Hort Innovation

Final Report

Development of Phenology Models and a Timing Guide for the Management of Red Scale in Australian Citrus

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Development of Phenology Models and a Timing Guide for the Management of Red Scale in Australia Citrus – CT15008

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Contents

Summary

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Red scale populations are normally kept below damaging levels by their natural enemies. Releases of the parasitoid *Aphytis* are sometimes made to enhance biological control. Chemical interventions are occasionally needed to reduce fruit contamination. Timing is important in red scale management. Many registered chemicals for red scale control are most effective against crawlers (new-born scale nymphs), whitecaps (newly settled crawlers) and other first instar scales, which do not have fully developed wax covers to protect them.

To help citrus growers time their red scale control, we collected data on the seasonal patterns of red scale populations in the southern citrus regions, conducted chemical timing trials and developed a timing guide for peak periods of adult males and crawlers. A red scale population model was also developed to investigate the underlying mechanisms for the observed seasonal patterns.

Seasonal patterns of adult males and crawlers in the Riverina and Sunraysia showed three periods of relatively high catches, occurring in spring, summer and autumn, respectively for adult males, and late spring to early summer, mid-summer to early autumn and autumn, respectively for crawlers. The three peak periods are produced by the overwintering, first and second post-winter generations. Red scale can complete at least four annual generations in the southern citrus production regions of Australia. Model simulations suggest the existence of a small fourth generation during the period from late autumn to early spring.

Individual seasonal patterns of adult males and crawlers varied considerably between monitoring sites and seasons, ranging from small, isolated peaks to broad, merged peaks. Despite the variations, red scale adult males and crawlers are more likely to peak during certain time periods. Crawlers are likely to be most abundant in November and least abundant during June to August. Adult males are likely to be most abundant in October and March and least abundant in May to August and November. The timing of spring male and crawler peaks can be predicted with a minimum accuracy of 75% by degree days. Degree day (DD) is a measure of heat units. Insects require certain numbers of degree days to complete development. Spring male peaks are predicted after 203–353 degree days have been accumulated between 11.7 °C and 38 °C since winter solstice and the spring crawler peaks after 582–732 DD have been accumulated. The timing of spring crawler peaks can also be predicted by the timing of the preceding adult male peaks using the DD gap of 272–422 DD.

Red scale populations increase from spring to autumn. Controlling the spring generation of crawlers will reduce the size of red scale populations in subsequent generations. *Aphytis* prefer to parasitise virgin females. The timing of spring adult male peaks can be used for *Aphytis* releases as it is also the time when virgin females are abundant. An online timing guide has been developed to help growers make the predictions and time their red scale management operations.

Public summary

Red scale is a key pest of citrus in Australia. It can infest all above ground surfaces of trees but in Australia it occurs most commonly on fruit, leaves, twigs, and branches up to two years old. Infested fruit produced for fresh fruit market is downgraded or sent for juicing. Red scale is a quarantine pest for citrus export to Korea.

Red scale populations are normally kept below damaging levels by their natural enemies. Releases of the parasitoid *Aphytis* are sometimes made to enhance biological control. Chemical interventions are occasionally needed to reduce fruit contamination and improve marketability. Timing is important in red scale management. Many registered chemicals for red scale control are most effective against crawlers (new-born scale nymphs), whitecaps (newly settled crawlers) and other first instar scales, which do not have fully developed wax covers to protect them.

To help citrus growers time their red scale control, we collected data on the seasonal patterns of red scale populations in the southern citrus regions, conducted chemical timing trials and developed a timing guide for peak periods of adult males and crawlers. A red scale population model was also developed to investigate the underlying mechanisms for the observed seasonal patterns.

Seasonal patterns of adult males and crawlers in the Riverina and Sunraysia showed three periods of relatively high catches, occurring in spring, summer and autumn, respectively for adult males, and late spring to early summer, mid-summer to early autumn and autumn, respectively for crawlers. The three peak periods are produced by the overwintering, first and second post-winter generations. Red scale can complete at least four annual generations in the southern citrus production regions of Australia. Model simulations suggest the existence of a small fourth generation during the period from late autumn to early spring.

Individual seasonal patterns of adult males and crawlers varied considerably between monitoring sites and seasons, ranging from small, isolated peaks to broad, merged peaks. Despite the variations, red scale adult males and crawlers are more likely to peak during certain time periods. Crawlers are likely to be most abundant in November and least abundant during June to August. Adult males are likely to be most abundant in October and March and least abundant in May to August and November. The timing of spring male and crawler peaks can be predicted using local temperatures.

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Keywords

Red scale, *Aonidiella aurantii*, crawler, male flights, timing, seasonal patterns, phenology models, prediction.

Introduction

Red scale, *Aonidiella aurantii* (Hemiptera: Coccomorpha: Diaspididae), is a major pest of Australian citrus. It can infest all above ground surfaces of trees but in Australia it occurs most commonly on fruit, leaves, twigs, and branches up to two years old (Figure 1). Infested fruit produced for fresh fruit market is downgraded or sent for juicing. Red scale is a quarantine pest for citrus export to Korea.

Figure 1. Red scale infested citrus fruit, leaf, and twigs/branches.

The life cycle of a red scale (Figure 2) starts as a crawler that emerges from underneath the cover of a female scale (Forster *et al*. 1995). The crawler soon settles and starts to secrete a wax cover, appearing as a whitecap. From then on, a female scale moults twice to become the second and third instars and then a mature female. A male scale moults once to become the second instar then pre-pupa, pupa and then an adult male. Adult male scales have wings and can fly.

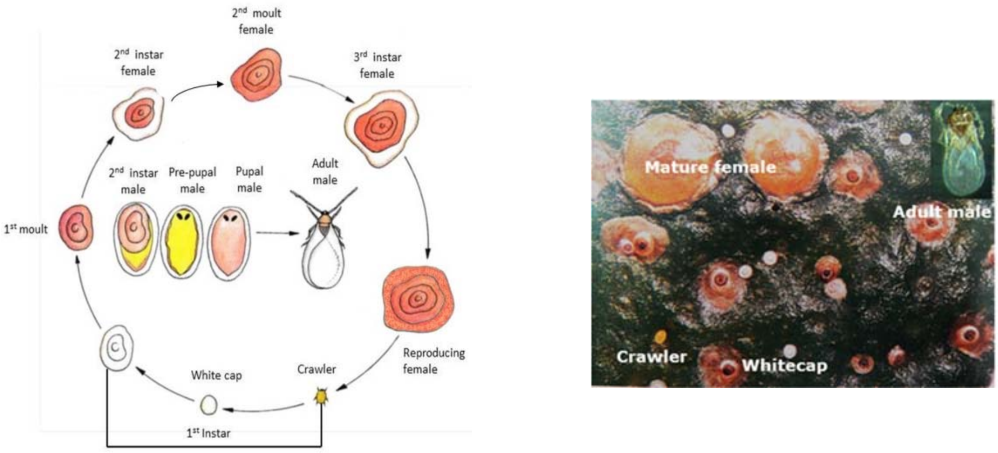

Figure 2. An illustration of red scale life cycle (Adapted from Forster *et al*. 1995) and a close-up view of different stages of red scale.

Crawlers are tiny (difficult to see with naked eyes) and yellowish. They crawl around to find suitable feeding sites. Within 24 hours, they settle down to feed and start producing wax covers. Before they moult into the second instar, their wax covers are thin and whitish and they are commonly referred to as 'whitecaps'. Crawlers and whitecaps are the most susceptible stages of the red scale life cycle to chemical control.

Red scale is attacked by many parasitoids and predators. In the southern citrus production regions, the main parasitoids are *Aphytis* spp. (Hymentoptera: Aphelinidae) and *Comperiella bifasciata* (Hymenoptera: Encyrtidae) and the main predator is *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) (Figure 3). *Aphytis* are ectoparasitoids, laying eggs on the body of the scale beneath the scale cover. There are three *Aphytis* species in Australia; *A. melinus, A. linganensis and A. chrysomphali* (Smith et al. 1997, Dao et al. 2017). *Aphytis* are commercially available and routinely used in red scale management in citrus. They prefer to parasitise virgin females and large second instar scales of both sexes (Yu and Luck 1988). *C. bifasciata* is an endoparasitoid, laying eggs inside scale bodies. It is capable of parasitising up to 80% of scales. *C. bifasciata* prefers to attack third instar female scales, but will oviposit in any stages except females that have already produced crawlers (Dreistadt 2012). Other red scale endoparasitoids include two *Encarsia* (Hymenoptera: Aphelinidae) species, *E. citrina* and *E. perniciosi*. Other red scale predators include *Halmus chalybeus* (Coleoptera: Coccinellidae) and *Chilocorus circumdatus* (Coleoptera: Coccinellidae).

Figure 3. Key natural red scale enemies in the southern citrus production regions. A: *Aphytis* spp. (adult on left, pupa on right), B: *Comperiella bifasciata* (adult on left, exit hole on the scale cover left by an emerged adult on right) and C: *Rhyzobius lophanthae* (adult on left, larva on right).

Red scale populations are normally kept below damaging levels by their natural enemies. *Aphytis* releases are sometimes made to enhance biological control. Chemical interventions are occasionally needed to reduce fruit contamination. Timing is important in red scale management. Many chemicals registered for red scale control, including horticultural mineral oil (HMO) and insect growth regulators, are most effective against crawlers and whitecaps, which do not have fully developed wax covers to protect them (Eberling 1936, Smith et al. 1997). Walker et al. (1990) compared a series of timings of chlorpyrifos sprays and noted significant differences in results. In southern Australia, up to two HMO sprays are recommended annually, with single sprays in either November-December or February-March, or one in November-December followed by another in February-March. *Aphytis* releases are recommended for October–March. The recommended windows may not be optimal for all sites, seasons and citrus varieties due to variations in red scale development.

To find the optimal timing for red scale control, we need to know when the vulnerable stages are most abundant. This can be determined by regular monitoring. Monitoring by direct visual inspections is labour intensive and requires good technical skills (Forster et al. 1995). Pheromone traps can substantially reduce monitoring costs and are widely used in red scale monitoring (Ervin et al. 1985, Grout and Richards 1989, Campos-Rivela et al. 2012). The timing of crawler peaks can be estimated by the timing of the preceding male flight peaks. In California, timing of the first generation crawler peak appeared about 306 degree-days after the first male flight peak and that of the first generation virgin females around the time of the first generation male flight peak (Kennett and Hoffman 1985). A similar time lag between a male flight peak and the following crawler peak was observed in Spain (Campos-Rivela et al. 2012).

Little is known about the phenology of red scale in Australia. Dao (2012) monitored red scale populations with pheromone traps on the central coast of NSW and showed that the scale had three annual generations. Spring peaks of male flights occurred between mid-September and mid-October, summer peaks between mid-December and mid-January and autumn peaks between mid-February and late March. Our preliminary pheromone trapping data from a citrus orchard in the Riverina in southwest NSW during 2013-2014 also showed the presence of three annual peaks of male flights with the spring peak being the most prominent.

To improve red scale management in Australia, this project investigated the seasonal patterns of red scale populations in the southern citrus regions and developed a timing guide on male flights and crawler abundance based on degree-days.

