, and first reproductive stages, respectively.

KEY WORDS Hemiberlesia rapax, greedy scale, temperature developmental thresholds

Developmental rate is calculated for each temperature and the linear relationship between rate and temperature provides an estimate of the lower developmental temperature. Nonlinear models can also be used to determine thresholds, although these are mainly used to investigate extremely high or low temperatures that are beyond the linear range. Several different approaches have been used to predict the proportions of insect stages completing development with time. Wagner et al. (1991) give a detailed literature review of this topic and describe a stochastic approach based on a single, temperature-independent distribution of normalized developmental times. This approach uses a standard normalized distribution obtained from a range of temperatures, and assumes the shape of the distributions is independent of temperature.

Greedy scale life history stages and the life cycle are well known (Blank et al. 1995a, 1995b, 1996). Reproduction is parthenogenetic, with males being unknown. There are two generations each year, with peak crawler release of the summer generation occurring in November to December and the winter generation in March to April. Greedy scale has three instars separated by a molt. The crawler is the only mobile stage and once this has settled all further development occurs with the scale firmly attached to the host plant. As soon as the crawler inserts its mouthparts into the plant and starts to feed it secretes white filaments in the shape of a disc to form the white cap stage. These filaments are cemented together by se-
creations of an anal fluid (Foldi 1990). The cap is gradually enlarged at the edge as the first instar grows. During molting the insect does not feed while new mouth parts and a new skin develop. The dorsum and venter of the cast skin splits and separates along the body margin. The dorsal portion is incorporated into the scale cover giving rise to the characteristic yellow and black caps of the second and third instars, respectively. The thin ventral portion remains under the body of the insect and contributes to the white dorsal pad, which remains firmly attached to the plant even after the death of the scale. After molting the immature adult, or third instar, continues to grow and changes from a flattened disc to a more off-center, mounded shape as eggs develop within the body. The mature scale lays eggs under the protective cover, and crawlers leave by way of an opening at the caudal end of the mother scale.

Greedy scale stage-specific phenology on kiwifruit and an alternative host plant taraire, Beilshniedia tarairi Bentham & Hooker, have been described using degree-day accumulation (Blank et al. 1995b, 1996). The developmental threshold of 9.3°C (SEM 0.34°C) for settlement to first reproduction was derived from the developmental data of Berry (1983) as plotted in Blank et al. (1995b). Other estimates were 7.6°C (SEM 0.99°C) and 9.1°C (SEM 0.74°C) for developmental thresholds from settlement to second instar and third instar, respectively. In contrast, Greaves et al. (1994) estimated the developmental thresholds to be 7.3, 6.7, and 7.5°C for the first, second, and third instars of greedy scale, respectively. These estimates, with the exception of the first instar, were significantly lower than the ones derived from Berry’s data. Critical evaluation of these two studies is difficult, because important information about the methods used was not presented and low numbers of scale were used.

To obtain an accurate estimate of the developmental threshold, at least 50 individuals must be reared at a minimum of four temperatures (Campbell et al. 1974). However, this assumes that the same insects can be assessed on each occasion. This cannot be accurately done for armored scale, which are hidden under a cover. Mortality of the sessile scale can only be determined by lifting the outer scale cover and observing the body of the insect. In addition, the accurate determination of the developmental stage requires that the outer scale cover be lifted so that the progress of the molt and presence of eggs can be observed. Lifting the cover compromises the integrity of the insect and may check or affect future development. Foldi (1990) found direct removal of the scale cover at different stages gave rise to aberrant scale cover formation. The destructive sampling of scale requires high numbers initially to sustain a frequent sampling regime. Wagner et al. (1991) also emphasizes the importance of sample size, replication (number of temperatures), and establishing insects concurrently using the offspring of a single population. The objective of this study was to determine the developmental thresholds of greedy scale stages.

**Materials and Methods**

Greedy scale were reared on potato tubers, *Solanum tuberosum* L. Ilam Hardy, held at seven constant temperatures. The greedy scale used for this study were from a laboratory colony continuously reared on potato tubers and butternut squash, *Cucurbita pepo* L., for 5 yr. The initial population of greedy scale was derived from kiwifruit plants grown near Whangarei (35° 44′ S, 174° 18′ E). Identification of mature scale in the colony was routinely checked using procedures described in Lo and Blank (1989) to ensure there was no contamination from closely related species. Potatoes were inoculated by placing fresh tubers on top of tubers, heavily infested with mature scale, for 24 hr at 22.9°C and 75% RH. Crawlers moved freely onto the new tubers. In addition, further crawlers were brushed onto the tubers on four occasions over this inoculation period. The tubers were removed from the colony and held for a further 24 hr at 22.9°C and 75% RH to encourage settlement before placing them in the seven temperature controlled rooms.