Degree-day (DD) is a measure of heat units. Insects need to accumulate sufficient DDs to complete development. Not all temperatures are conducive to insect development. DD is accumulated from a fixed date (Biofix date) between a lower threshold temperature and an upper threshold temperature until the target DD is reached. The target DD differs with insect species and developmental stages. Because of temperature differences, the same target DD may be reached on different dates in different seasons. Timing predictions based on DD are more robust than those based on calendar dates as they have accounted for the effects of temperature on insect development.

Methodology

Seasonal patterns

Male flights and crawler numbers were monitored during 2015–2018 at three sites in the Riverina and four sites in the Sunraysia (Appendix-1). Male flights were monitored with pheromone traps and crawler numbers with Scotch® double-sided tape. An additional dataset of seasonal patterns of red scale males and crawlers was collected during 2013–14 using the same method in a citrus orchard at the Yanco Agricultural Institute. This dataset was used together with data collected during the project to analyse the seasonal patterns and develop a timing guide.

Individual seasonal patterns were normalised by their respective mean catches and plotted over accumulated DDs to check for DD periods when male and crawler catches were relatively high. Where such periods were found, the weighted mean DD was estimated for each period. DDs were estimated using the lower threshold temperature of 11.7 °C and the upper threshold temperature of 38 °C and accumulated from winter solstice.

Prediction

Predicting the exact time for an individual red scale adult male or crawler peak is difficult due to site and seasonal variations. A more realistic approach is to predict the time period when a peak is likely to occur. Given a wide enough time interval, any peaks can be predicted with confidence; however such predictions may be impractical because the predicted time interval is too wide. By contrast, a small interval containing the target peak provides more information about the location of the peak but is more difficult to estimate. A compromise is to find the smallest time interval when a target peak can be predicted with a desired level of confidence.

A series of candidate DD intervals were tested in the predictions of male and crawler peaks. The upper limit of a candidate DD interval was the mean DD plus a fixed DD width and the lower limit was the mean DD minus the same fixed DD width (Appendix-2). Fixed DD widths tested were 25, 50, 75 and 100 DD. The DD intervals were tested on catch data from individual sites and seasons to see how well they predicted the positions of spring, summer and autumn peaks.

Population model

A variety of seasonal patterns of red scale populations were observed in the southern citrus production regions (Appendix-1). To investigate the underlying mechanisms, a temperature-driven, multi-cohort population model that simulates the development process of individual scales in a population was developed (Appendix-3). Where available, published data were used to estimate the model parameters. The effects of overwintering age structure, variations in the development rates of individual scales and extreme temperatures on red scale seasonal patterns were investigated.

Timing trials

Three timing trials were conducted in blocks of Valencia orange trees in Leeton in the Riverina, one each in the 2015/16 and 2016/17 seasons and one in 2018 (Appendix-4). In each trial, four spray timings were compared with an unsprayed control. The 2015/16 and 2016/17 trials compared spray timings in spring and the 2018 trial compared spray timings in autumn. To reflect citrus industry's standard practice for red scale control, chlorpyrifos (50 mL/100 L) plus oil (1% Biopest) was used as the chemical spray. Effects of spray timing were assessed by the proportions of infested fruit and leaves 3-6 months after the sprays.

Outputs

- Eighteen datasets (6 sites by 3 seasons) have been collected on the seasonal patterns of red scale adult males and crawlers in the southern citrus production regions (Appendix-1).
- Peak periods of crawlers and adult males in spring, summer and autumn have been identified (Appendix-2).
- A population model has been developed to investigate the mechanisms for the observed seasonal patterns (Appendix-3).
- Three timing trials have been conducted comparing efficacy of red scale control on different dates (Appendix-4).
- An online timing guide has been developed allowing growers to predict spring crawler and adult male peaks using local temperatures (Appendix-5).
- 7 Grower meetings, 4 in the Riverina and one each in the Sunraysia, Riverland and WA (Appendix-6).
- 5 articles in industry magazines/newsletters (Appendix-6).

Outcomes

- Increased knowledge of red scale phenology in the southern citrus production regions.
  - Seasonal patterns of red scale adult males and crawlers have three annual peak periods. For adult males, the three peak periods are found in spring, summer and autumn. For crawlers, the three peak periods are found during late spring to early summer, mid-summer to early autumn and autumn (Appendix-1).
  - The tri-modal seasonal pattern was also shown by the simulation model (Appendix-3). The three peak periods were produced by the overwintering, first and second post-winter generations respectively (Appendix-1 and 3).
  - There are likely multiple age groups in any generation of red scale. As a result, different generations become increasingly overlapped as the season progresses, leading to largely merged peaks late in the season (Appendix-3).
  - In addition to population age structure, variations in individual development rates, extreme temperatures and orchard operations might have also contributed to the observed seasonal patterns (Appendix-3).
- Australian citrus industry is provided with an online timing guide for red scale controls.
  - Average monthly activities of red scale crawlers and adult males have been estimated for the southern citrus production regions (Appendix-1).
  - Spring crawler peaks and adult male peaks can be predicted with a minimum accuracy of 75% using local temperatures (Appendix-2).
  - Field trials have confirmed the importance of timing in red scale control (Appendix-4).
  - An easy-to-use, online timing guide has been developed for growers and pest consultants to make the predictions (Appendix-5).

Monitoring and evaluation

What is already known about red scale phenology in Australia?

At the start of this project, knowledge of red scale phenology in Australia was mostly descriptive. No published information was available on the seasonal patterns of red scale populations in the major citrus production regions of Australia.

Are there distinct annual peaks of male flights and crawler numbers in Australia?

Distinct annual peaks of male flights and crawler numbers have been found but their locations in the seasonal patterns varied with site and year. Spring peaks occurred more frequently than summer and autumn peaks.

Can the timing of peak male flights and crawler numbers be predicted with confidence?

The timing of spring male and crawler peaks can be predicted with a minimum accuracy of 75% using local temperatures. Using the winter solstice as the starting date for DD accumulations and the lower and the upper developmental threshold temperatures of 11.7 °C and 38 °C, spring male peaks are predicted at 203–353 DD and the spring crawler peaks at 582–732 DD. The timing of spring crawler peaks can also be predicted with at least 75% accuracy by using the timing of the preceding adult male peaks and the DD gap of 272–422 DD.

How much does spray timing affect the effectiveness of red scale control?

Spray timing showed a significant influence on the effectiveness of red scale control in two of the three timing trials. In one trial, there was a 2.5-fold difference in the proportion of red scale infested fruit and a 4-fold difference in the proportion of heavily infested fruit (> 10 scale/fruit). In the other trial, there was an over 8-fold difference in the proportion of heavily infested fruit. In both trials, mid-November timing achieved the best control of red scale.

How to encourage adoption of the timing guide?

An interactive, online timing guide has been developed to help growers time their red scale control. The online timing guide is easy to use. Growers simply select the nearest weather station and the predicted periods of spring crawler peaks are shown. The online timing guide also provides monthly charts of crawler activity in the southern citrus production regions. The online timing guide can be accessed at https://redscale.shinyapps.io/predict/.

Recommendations

Red scale populations are normally kept below damaging levels by their natural enemies. Chemical interventions are occasionally needed to reduce fruit contamination. Many registered chemicals for red scale control are most effective against crawlers (new-born nymphs) and whitecaps (recently settled crawlers), which do not have fully developed wax cover to protect them. It is therefore important to time sprays when crawlers and whitecaps are most abundant. There are multiple crawler peaks in a year. Spring crawler peaks are more predictable than the other seasonal peaks. Late season crawler peaks tend to merge into large, flat peaks with no distinct peak positions.

It is recommended that red scale controls be timed at spring crawler peaks. These peaks are produced by the overwintering generation. Red scale populations are relatively low after the winter months. Controlling the first post-winter generation will also reduce red scale populations in later generations. Timing of spring crawler peaks can be predicted using local temperatures. An online timing guide has been developed to help with the predictions (https://redscale.shinyapps.io/predict). As a general guide, crawler numbers in the southern citrus production regions are highest in November and lowest from June to September. Please note that chemical control might not be needed if infestation level is low and parasitism level is high. Check with your pest consultant regarding the necessity of sprays.

Another commonly used management option for red scale is to release *Aphytis*. *Aphytis* is a group of small wasps that parasitise red scale. They prefer to parasitise virgin female scales (unmated 3rd instar females). The online timing guide can be used to predict the time when spring male flight peaks, which occurs at a similar time when spring virgin female peaks. Releasing *Aphytis* at this time will accelerate the build-up of *Aphytis* numbers following a decline of *Aphytis* populations during winter. *Aphytis* can be obtained from Bugs for Bugs (https://bugsforbugs.com.au/) and Biological Services (http://www.biologicalservices.com.au/).

Pheromone traps are useful tools for monitoring red scale populations. They can be used to detect male flights, which in turn, can be used to predict crawler peaks and therefore timing of red scale controls. The size of trap catches provides a measure of local red scale infestation levels. Currently pheromone traps are not widely used in Australia. Extension activities are needed to encourage the adoption of pheromone traps and enhance red scale IPM.

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Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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Appendices

Appendix-1	Seasonal patterns of red scale males and crawlers
Appendix-2	Prediction of male flight peaks and crawler peaks
Appendix-3	Population model
Appendix-4	Timing trials
Appendix-5	Timing guide
Appendix-6	Communications

Appendix-1 Seasonal patterns of red scale males and crawlers

Materials and Methods

Male flights and crawler numbers were monitored during 2015–2018 at three sites in the Riverina and four sites in the Sunraysia (Table A1-1). Monitoring at the Riverina sites started in late September 2015 and finished in late February 2018. Starting dates at the Sunraysia site were late September 2015 at two sites, mid-November 2015 at one site and late June 2016 at another site. It was difficult to find navel orange blocks with moderate to high red scale infestations in the Sunraysia and the monitoring sites had to be changed in 2015 and 2016 to avoid long periods of near zero catches, resulting in different starting dates at the monitoring sites.

Table A1-1. Description of monitoring sites.

Region	Site [1]	GPS	Variety	Age(yrs.)	Infestation [2]	Period [3]
Riverina	YAI	-34.6308° 146.4267°	Washington Navel	55	Medium	25/09/2015 26/04/2018
	AN	-34.5376° 146.3775°	Valencia	6–7	High	25/09/2015 26/04/2018
	TN	-34.5236° 146.2897°	Valencia	42	High	25/09/2015 26/04/2018
Sunraysia	CK	-34.1612° 142.1857°	Valencia	33	Medium	28/10/2015 21/3/2018
	CRX	-34.4439° 142.3185°	Late Lane	7	Low	28/10/2015 21/3/2018
	SCF	-34.4984° 142.3502°	Barnfield Navel	26	Low	10/11/2015 21/3/2018
	DV	-34.1797° 142.0403°	Late Lane	NA	Low	30/6/2016 21/3/2018

[1] Code names used to identify different monitoring sites; [2] low: < 30%, medium: 31–70%, high: > 70% fruit infested; [3] Start date to end date.

Male flights were monitored with pheromone traps (Moreno and Kennett 1985). The trap consisted of a folded-over yellow sticky trap (75 × 110 mm, Bugs for Bugs, Mundubbera, QLD) with a rubber septum impregnated with red scale sex pheromone (ENTOSOL Australia Pty Ltd, Roselands, NSW) attached to a wire hook. Ten pheromone traps were used at each site. The traps were placed at approximately 1.5 m above ground in the outer canopy of 10 separate trees at least 20 m apart. Pheromone lures were replaced every four weeks.

Crawler numbers were monitored with double-sided tape wrapped tightly around twigs adjacent to red scale-infested fruit or leaves (Walker et al. 1990). Two twigs from each of the 10 trees selected for pheromone trap monitoring were taped.

Pheromone traps and sticky tapes were replaced weekly from October to May and fortnightly from June to September. Collected sticky traps were wrapped with Glad® ClingWrap and were sandwiched between two microscope slides before being examined. The numbers of adult red scale males caught on sticky traps were counted under a stereo microscope. The numbers of red scale crawlers caught on the sticky tapes were counted under a compound microscope.

An additional dataset of seasonal patterns of red scale males and crawlers was collected during 2013–14 using the same method in a citrus orchard at the Yanco Agricultural Institute. This dataset was used together with data collected during the project to analyse the seasonal patterns and develop a timing guide.

Numbers of adult red scale males and crawlers caught in all traps in the same trapping interval were averaged to estimate the mean catch on each monitoring date. Associations between seasonal patterns of mean daily catches of males and crawlers at different sites were analysed by the function 'cor.test' in R (R Core Team 2012) for each monitoring region (Riverina or Sunraysia) and season.

To compare trap catches across sites and seasons, the mean catch-by-date data were normalised by the respective mean catches from individual sites and seasons. Accumulated DDs from the winter solstice (Biofix date) to each monitoring date were estimated using the lower threshold temperature of 11.7 °C and the upper threshold temperature of 38 °C (Grout and Richards 1989). Daily temperatures at the Yanco Agricultural Institute and Mildura Airport were downloaded from the Australian Bureau of Meteorology website (www.bom.gov.au) and used to estimate the DDs. The distance from a monitoring site to the nearest weather stations was < 50 km. Daily maximum and minimum temperatures were converted to hourly temperatures using the single-sine method with horizontal cut-off (Roltsch et al. 1999).

Normalised mean catch-by-date data from all monitoring sites and seasons were pooled and plotted over accumulated DDs to check for DD periods when male and crawler catches were relatively high. Where such periods were found, the weighted mean DD was estimated for each period using the following equation:

$$DD_{wt} = \sum dd_i \, y_i / \sum y_i \qquad \text{(Equation A1-1)}$$

where DD_{wt} is the weighted mean DD for a period, dd_i and y_i are the DD and mean catch on an individual date respectively. Summation was done over all DDs and mean catches within the target DD period.

To find the positions, sizes and widths of male flight and crawler peaks, individual male flight peaks and crawler peaks were identified for each site and season. Male or crawler catch was considered to have reached a peak period if the rate of increase had exceeded a given threshold over the previous catch and was higher than the seasonal median catch. A peak period was considered to have ended if the rate of decrease had exceeded the threshold and was lower than the seasonal median catch. The threshold used for the rate of change was 200%. For each peak, the following statistics were estimated: peak value, peak DD (DD at peak catch), start and end DDs and weighted DD (Equation-A1-1).

Individual peaks from different sites and seasons were then pooled to analyse their positions. To avoid bias, peaks from partial monitoring seasons were excluded in the analyses. The total number of male flights and crawler peaks were calculated for each month. Distributions of male and crawler peaks were then analysed to find out DD intervals where the peaks were concentrated. Finally, mean weighted DDs were estimated for each DD interval of concentrated peaks.

Results

Male flights

Seasonal patterns of male flight showed distinct spring peaks in the Riverina in 2013 and 2015 and in the Sunraysia in 2016 (Figure A1-1). Male flight peaks were also detected in spring in the Riverina in 2016 and 2017 and in the Sunraysia in spring 2017. However, these peaks were either very small or did not show up at all monitoring sites. The presence or absence of spring male flight peaks in the Sunraysia in 2015 cannot be verified due to the late commencement of monitoring. The earliest spring peak was detected in mid to late September and the latest in early November but most were found in mid-October. Prominent male flight peaks were detected in summer each year in the Riverina. Prominent autumn peaks were detected in 2014 and 2017 in the Riverina. Less prominent male flight peaks were detected in the summer of 2013/14 in the Riverina and in autumn of each year in the Sunraysia. The earliest summer peak was detected in mid-December and the latest in mid-February. The earliest autumn peak was detected in mid-March and the latest in mid-May. The gap between the spring peaks and summer peaks was 2.5–3 months and the gap between the summer peaks and autumn peaks was 2–2.5 months. Where spring, summer and autumn male flight peaks were all present in the same seasons, spring and autumn peaks were considerably larger than the summer peaks.

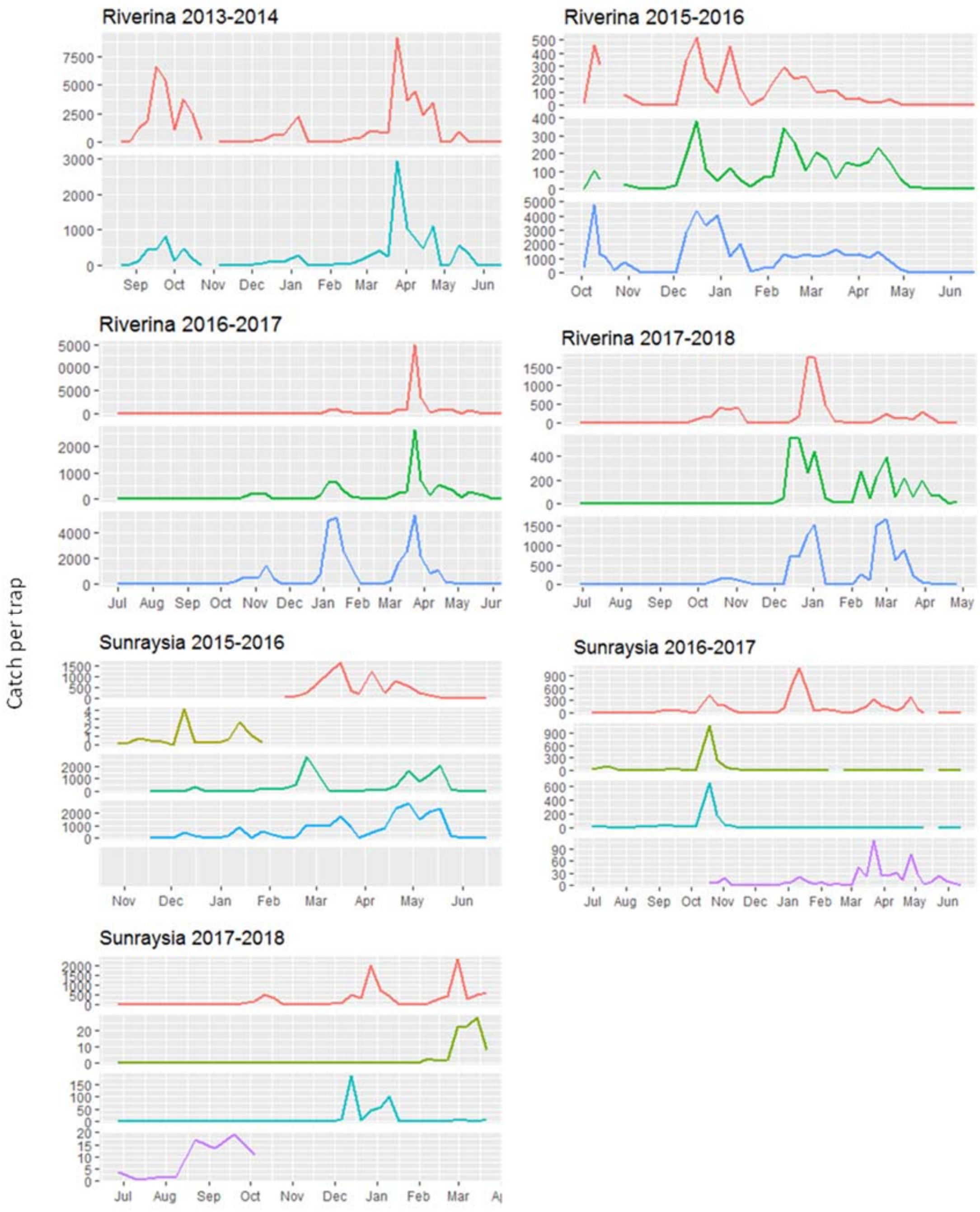

Figure A1-1. Seasonal patterns of red scale male flights at the monitoring sites in the Riverina and Sunraysia. Patterns at different monitoring sites in the same region and season are indicated by different colours.

There were significant correlations in male catches between any two sites in the Riverina in any monitoring seasons (*$P < 0.05$*) (Table A1-2). Significant site correlations were also found in male catches between some of the sites in the Sunraysia in each of the three monitoring seasons. Where a significant correlation was found, the correlation coefficient was positive, indicating a positive association between catches at the two sites being compared.

Table A1-2. Site correlations in male catches estimated using Pearson's product moment correlation coefficient. Only sites with the same data periods were compared.

Region	Season	Comparison	Correlation	*t*	*D.F.*	*P*
Riverina	2013/14	Site Y1 ~ Site Y2	0.85	10.20	40	< 0.0001
	2015/16	Site R1 ~ Site R2	0.60	4.45	35	< 0.0001
		Site R1 ~ Site R3	0.73	6.30	35	< 0.0001
		Site R2 ~ Site R3	0.54	3.57	35	0.0006
	2016/17	Site R1 ~ Site R2	0.80	10.13	22	< 0.0001
		Site R1 ~ Site R3	0.89	14.19	22	< 0.0001
		Site R2 ~ Site R3	0.97	19.63	22	< 0.0001
	2017/18	Site R1 ~ Site R2	0.41	2.64	35	< 0.0124
		Site R1 ~ Site R3	0.59	4.28	35	0.0001
		Site R2 ~ Site R3	0.76	6.88	35	< 0.0001
Sunraysia	2015/16	Site S2 ~ Site S3	0.62	4.11	27	0.0003
	2016/17	Site S1 ~ Site S2	0.21	1.35	38	0.1859
		Site S1 ~ Site S3	0.21	1.36	39	0.1802
		Site S2 ~ Site S3	0.99	52.71	38	< 0.0001
	2017/18	Site S1 ~ Site S2	0.43	2.58	30	0.0151
		Site S1 ~ Site S3	0.27	1.51	30	0.1422
		Site S2 ~ Site S3	-0.08	-0.44	30	0.6598

When all seasonal patterns were superimposed on one another using normalised mean catches and degree-days (DD), three broad peaks were discernible, the first between 200 and 400 DD, the second between 850 - 1350 DD, and the third between 2000 and 2300 DD (Figure A1-2), with peak positions at 298, 1097, and 2155 DD, respectively (Table A1-3). The DD intervals corresponded to September - October, December - January, and March - April, respectively, matching the positions of spring, summer and autumn peaks observed in individual seasonal patterns (Figure A1-1). Normalised mean male catch was highest in the third peak period and lowest the first peak period (Table A1-3).