The temperatures tested were within the range normally encountered by greedy scale in northern regions of New Zealand where kiwifruit is grown. Extremely high temperatures were avoided because these can reduce development rate (Campbell et al. 1974, McClain et al. 1990). Lower temperatures were included to determine if development would occur near the lower temperature threshold. Relative hu-
mididity was left uncontrolled because previous rearing experience had shown that maintenance of humidity above ambient encouraged rots to develop on the tubers. Temperature rooms were kept unlit because development of settled scale was unaffected by light. Four tubers, each with high numbers (>350) of newly settled white caps, giving a total of 1,400 scale, were placed inside a plastic aquarium in each of the temperature control rooms. A second group of tubers was set up in the same way, but 24 h later. Each temperature thus had two groups of tubers, which were assessed separately.

Scale developmental stage was assessed at regular intervals. The interval between assessments varied for each temperature and stage, and was determined from preliminary studies and experience. The interval was reduced when a proportion of the population was undergoing a transition between stages. The number of assessments ranged from 17 to 22 for each temperature, except at 9°C when only 10 assessments were completed. At each assessment, the first five scale per tuber found to be alive, giving a total of 20 scale per temperature for each group, were examined under a microscope to determine the developmental stage. This involved the removal of the cap using a pin and examination of the underside of the cap as well as the body of the scale. When necessary the scale body was pierced with a pin to determine the presence of body fluids as evidence of live scale, a newly shed skin, or eggs within the body. Five developmental stages were identified (Table 1). Stages 2 and 3 were considered completed once the old skin could be easily separated from the body of the scale. The shed skin did not have to be incorporated into the inner surface of the cap. Assessments were carried out at ambient temperature and tubers were returned to their controlled temperature rooms as quickly as possible.

Scale for future mortality assessments were identified, within 5 d from inoculation, by drawing a circle around 10 lots of 20 settled white caps on each tuber. A mortality assessment using 80 scale (20 scale × 4 tubers) from each temperature was carried out when the assessment of the developmental stage showed >90% or greater of live scale had passed through to the next stage. There was some overlap between stages 4 and 5, because by then development within the population was more spread out. Scale were considered dead when their normally yellow and turgid bodies became dark brown, shriveled, and dry. To simplify presentation the average mortality for the completion of each developmental stage was determined by combining data from the two groups, as well as several assessments taken over a similar period. Where scale had commenced releasing crawlers the newly settled live white caps and yellow caps were omitted from mortality assessments.

Temperature probes were positioned near the tubers within the temperature control rooms and air temperature was recorded at hourly intervals on a data logger. The temperature probes were calibrated.
against a mercury reference thermometer. The average corrected temperatures were calculated for the duration of each scale stage.

The mean developmental stage was calculated using all 20 scale assessed at each assessment date, for each temperature. Mean developmental stages for each of the two scale groups were calculated separately. An asymptotic curve was fitted to these data using the Bayesian smoothing program Flexi (Wheeler and Updell 1994). Flexi was used to estimate the mean number of days and standard error for 50% of the population to reach each developmental stage (i.e., 1.5, 2.5, 3.5, and 4.5). For example, the 50 or 90% point between the stage 1 and 2 would be 1.5 or 1.9, respectively. Graphs of mean developmental stage against time are presented to show how these developmental times were estimated.

Standard normalized distributions were determined following similar procedures to Wagner et al. (1984). The predicted times when 1, 5, 10, 15, . . . , 90, 95, 99, 100% of the scale complete development were calculated for each temperature using Flexi. Each of the 22 developmental times was divided by the median time to produce a normalized distribution for each temperature. The mean normalized distribution was then calculated for each stage. Linear regression was used to fit a straight line to the data after logit transformation of the percentages and logarithmic transformation of time.

The developmental rate (D) of scale at the different temperatures was determined as the reciprocal of the developmental time (t) to complete each stage.

\[ D = \frac{1}{t} \]

Developmental rate was corrected for the 2 d at 22.9°C used during inoculation and settlement of the crawlers. Where

\[ D_{22.9°C} = 0.0502 \text{ d}^{-1} \]

\[ D_{corrected} = \frac{(t \times D)_{uncorrected} - (2 \times 0.0502)}{(t - 2)} \]

This ensured that the developmental rate was measured from the start of crawler inoculation. A linear regression of developmental rate against temperature (T) was used to estimate, by extrapolation, the temperature intercept or developmental threshold \( T_0 \).

\[ T_0 = \frac{a}{b} \]

The standard error of the x intercept \( T_0 \) was determined using the equation as described by Campbell et al. (1974).
Where $D$ is the mean developmental rate, $a$ the intercept, $s^2$ the residual mean square of the developmental rate, $b$ the slope and $N$ the number of temperatures.