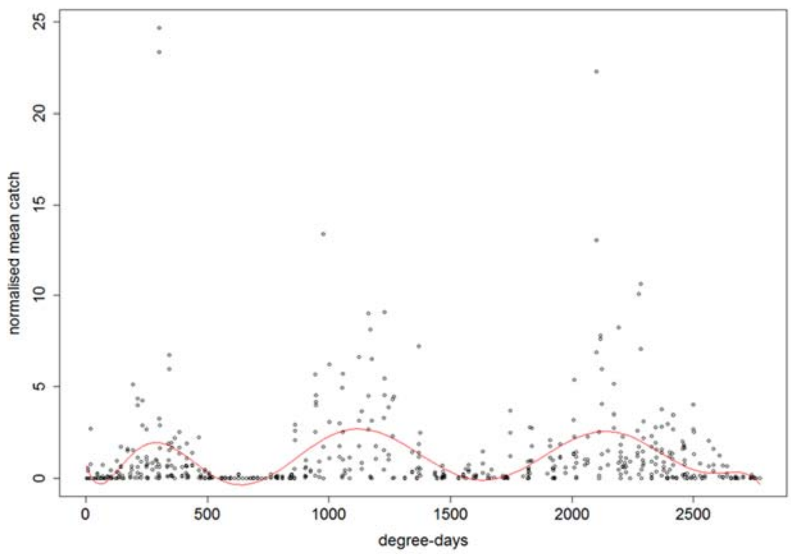

Figure A1-2. Normalised mean catches of red scale males by degree days across all monitoring sites and seasons. Red line was obtained by fitting the observed data points to a polynomial function.

Table A1-3. Degree day (DD) range, date range, normalised mean catch and weighted mean DD in the pooled seasonal patterns of males.

Peak ID	DD range	Date range	Mean catch	Peak DD
1	200–400	19 Sep–17 Nov	1.9	298
2	850–1,450	8 Dec–7 Jan	2.2	1,097
3	2,000–2,300	16 Mar–12 Apr	2.5	2,155

A total of 71 Individual male flight peaks were detected in the seasonal patterns across all sites and seasons. After removing those from partial monitoring seasons and those that were too small (< 1 per trap), 57 peaks remained. The remaining peaks were concentrated over the DD intervals of 150–400 DD, 900–1,200 DD and 2,050–2,300 DD, with scattered peaks elsewhere (Figure A1-3). Locations of the three groups of male flight peaks corresponded to spring, summer and autumn respectively. There was a lengthy absence of male flight peaks between 1,150 and 1,800 DD. The mean DDs of the spring, summer and autumn peaks were 278, 1,127 and 2,171 DD respectively. The duration of the peaks increased from 187 DD in spring, to 424 in summer and 559 DD in autumn.

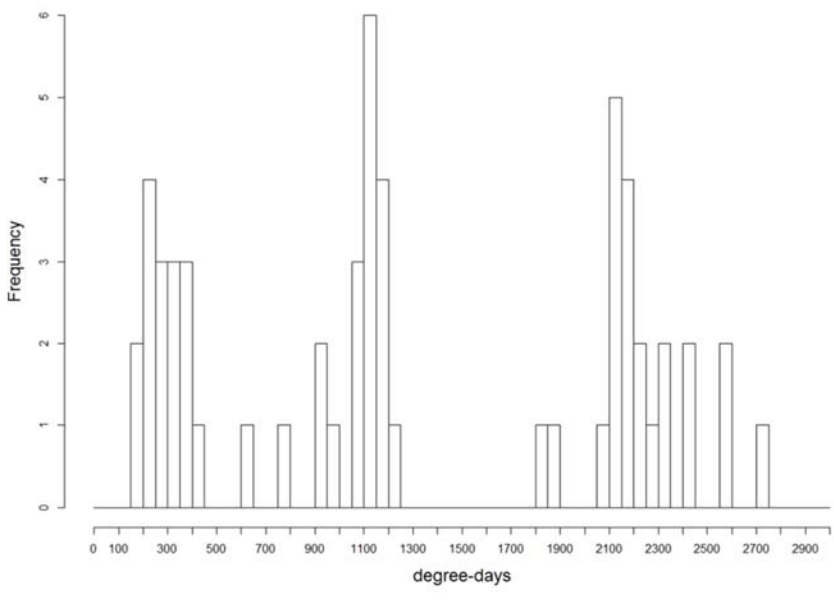

Figure A1-3 Distribution of individual male flight peaks over degree days.

Males were caught in all months of the year although significantly higher catches were made in October, December, January and March compared with the other months (*P < 0.05*) (Figure A1-4). The highest numbers of male flight peaks were found in October, January and March. No male flight peaks were found from June to August.

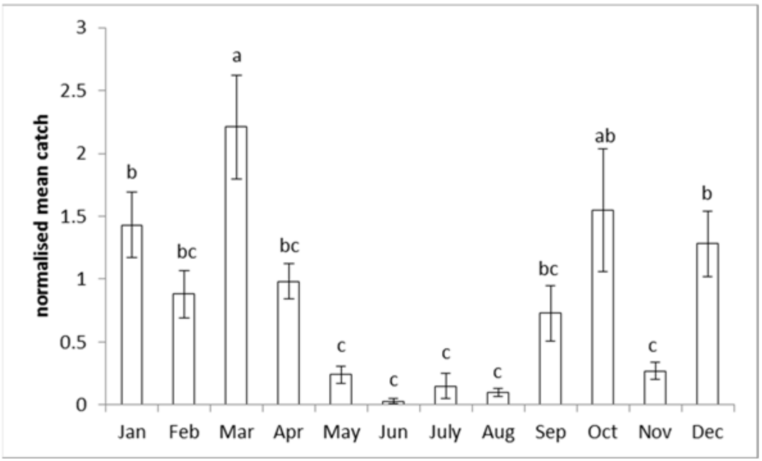

Figure A1-4. Normalised mean catch of red scale males by month. Bars labelled with no matching letter(s) are significantly different at P = 0.05 by Fisher's LSD test following the detection of a significant treatment (month) effect by ANOVA. Wire bBars show standard errors.

Crawler abundance

Most seasonal patterns of crawlers did not show well-defined spring, summer and autumn peaks (Figure A1-5). A broad crawler peak was found between mid-spring and early-summer at the two monitoring sites in the Riverina in 2013. The same period also had elevated crawler catches at one or more monitoring sites in the Riverina in 2015 and 2017 and at the monitoring sites in the Sunraysia in 2016 and 2017. However, the elevated crawler catches did not show up as well-defined, standalone peaks. In addition, a distinct crawler peak was found in mid-summer in 2016 at one of the three monitoring sites in the Riverina. Elsewhere, elevated crawler catches were found in autumn 2014 and 2017, between late summer and late autumn in 2016 and in late summer in 2018. The locations and duration of the periods of the elevated crawler catches differed with monitoring sites. It is interesting to note that most of the elevated crawler catches lasted over two months.

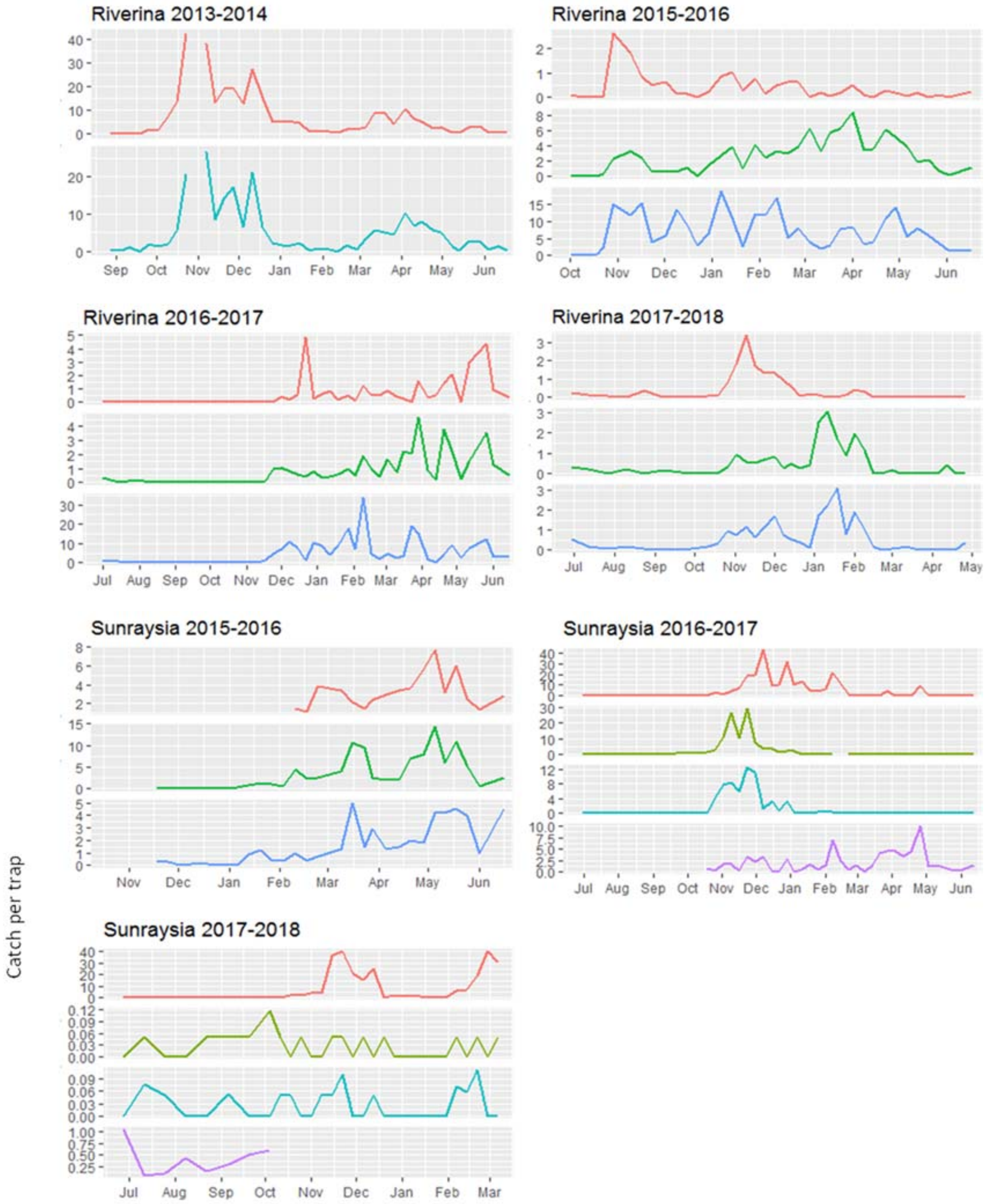

Figure A1-5. Seasonal patterns of red scale crawlers at monitoring sites in the Riverina and Sunraysia. Patterns at different monitoring sites in the same region and season are indicated by different colours.

Crawler catches were significantly correlated between some of the sites in the Riverina in all three monitoring seasons and between some of the sites in the Sunraysia in the 2015/16 and 2016/17 monitoring seasons ($P < 0.05$) (Table A1-4). Where a significant correlation was found, the correlation coefficient was positive, indicating a positive association between catches at the two sites being compared.

Table A1-4. Site correlations in crawler catches estimated using Pearson's product moment correlation coefficient. Only sites with the same data periods were compared.

Region	Season	Comparison	Correlation	*t*	*D.F.*	*P*
Riverina	2013/14	Site Y1 ~ Site Y2	0.94	16.89	39	< 0.0001
	2015/16	Site R1 ~ Site R2	0.12	0.68	34	0.5000
		Site R1 ~ Site R3	0.56	3.99	34	0.0003
		Site R2 ~ Site R3	0.33	2.03	34	0.0507
	2016/17	Site R1 ~ Site R2	0.51	3.71	40	0.0006
		Site R1 ~ Site R3	0.25	1.66	40	0.1051
		Site R2 ~ Site R3	0.55	4.13	40	0.0001
	2017/18	Site R1 ~ Site R2	0.12	0.73	35	0.4702
		Site R1 ~ Site R3	0.28	1.73	35	0.0918
		Site R2 ~ Site R3	0.85	9.74	35	< 0.0001
Sunraysia	2015/16	Site S2 ~ Site S3	0.75	5.90	27	< 0.0001
	2016/17	Site S1 ~ Site S2	0.26	1.64	38	0.1087
		Site S1 ~ Site S3	0.35	2.36	39	0.0231
		Site S2 ~ Site S3	0.87	10.92	38	< 0.0001
	2017/18	Site S1 ~ Site S2	0.04	0.22	29	0.8293
		Site S1 ~ Site S3	0.24	1.33	29	0.1940
		Site S2 ~ Site S3	0.21	1.14	29	0.2642

Similar to the male flight patterns, three broad peaks of crawler catches were also detected in the plot of normalised mean catches and DD (Figure A1-6). The second and third peaks were not as clear as the first one and can be considered as a single peak. The three peaks were found between 300 and 1,000 DD, 1,200 and 1,700 DD and 2,000 and 2,500 DD, peaking at 615, 1,444 and 2,280 DD respectively (Table A1-5). The DD range of the first peak region corresponded to the monthly interval of November–December, the same as the first crawler peak shown in the individual seasonal patterns. Contrary to male flight, normalised mean crawler catch was highest in the first peak period and lowest in the third peak period (Table A1-5).

A total of 44 Individual crawler peaks were detected in the seasonal patterns across all sites and seasons. After removing those from partial monitoring seasons and those that were too small (< 1 per trap), 36 peaks remained. These were concentrated over the DD intervals of 500–900 DD and 2,100–2,450 DD (Figure A1-7). A series of less concentrated crawler peaks were also found between the two groups of peaks (1,150–1,950 DD). Locations of the three groups of crawler peaks corresponded to the periods of late spring to early summer, mid-summer to early autumn and autumn respectively. For simplicity, the three groups of crawler peaks will be referred to hereafter as spring, summer and autumn peaks. The mean DDs of the three groups of crawler peaks were 657, 1,517 and 2,285 DD, respectively. Duration of the peaks was 455 DD in spring, 443 DD in summer and 277 DD in autumn. Mean DDs of the spring, summer and autumn crawler peaks lagged behind the corresponding male peaks by 347, 387 and 95 DD, respectively.

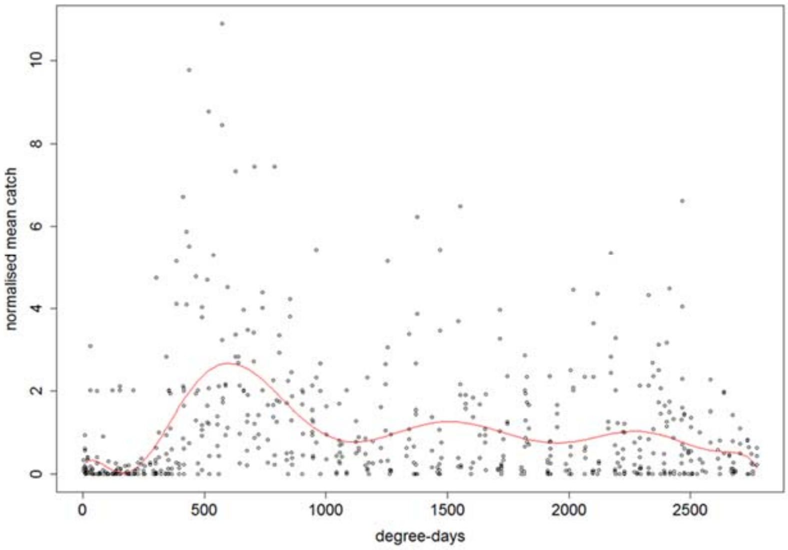

Figure A1-6. Normalised mean catches of red scale crawlers by degree days across all monitoring sites and seasons. The red line was obtained by fitting the observed data points to a polynomial function. It draws an outline of the three periods in a season when male catches were relatively high.

Table A1-5. Degree day (DD) range, date range, normalised mean catch and weighted mean DD in the pooled seasonal patterns of crawlers.

Peak ID	DD range	Date range	Mean catch	Peak DD
1	300–1,000	19 Oct–17 Nov	1.9	615
2	1,200–1,700	10 Jan–16 Feb	1.2	1444
3	2,000–2,500	21 Feb–15 Jun	0.9	2280

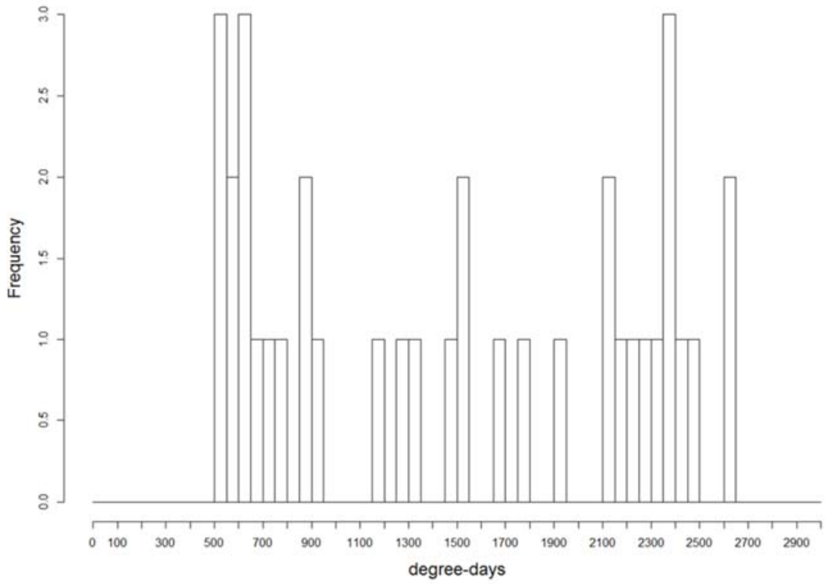

Figure A1-7. Distribution of individual crawler peaks over degree days.

Crawlers were caught in all months of the year (Figure A1-8). Significantly higher catches occurred in November compared with other months (*P < 0.05*). June to September had the lowest catches and December to May had intermediate catches. The highest numbers of male flight peaks were found in November, December and April. No crawler peaks were found in June, July or August.

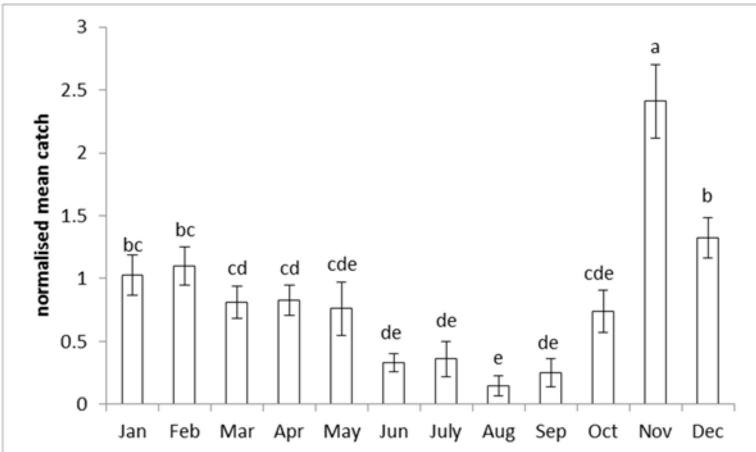

Figure A1-8. Normalised mean catches of red scale crawlers by month. Bars labelled with no matching letter(s) are significantly different at P = 0.05 by Fisher's LSD test following the detection of a significant treatment (month) effect by ANOVA. Wire bars show standard errors.

Discussion

Seasonal patterns of male flight and crawler catches in the Riverina and Sunraysia showed three periods of relatively high catches. This tri-modal pattern was also seen in the distribution of individual male flight peaks, with a concentration of peaks inside the same DD intervals. Crawler peaks were concentrated over two DD intervals, but a less concentrated group of crawler peaks were also present between the two DD intervals. For male flights, the three concentrated peaks occurred in spring, summer and autumn, respectively. The three groups of crawler peaks occurred during the periods of late spring to early summer, mid-summer to early autumn and autumn. Pooled data of male flight and crawler catches revealed three broad peaks. The tri-modal pattern of the seasonal patterns of red scale male and crawler catches suggest the presence of three annual generations of red scale populations at the monitoring sites. Dao (2012) reported three annual generations for red scale populations on the central coast of NSW. Annual accumulated DD at the monitoring sites during the data periods ranged from 2,499 to 2,884 DD, which was similar to that in Sydney. The estimated DD requirement for a red scale generation is 577–615 DD (Kennett and Hoffmann 1985, Grout and Richards 1989, Campos-Rivela et al. 2012). Based on this generational DD requirement, red scale can complete at least four generations per year at the monitoring sites. Model simulations from this project showed three major annual peaks for crawlers, with another small crawler peak visible in late winter. This suggests the existence of four annual generations, although the population size in the fourth generation is relatively small compared with that in the other generations. This is perhaps why it was not detection in the observed seasonal patterns (Appendix-3).

Crawler peaks tend to be less prominent than male flight peaks. This is likely due to the different efficiencies of the male traps and crawler traps. Male traps use pheromone lures to actively attract and trap the males, whereas the crawler traps are passive traps, which rely on crawlers randomly walking up to the sticky tapes to trap them.

Individual seasonal patterns of adult males and crawlers varied considerably between monitoring sites and seasons ranging from small, isolated peaks to broad, merged peaks. Several reasons can explain the variations. First, the monitoring sites were located in commercial citrus blocks. Insecticides may have been used more often at some sites in some seasons than in others in other seasons. Secondly, age structures of red scale populations possibly varied at the different sites and in different seasons due to variations in individual development rates, random mortalities, management operations and/or extreme temperatures.

The gradual increase in the duration of male flight peaks from spring to autumn is an indication of red scale populations generations become increasingly overlapped as the season progresses. The overlapping trend is likely responsible for the broad and less well-defined peaks observed late in the season.