**Results**

Monitoring of the temperature controlled rooms showed some drift in temperatures during the long periods, of up to 335 d, over which the trials were run. Average hourly temperatures for each scale development stage had a mean combined standard error of 0.03°C and a range of 0.01–0.08°C (Table 2). Average temperatures for the period from settlement until the completion of each stage varied by <0.3°C, except for the initial 14.4°C treatment, which increased to an average of 15.9°C after 150 d during the development of stages 4 and 5. For ease of presentation, temperature treatments will be identified using the average temperature from settlement to stage 5.

No development of settled white caps occurred at 9°C and most scale were dead after 135 d. Mortality assessments showed that all scale held at 9°C were dead after 202 d (Table 3). At 12.3°C, 6–9% of the scale population developed though to stage 3, but none survived through to stage 4. A greater proportion of the population successfully reached the reproductive stage as temperature increased, with 1, 3, 17, 25, and 43% reaching stage 5 at 13.7, 15.0, 16.7, 22.7, and 24.6°C, respectively. Mortality was higher for stages 1 and 2 compared with later stages, at temperatures of 16.7°C and below. Mortality of stage 3 was also relatively high at 22.7 and 24.6°C.

Development of >90% of the population through to stages 2, 3, 4, and 5 was completed after 30, 45, 70, and 80 d, respectively, at the two highest temperatures of 22.7 and 24.6°C (Figs. 1 and 2). At lower temperatures, development proceeded much more slowly. For example, at 13.7°C it took ~320 d for most of the scale
population to reach stage 5. At 12.5°C, development to stage 4 was completed by 30% of the population after 320 days, but no live scale were found at the next assessment. Two treatments were found to have similar temperature regimes during the development of stages 4 and 5. This was due to the increase in the initial 14.4°C treatment to 15.9°C. A crossover in developmental times occurred about the time this temperature change occurred. The development times for 50% of the live scale population to reach each development stage increased exponentially as temperatures declined (Table 4). The estimates for these development times showed a high level of precision with coefficients of variation all <2.9%.

Standard normalized distributions derived from each temperature all gave similar S-shaped curves, which were closely aligned to the mean, especially over the frequency range from 0.2 to 0.8 (Fig. 3). Significant linear regressions for all stages were obtained after logit transformation of the proportion and logarithmic transformation of normalized time (stage 2: $F = 10.450; \text{df} = 1, 19; P < 0.001$; stage 3: $F = 9.586; \text{df} = 1, 19; P < 0.001$; stage 4: $F = 40.823; \text{df} = 1, 19; P < 0.001$; stage 5: $F = 3.116; \text{df} = 1, 19; P < 0.001$) (Fig. 4). Observed data points gave close agreement with the linear model derived using predicted percentiles. The slopes of the linear regression lines were significantly different ($P < 0.05$; $df = 3, 76; F = 519$), although all the third instar stages (stages 3–5) as a group had relatively similar slopes that were different from the second instar.

Plots of developmental rate against temperature were linear over the range of temperatures tested and there was no reason to investigate nonlinear models (Fig. 5). Correcting for the 48-h inoculation and settlement temperature increased developmental thresholds by 0.51, 0.23, 0.16, and 0.13°C for stages 2, 3, 4, and 5, respectively. Corrected developmental thresholds for immature stages were lower than for mature scale and increased from 9.6, 9.7, 10.3, and 10.6°C for stages 2, 3, 4, and 5, respectively (Table 5). Stages 1 and 2 occupied 26 and 56%, respectively, of the 992 DD to reach stage 5.

Discussion

This study developed rigorous procedures for the determination of diaspidid developmental thresholds. This was necessary because of the need to compare these findings with earlier estimates of developmental thresholds for greedy scale. Procedures were used that avoided potential errors in the estimation of developmental times. These included ensuring adequate numbers of scale, destructive sampling of scale, clear definitions of mortality and stages, and correcting for time during inoculation and settlement. In addition, care was taken to measure the actual temperatures that scale were exposed to within the temperature con-
trolled rooms. This study also developed a useful method for the accurate determination and presentation of developmental times using Flexi. This presentation was similar to the cumulative probability distributions of Wagner et al. (1984), which gave plots of the percentiles for each temperature distribution. However, Flexi presents actual data points as well as the curve giving the best fit so that the accuracy of the distributions can be visualized more easily. The procedure of assessing 20 live scale at each sampling also overcame potential problems caused by different sample sizes and the differential mortality of insects held at the temperature extremes (Wagner et al. 1984). Both of these factors influence the shape distributions of normalized developmental time. The close fit of the normalized distributions obtained in this study was caused in part by the same sample size being used throughout the trial.

The linear transformations of the mean normalized distributions are useful models for predicting what proportion of the scale population has reached a particular development stage in terms of normalized time, independent of temperature. These models are most accurate for predicting proportions of stages between 20 and 80%. However, it is also of interest to predict the first appearance or last presence of a particular stage. Given that a sample size of 20 was used to generate this data set, then a 5% (1 out of 20) and 95% (19 out of 20) proportion may best represent these predictions.