For growers who do not use pheromone traps to monitor red scale, the monthly chart of crawler activities provides a general guide on the times when crawlers are likely to be most abundant. Generally, crawlers will be most abundant in November and least abundant in the winter months. Moderate and similar numbers of crawlers are likely to be present in the other months. The November crawler peak is likely to be the result of reproduction by adults of the overwintering generation, which have matured a month before, as seen in the male flight peak in October. After a relatively quiet period from May to August, male flight starts to pick up in September and increases again in October, signalling the maturing of the overwintering generation. Relatively low numbers of males and crawlers in winter are most likely due to low temperatures, which slow red scale development, cause additional crawler mortality and suppress male flight.

Despite considerable variations, red scale populations at different sites in the same region have shown a tendency to rise and fall around a similar time, as indicated by the detections of significant site correlations in male and crawler catches. Although the synchrony levels do not appear to be high, they provide the basis for area-wide predictions of red scale phenology using degree days.

Appendix-2 Prediction of male flight peaks and crawler peaks

Materials and Methods

Pooled seasonal patterns of red scale adult males and crawlers showed three periods of relatively high catches (Appendix-1). For adult males, the three periods were in spring, summer and autumn respectively. The three peak periods for crawlers were slightly behind those of the adult males. Predicting the exact time for an individual red scale adult male or crawler peak is difficult due to site and seasonal variations (Grout and Richards 1989). A more realistic approach is to predict the time period when a peak is likely to occur. Given a wide enough time interval, any peaks can be predicted with confidence; however such predictions may be impractical because the predicted time interval is too wide. By contrast, a small interval containing the target peak provides more information about the location of the peak but is more difficult to estimate. A compromise is to find the smallest time interval when a target peak can be predicted with a desired level of confidence (smallest effective prediction intervals).

A series of candidate DD intervals were tested in the predictions of male and crawler peaks in spring, summer and autumn. The upper limit of a candidate DD interval was the mean DD plus a fixed DD width and the lower limit was the mean DD minus the same fixed DD width. Fixed DD widths tested were 25, 50, 75 and 100 DD. The DD intervals were tested on catch data from individual sites and seasons to see how well they predicted the locations of spring, summer and autumn peaks.

A red scale male or crawler peak usually spans multiple dates or DD points (Figure A2-1). A peak was considered to have been correctly predicted if either its peak DD or weighted peak DD was inside a candidate DD interval (criterion-1), or any part of the peak was inside the candidate DD interval (criterion-2). The proportion of correct predictions by each of the candidate DD intervals using the two criteria were calculated. The smallest DD intervals achieving a correct prediction rate of 75% or higher were considered as the best DD intervals for prediction.

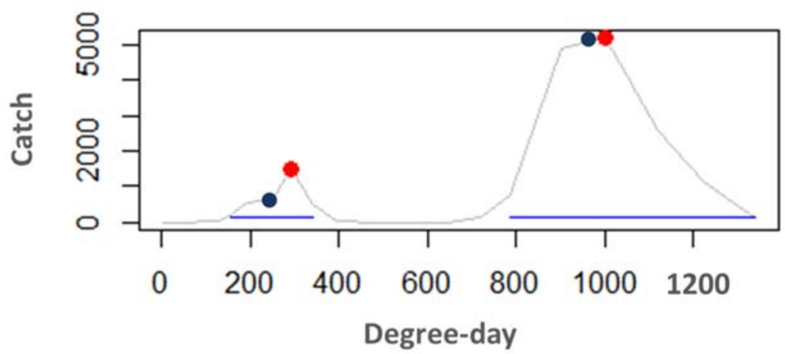

Figure A2-1. Seasonal pattern red scale male catches at one monitoring site in one season showing the peak position (highest point, shown as red dots), weighted peak position (shown as blue dots) and span of the peak (shown as blue lines).

To predict a crawler peak by a preceding male peak, the mean DD gaps between male flight peaks and the matching crawler peaks were calculated for spring, summer and autumn. A male flight peak and a crawler peak were considered to be matched if they were both from the same site, year and season of the year. Where more than one male or crawler peak were present in the same season and at the same site, the largest male or crawler peaks were used in calculating the DD gap. After the mean DD gap was determined, a series of candidate DD intervals were tested in the predictions of crawler peaks. The lower limit of a candidate interval was: male peak DD + mean DD gap - a fixed DD width. The upper limit of a candidate interval was male peak DD + means DD gap + a fixed DD width. Fixed DD widths tested were 25, 50, 75 and 100 DD. The proportions of correct predictions of crawler peaks were made using criterion-1.

To test the validity of the prediction methods described above, data collected in the 2013/14, 2015/16 and 2016/17 seasons were used to estimate the mean DD positions of the spring, summer and autumn peaks and data collected in the 2017/18 season were used for validation.

Results

Male peaks by DD

Spring male peaks were predicted at a correct rate of 88% by the 100 DD interval of the mean DD (mean DD ± 100 DD) using criterion-1 (Table A2-1). Using criterion-2, the correct prediction rate ranged from 88% by the smallest DD interval (mean DD ± 25 DD) and 100% by the two larger DD intervals (mean DD ± 75 DD and mean DD ± 100 DD). Summer and autumn peaks can also be predicted but not as accurately as spring peaks. The highest correct prediction rate achieved for summer peaks was 79% by criterion-1 and 95% by criterion-2. However, the width of the smallest DD interval achieving a correct prediction rate of 75% or higher by both criteria was the same as that for spring peaks (mean DD ± 100 DD). None of the four DD intervals tested achieved a correct prediction rate of 75% or higher for autumn peaks by criterion-1, however, even the smallest interval (mean DD ± 25 DD) achieved a correct prediction rate of 94% for autumn peaks using criterion-2.

Table A2-1. Percentages of spring, summer and autumn male peaks correctly predicted by DD intervals of their mean DDs.

					% Correct	
Peak group	N [1]	Mean DD	±	DD interval	Criterion-1 [2]	Criterion-2 [3]
Spring	16	278	25	253–303	31%	88%
			50	228–328	50%	94%
			75	203–353	69%	100%
			100	178–378	88%	100%
Summer	19	1,127	25	1,102–1,152	32%	74%
			50	1,077–1,177	42%	79%
			75	1,052–1,202	68%	95%
			100	1,027–1,227	79%	95%
Autumn	17	2,171	25	2,146–2,196	29%	94%
			50	2,121–2,221	47%	94%
			75	2,096–2,246	59%	94%
			100	2,071–2,271	65%	94%

[1] N is the number of male peaks

[2] Criterion-1: a peak was considered to have been correctly predicted if either its peak DD or weighted peak DD was inside a target DD interval

[3] Criterion-2: a peak was considered to have been correctly predicted if any part of the peak was inside a target DD interval.

Crawler peaks by DD

With a best prediction rate of 59%, none of the four DD intervals tested satisfactorily predicted the crawler peaks in spring, summer or autumn using criterion-1 (Table A2-2). However, satisfactory predictions were achieved for all seasonal crawler peaks using criterion-2. The correct prediction rate was 80–100% for spring crawler peaks and 94% for autumn crawler peaks. Summer crawler peaks were also predicted at a correct rate of 80% by the two larger DD intervals (mean DD ± 75 or 100 DD) using criterion-2.

Table A2-2. Percentages of spring, summer and autumn crawler peaks correctly predicted by DD intervals of their mean DDs.

					% Correct	
Peak group	N	Mean DD	±	DD interval	Criterion-1 [1]	Criterion-2 [2]
Spring	10	657	25	632–682	30%	80%
			50	607–707	30%	80%
			75	582–732	40%	100%
			100	557–757	50%	100%
Summer	10	1,517	25	1,492–1,542	20%	50%
			50	1,467–1,567	30%	70%
			75	1,442–1,592	40%	80%
			100	1,417–1,617	40%	80%
Autumn	12	2,285	25	2,146–2,196	29%	94%
			50	2,121–2,221	47%	94%
			75	2,096–2,246	59%	94%
			100	2,071–2,271	59%	94%

[1] N is the number of crawler peaks. [2] Criterion-1: a peak was considered to have been correctly predicted if either its peak DD or weighted peak DD was inside a target DD interval. [3] Criterion-2: a peak was considered to have been correctly predicted if any part of the peak was inside a target DD interval.

Crawler peaks by preceding male peaks

The mean DD gaps between male peaks and crawler peaks were 347, 387 and 120 DD, respectively, for spring, summer and autumn peaks (Table A2-3). Spring crawler peaks were correctly predicted by 94% of the preceding spring male peaks using the DD gap interval of 272–422 DD (347 ± 75 DD) or 247–447 DD (347 ± 100 DD). Summer and autumn crawler peaks could not be satisfactorily predicted by the corresponding male peaks using any of the DD gap intervals tested.

Table A2-3. Percentages of correct predictions of crawler peaks by DD intervals of the mean DD gaps from a male flight peak to the following crawler peak.

Peak group	N [1]	Mean DD gap	±	Target gap [2]	Correct % [3]
Spring	16	347	25	322–372	25%
			50	297–397	44%
			75	272–422	94%
			100	247–447	94%
Summer	19	387	25	362–412	26%
			50	337–437	32%
			75	312–462	37%
			100	287–487	37%
Autumn	17	120	25	95–145	6%
			50	70–170	29%
			75	45–195	35%
			100	20–220	53%

[1] N is the number of male peaks. [2] The test interval for a crawler peak was the target gap plus the peak DD of the preceding male peak (e.g. if the target gap is 322–372 and the male peak DD is 250, then the test interval is 572–622). [3] Based on criterion-1.

Validation

The mean DDs of spring, summer and autumn male peaks estimated from data collected in the 2013/14, 2015/16 and 2016/17 seasons were 269, 1,124 and 2,152 DD, respectively. The estimated DDs for spring, summer and crawler peaks were 638, 1,577 and 2,301 DD, respectively. Both sets of mean DDs were close to those estimated from the full datasets (Table A2-1 and Table A2-2). The validation data (2017/18 data) had four male peaks and two crawler peaks in spring, five males peaks and three crawler peaks in summer and five male peaks and zero crawler peaks in autumn. Satisfactory predictions of the male and crawler peaks from the validation data were made using fixed DD intervals and the second criterion for correct predictions. All four spring male peaks were correctly predicted by a 50 DD interval around the mean spring male DD (219–319 DD). Both spring crawler peaks were correctly predicted by a 25 DD interval around the mean spring crawler DD (613–663 DD). Four of the five summer male peaks and all five autumn male peaks were correctly predicted by 25 DD intervals around summer and autumn mean DDs (summer: 1,099–1,149 DD; autumn: 2,127–2,177 DD). Two of the three summer crawler peaks were correctly predicted by a 25 DD interval around the mean summer crawler DD (1,552–1,602 DD).

Three of the four spring male peaks in the validation data correctly predicted the presence of follow-up crawler peaks by the DD gap interval of 262–412 DD (337 ± 75 DD) using the first criterion. Summer crawler peaks were not satisfactorily predicted by the corresponding male peaks in the validation data using any of the DD gap intervals tested (correction rate < 75%).

No autumn crawler peaks were available for validating in the validation data.