These developmental models can be combined with the developmental rate model to predict the proportions of a population completing development through time under variable temperatures of up to 25°C (Wagner et al. 1984).

The developmental thresholds obtained for greedy scale stages in this study were closer to those derived from Berry’s data than those of Greaves et al. (1994). Berry (1983) inoculated crawlers onto tubers, which were placed directly into temperature controlled rooms, without holding for 24 h at an optimum temperature for settlement. Developmental times for all stages were determined at four temperatures by observation of external features without removal of the cap. Berry found some scale molted through to yellow cap stage at 9°C, but after this there was no further development. The large 95% CL ranging from 80 to 115 d for this estimate, in combination with a very low settlement of 8% at 9°C, determined in another experiment, suggested that very few scale actually survived to stage 2.

The developmental thresholds of 7.8°C and below obtained by Greaves et al. (1994) were in contrast to both this and Berry’s findings that scale did not develop at 9°C. Greaves et al. (1994) estimated developmental thresholds using the developmental times for each individual stage, rather than an accumulative developmental time from crawler settlement to a particular stage. However, further analysis of develop-
mental times in both this study and Greaves et al. (1994) found little difference in developmental thresholds using either method. Greaves et al. (1994) transferred crawlers with a fine brush and held tubers at 25°C for 24 h before placing them in six controlled temperatures. Assessments were made by continuous observation of the same scale. At the highest temperature of 28°C, abnormality occurred at stage 2, and all third instars died without producing eggs.

Characterization of the scale developmental stage principally by observation of the external appearance of the cap would overestimate developmental times. First, because of the difficulty of recognizing dead scale without complete removal of the cap, these may be included in the assessment of live stages. The developmental stage of dead scale is fixed in time and will give an overestimate of development, as live scale progress to more advanced stages. Second, determination of the completion of a molt by external observation would result in a longer developmental time than internal examination. The difference in time arises from when the shed skin first appeared, to when it became incorporated into the underside of the cover. External observation would thus give a lower developmental threshold, compared with determining the stage by lifting the cap.

The lower thresholds found for immature, compared with mature greedy scale would enhance synchronization of the winter scale generation. This mechanism would enable immature scale to continue development during cool temperatures in autumn, winter, and spring, when the mature scale are quiescent. The slightly faster development of immature scale would thus enable them to catch up to mature scale. The advantage of the higher temperature threshold for the mature scale would be that oviposition and crawler release are more likely to occur under the warmer conditions favorable for crawler dispersal and successful settlement (Berry 1983). Developmental times and thresholds for stages 4 and 5 were similar, indicating there was little delay between egg formation and the onset of oviposition. These findings confirm that there is little need to distinguish between these two stages when studying scale populations in the field. However, the presence of internal eggs could be used as an advance warning of crawler release.

Temperatures at the higher end of the range between 16.7 and 24.6°C appeared most favorable for greedy scale development to the reproductive stage. The finding that greedy scale development is restricted at low temperatures and that no scale survived after long periods at 9°C has important implications for a pest that has been considered a quarantine issue in some markets. Given that all export kiwifruit are held in cool-storage after harvest and during transit to world markets, there is a need to quantify the effects of commercial cool-storage on scale mortality.

The estimate of 992 DD obtained in this study may slightly underestimate generation interval, because developmental time was measured to the first rather than the middle of the reproduction stage. This laboratory estimate of generation interval can be compared with estimates derived from field studies of 1,056 and 1,022 DD on taraire and kiwifruit, respectively, but some adjustment is necessary for the 9.3°C developmental threshold used (Blank et al. 1995b, 1996). The closeness of these estimates gives some confidence that the developmental thresholds ob-

**Table 5. Regression relationships between developmental rate and temperature used to estimate by extrapolation developmental thresholds for greedy scale stages**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Regression</th>
<th>Developmental threshold</th>
<th>Developmental time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope SE</td>
<td>Intercept SE</td>
<td>r²</td>
</tr>
<tr>
<td>2</td>
<td>0.0039</td>
<td>0.00029</td>
<td>-0.037</td>
</tr>
<tr>
<td>3</td>
<td>0.0018</td>
<td>0.00008</td>
<td>-0.017</td>
</tr>
<tr>
<td>4</td>
<td>0.0011</td>
<td>0.00012</td>
<td>-0.012</td>
</tr>
<tr>
<td>5</td>
<td>0.0010</td>
<td>0.00008</td>
<td>-0.011</td>
</tr>
</tbody>
</table>

Developmental times in degree-days are given for each stage.
tained from rearing scale on potato tubers can be used to develop a phenological model for greedy scale on kiwifruit and other host plants.

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References Cited


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