Discussion

On any day of the year, red scale of different developmental stages are found on the same leaf and same fruit. However, there are periods when individuals of certain stages are relatively more abundant and periods when these individuals are relatively scarce (Kennet and Hoffman 1985; Campos-Rivela et al. 2012; Dao 2012). Predicting the exact time when individuals of a target stage will peak is difficult due to site and seasonal variations and multi-cohort structures in red scale populations (Grout and Richards 1989). A more realistic approach is to predict the time period when the target peak is likely to occur. Given a wide enough time interval, any peaks can be predicted with confidence; however such predictions may be impractical because the predicted time interval is too wide. By contrast, a small interval containing the target peak provides more information about the location of the peak but is more difficult to estimate. A compromise is to find the smallest time intervals when the target peak can be predicted with a desired level of confidence (smallest effective prediction intervals). In this study, various DD intervals from 50 to 200 DD were tested in the predictions of red scale crawler and male peaks in spring, summer and autumn. The target level of confidence was 75% correct predictions. Two criteria were used to judge if a peak was or was not correctly predicted. The first criterion for a correct prediction was that either the highest point of the peak period or the weighted mean position of the peak period was inside the test DD interval. The second criterion was more relaxed. A peak was considered to have been correctly predicted if any part of the peak was inside the test DD interval.

Using the more stringent first criterion, effective prediction intervals were found for male peaks in spring and summer but not for other peaks. Both intervals were 200 DD wide. Using the more relaxed second criterion, effective prediction intervals were found for all peaks. The smallest intervals were 50 DD for male peaks in spring, male peaks in autumn, crawler peaks in spring and crawler peaks in autumn, 100 DD wide for male peaks in summer and 150 DD wide for crawler peaks in autumn. The highest correct prediction rate was 100%.

The DD gaps for spring (347 DD) and summer peaks (387 DD) are close to that used in the Californian degree-day calculator for red scale (305 DD) (KAC Citrus Entomology 2018). The DD gap for autumn peaks (120 DD) is close to that estimated by Campos-Rivela et al. (2012) (184 DD). Spring crawler peaks were correctly predicted by 94% of the preceding spring male peaks using a 150 DD gap interval between male and crawler DD. Crawler peaks in summer and autumn could not be predicted with the minimal accuracy of 75% by the corresponding male peaks using any of the DD gap intervals tested.

Validation results confirmed the validity of using fixed DD intervals and DD gap intervals to predict male and crawler peaks.

In summary, male and crawler peaks can be predicted with a minimal accuracy of 75% by using DD intervals of 50 to 200 DD. Spring peaks can be predicted with smaller intervals than summer and autumn peaks. Male peaks can be predicted with smaller intervals than crawler peaks. For both males and crawlers in all seasons, DD intervals of 150 DD centred on the mean DDs for the target stage and season, were sufficient for the predictions. A 150 DD interval equates to 18 days at 20 °C, which is not excessively long considering the large variations in red scale seasonal patterns. The predictions may not predict the exact points of time when a peak occurs but are likely to cover a significant part of the peak period.

Appendix-3 Population model

Materials and Methods

A temperature-driven, multi-cohort population model that simulates the development process of individual scales in a population was developed. The purpose of the model was not to replicate individual observed seasonal patterns, but rather to investigate the underlying mechanisms for the observed patterns. Model parameters included stage-specific threshold temperatures for development, production of crawlers, emergence of adult males, stage-specific DD requirement, stage-specific mortality rates due to predation, parasitism and extreme temperatures and fecundity. Where available, published data were used to estimate the model parameters. Daily temperature data from the Bureau of Meteorology were used to drive the model process.

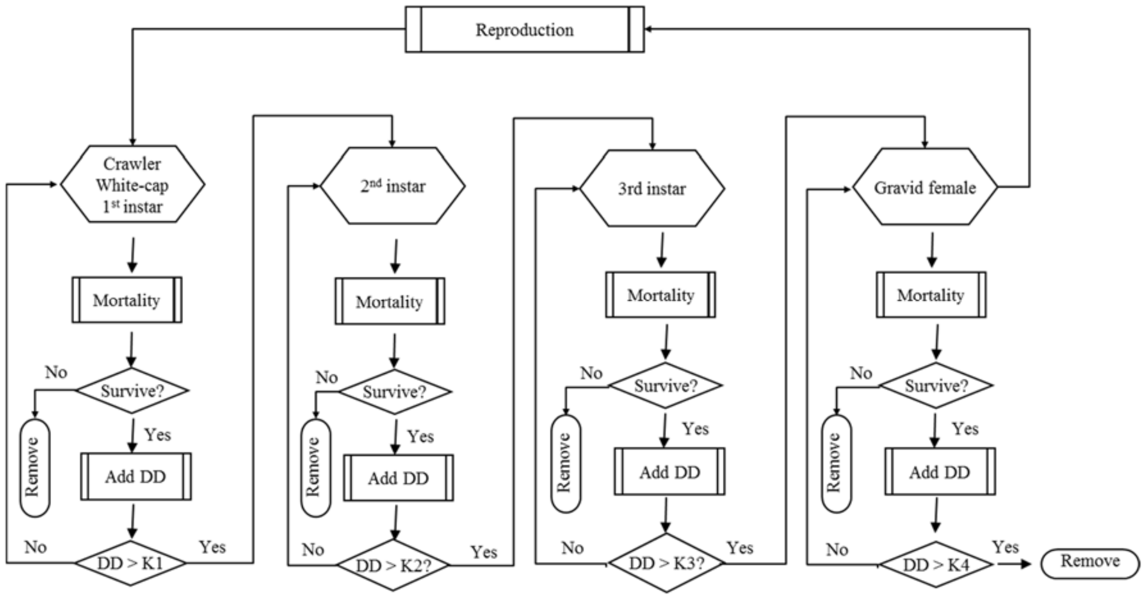

Figure A3-1. A diagram of the model structure for female scales.

The female life cycle was split into the first instar (crawler birth to completion of first the moult), second instar (completion of the first moult to completion of the second moult), third instar (completion of the second moult to mature females) and reproductive females. The male life cycle was split into the first instar (crawler birth to completion of first the moult), second instar (completion of the first moult to adult male emergence) and adult males. Red scale sex differentiation starts in the second instar (Smith et al. 1997). For convenience of tracking the development of individual male and female scales, sex differentiation was made to start on the day of crawler birth. A diagram of the model structure for female scales is shown in Figure A3-1. The model structure for male scales is similar to this except there are fewer stages and reproduction is replaced by male flight. The model tracks the daily development and survival of individual scales. At the end of each day, an individual is first checked to determine if it has survived the current stage. If it has, then the model will check to see if it has accumulated sufficient DD to advance to the next stage. DDs are accumulated daily for each individual, starting from the date of winter solstice. A mature female produces crawlers daily at a given rate throughout its life. The lifespan is determined by whether or not the female has accumulated a given number of DD. Crawlers only live for one day. Model outputs are produced after 100 simulations, at the end of which the average number of crawlers, adult male scales (representing male flight activity) and virgin females is estimated.

DD requirements for completion of stage-specific development of red scale were estimated based on data from Yu and Luck (1988). The estimated requirements for the first, second and third instar females were 175, 180 and 313 DD respectively and that for the completion of the combined pre-pupa and pupa stages of males was 209 DD. DD requirements for first instar males was the same as that for the first instar females as males and females are inseparable at this stage. Adult females were set to die after 200 DD and adult males after 100 DD. DD accumulation started at the winter solstice with the upper threshold temperature set at 38°C and the lower threshold temperature at 11.7°C. Variability in individual development rate was investigated by treating the DD requirement as a normally distributed random variable with a standard deviation equal to 10% of the mean DD requirement.

Individuals of all stages were subjected to random daily predation rates with a mean of 0.06 and a standard deviation of 0.03. This predation rate was chosen to ensure simulated populations do not either crash to extinction or soar into an infinite size. An extra daily mortality rate of 0.5 was introduced to crawlers to account for the high rate of failure to settle (Willard 1973, Zhao 1987). Temperature extremes can cause additional mortality in red scale (Morse et al. 1985). This effect was investigated in some model simulations by introducing a daily mortality rate of 0.25 when the temperature was ≥ 40 °C or ≤ 0 °C.

Fecundity was modelled as a function of temperature according to Willard (1972):

$$F = 4.2843 - 0.8497T + 0.0506T^2 - 0.0008T^3 \qquad \text{(Equation A3-1)}$$

Where F is the number of crawlers produced per female per day and T is temperature.

The proportion of females was set at 0.3 (Stofberg 1937).

Results

Initial age structure

The age structure of the red scale population at winter solstice (initial age structure) influences the simulated seasonal patterns. While the initial age structure of males affected only the positions of the first post-winter male peaks, the initial age structure of females affected the positions of both crawler peaks and male flight peaks. Observations at the winter solstice showed red scale at various developmental stages in citrus trees in Yanco, NSW. As such, the investigation focused on multi-cohort age structures for the female population. The initial age structure for the male population was fixed to equal proportions of first instar scales and scales in other immature males (second instar, pre-pupa and pupa).

All two-cohort age structures of female scales produced seasonal patterns with more or less discrete groups of peaks. When the initial age structure for the female population was set to equal proportions of first instar scales and gravid females, three major crawler peaks were clearly seen in the simulated seasonal pattern (Figure A3-2, top). These peaks were produced respectively by the first, second and third post-winter generations. A small crawler peak produced by the overwintering generation was also visible. The simulated seasonal pattern for adult males also showed three major peaks. The first peak consisted of two sub-peaks produced by the overwintering generation. The second and third peaks were produced by the first and second post-winter generations respectively. In terms of the locations of the peaks, the simulated crawler peaks matched nicely with the observed seasonal patterns, with all simulated peaks occurring entirely within the DD ranges of the observed peak periods for crawlers (Figure A3-2). For adult males, the second simulated major peak occurred entirely within the DD range of the second observed peak period but the first and third major peaks were only partially inside the observed peak periods. Similar simulated patterns were obtained when the initial female age structure was set to equal proportions of first and second instars.

Other 2-cohort initial female age structures produced seasonal patterns that were less well matched by the observed seasonal patterns; some had peaks outside the DD ranges of the observed peak periods. A 3-cohort initial female age structure of equal proportions of first and second instars and gravid females produced similar patterns as the 2-cohort age structure of first instar scale and gravid females except that the simulated third crawler peak and the second adult male peak were only partially inside the observed peak periods (Figure A3-2, middle). The 4-cohort initial female age structure produced seasonal patterns with flat, merged peaks (Figure A3-2, bottom).

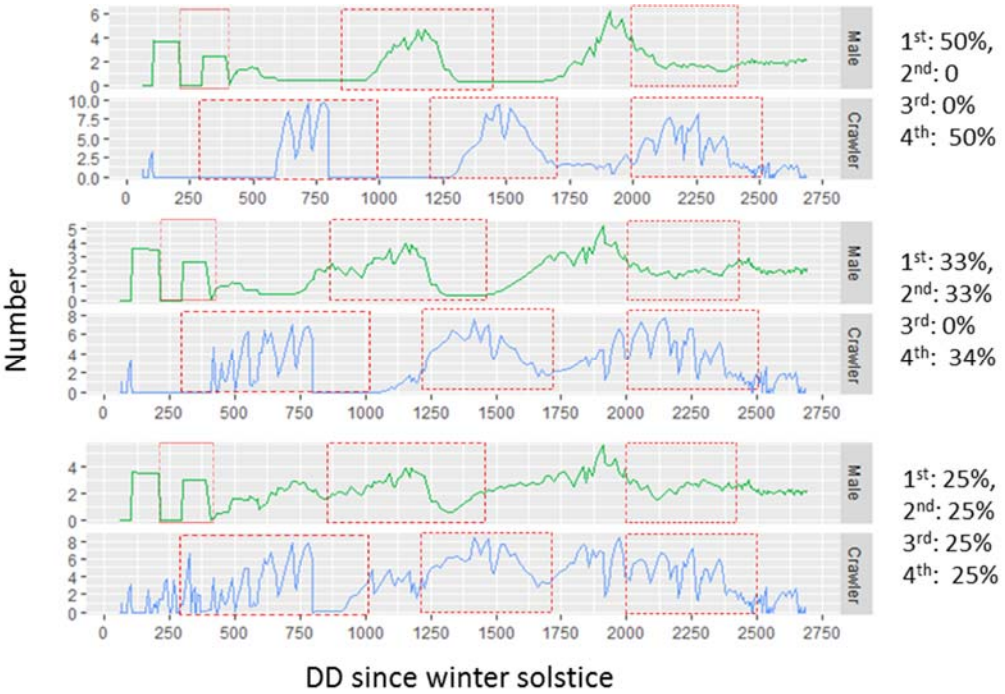

Figure A3-2. Simulated seasonal patterns of red scale adult males and crawlers assuming a 2-cohort initial age structure of equal proportions of gravid females and first instars, a 3-cohort initial age structure of equal proportions of first and second instars and gravid females and a 4-cohort initial age structure of equal proportions of first, second and third instars and gravid females for the female population. Daily temperatures in Yanco, NSW, in 2013/14 were used in the simulations. The initial age structure for the male population was set to equal proportions of first instars and other immature stages (second instar, pre-pupa and pupa). Dashed boxes show DD intervals for periods of relatively high catches in the observed seasonal patterns.

Effects of variations in individual development rate

Due to inherent genetic variations and differences in microclimates, not all individuals develop at the same rate. To show the effect of variations in individual development rates, random variation was introduced to the mean DD requirement for each developmental stage. Compared with the baseline pattern produced by the 2-cohort initial female age structure of equal proportion of first instar scales and gravid females, the introduction of 10% variation in DD requirements resulted in merging of neighbouring peaks with no distinct peaks after 750 DD (Figure A3-3).

Effects of extreme temperatures

In addition to its effects on red scale development, extreme temperatures can cause additional mortality to red scale. Adult males might only fly when the temperature is above a minimum threshold temperature. With the introduction of an extra daily mortality rate of 0.25 for red scales of all stages when temperatures were ≥ 40 °C, an extra daily mortality rate of 0.25 for crawlers when temperatures were ≤ 0 °C and a restriction of male flight to temperatures ≥ 12 °C, the adult male peaks produced by the overwintering generation disappeared (Figure A3-3). The size of the third major crawler peak was also greatly reduced, leaving the seasonal pattern with only two major peaks.

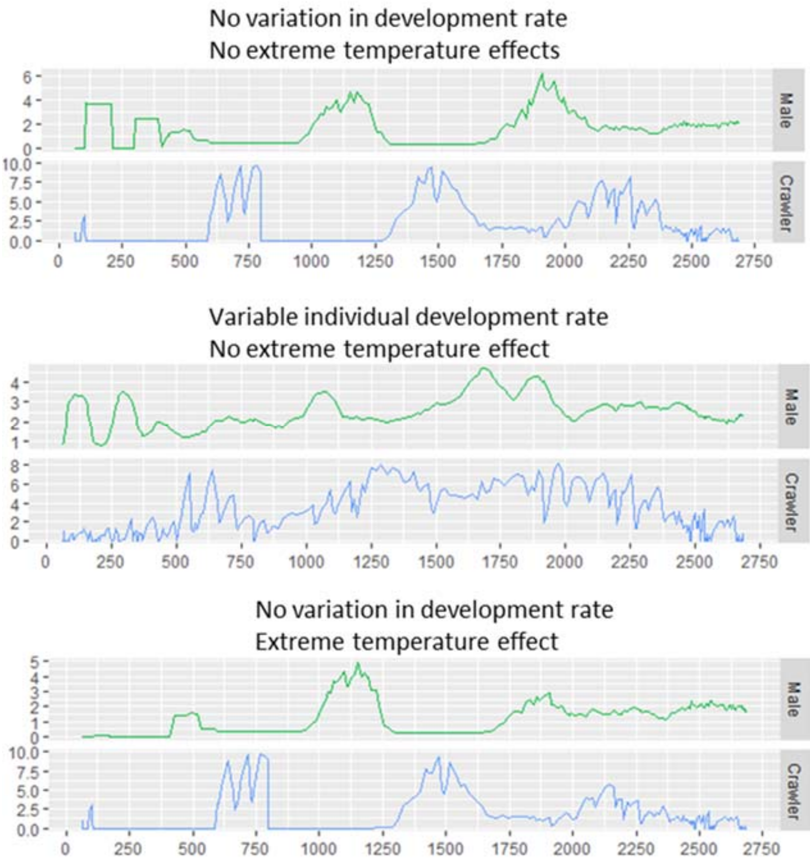

Figure A3-3. Simulated seasonal patterns of red scale adult males and crawlers assuming a 2-cohort initial age structure of equal proportions of gravid females and first instars for the female populations, with no variations in individual development rate and no extreme temperature effects (top), random variations in individual development rate but no extreme temperature effects (middle) and random variations in individual development rate plus extreme temperature effects (bottom). Daily temperatures in Yanco, NSW, in 2013/14 were used in the simulations. The initial age structure for the male population was set to equal proportions of first instars and other immature stages (second instar, pre-pupa and pupa).

Seasonal patterns of virgin female scales

For reproduction success, red scale adult males should peak when virgin female scales are abundant. The synchrony between the seasonal patterns of adult males and virgin female scales are clearly seen in the simulated seasonal patterns (Figure A3-4).

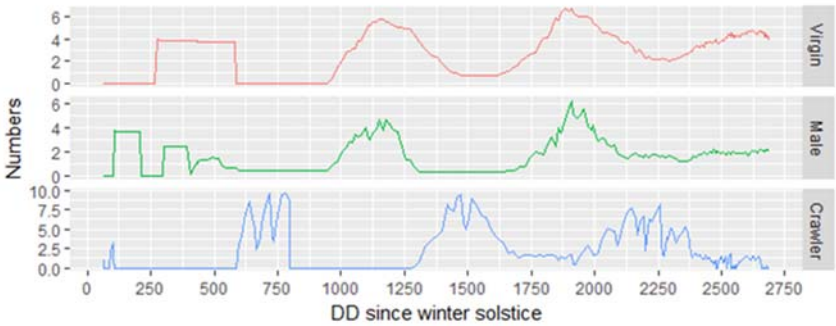

Figure A3-4. Simulated seasonal patterns of red scale virgin females, adult males and crawlers assuming a 2-cohort initial age structure of equal proportions of gravid females and first instars for the female population and a 2-cohort initial age structure of equal proportions of first instars and other immature stages (second instar, pre-pupa and pupa) for the male population. Daily temperatures in Yanco, NSW, in 2013/14 were used in the simulations. The initial age structure for the male population was set to equal proportions of first instars and other immature stages (second instar, pre-pupa and pupa).

Discussion

A temperature-driven process model has been developed to investigate the seasonal patterns of red scale populations in the southern citrus production regions of Australia. The model is flexible in that it allows for random variations in individual development rates, mortality rate and fecundity. The purpose of the model was not to replicate individual observed seasonal patterns, but rather to investigate the underlying mechanisms for the observed patterns.

A variety of seasonal patterns can be produced by the model, ranging from those with discrete peaks to those with merged peaks. The initial age structure of female scales influences the simulated seasonal patterns, with 2-cohort age structures producing discrete generation peaks and 4-cohort age structures producing flat and merged peaks, indicating generation overlap. These results are not surprising as more age groups in the starting population means earlier overlapping of individuals of the same age from different generations. Grout and Richards (1989) reported multi-cohort age structures for red scale populations in South Africa. Multi-cohort age structures are likely true for Australian red scale populations considering similar climates in the two countries. In addition to the initial age structures, model simulations showed that variations in individual development rates can also result in highly merged peaks. Highly merged peaks were also seen in some of the observed seasonal patterns particularly in crawlers (Appendix-1).

Simulated seasonal patterns with discrete peaks showed three annual peaks. The three annual peaks were produced respectively by the by the overwintering, first and second post-winter generations. The results matched the observed seasonal patterns of male and crawler catches, where three annual peak periods were visible (Appendix-1). This supports the findings of Dao (2012), who reported three annual generations of red scale populations on the NSW central coast. However, the southern citrus production regions and NSW central coast both have enough heat units for red scale to complete at least four annual generations (Appendix-1). So where is the missing fourth generation? Some of the simulated seasonal patterns have actually shown small crawler peaks in late winter in addition to the three major crawler peaks. It appears there is another red scale generation during late autumn and winter. This generation was not detected, probably because the population size is relatively small compared with the other generations. Population peaks from this generation may have also been partially hidden among those from the previous generation due to generation overlapping.

The age structure of female scales in the overwintering generation appears to be a key factor in shaping the seasonal patterns. With the 2-cohort age structure of gravid females and first instars, the model is capable of reproducing annual crawler peaks right where the observed annual peak periods for crawlers were found. It is possible that red scale populations in the southern citrus production regions mostly overwinter as gravid females and first instars. However, other overwintering age structures cannot be ruled out. Model simulations show that red scale seasonal patterns can be changed significantly by variations in the development rate of individual scales and extreme temperatures.

Finally, the model demonstrated the synchrony of adult male and virgin female peaks. This synchrony is needed for reproductive success of red scale before neighbouring generations start to overlap late in the season. For heavily overlapped generations, the synchrony is not as critical because virgin females will be present over an extended period of time.

Appendix-4 Timing trials

Materials and Methods

Three timing trials were conducted in blocks of Valencia orange trees in Leeton in the Riverina, one each in the 2015–16 and 2016–17 seasons and one in 2018. In each trial, four spray timings were compared with an unsprayed control in a completely randomised design with 5–6 replicates. A plot consisted of a central tree plus its four immediate neighbours (2015–16 and 2016–17 trials) or a single tree (2018 trial). The plots were selected such that the central trees in all plots within the same block all had similar levels of red scale infestation. Neighbouring plots in the same row were separated by at least one tree and in different rows by a minimum of one row. To reflect citrus industry's standard practice for red scale control, chlorpyrifos (50 mL/100 L) plus oil (1% Biopest) was used as the chemical spray. A single spray was applied to all five trees in each plot with a motorised boom sprayer at the water rate of 14 L/tree.

The timing treatments investigated were as follows:

1. 2015–16: 10, 16 and 23 November then 2 December
2. 2016–17: 3, 17 and 31 October then 14 November
3. 2018: 2 and 16 of May then 1 and 13 June.

Red scale infestation was checked in situ before and after sprays on randomly selected fruit and leaves. Sample sizes were 50 leaves and 10 fruit per plot in the pre-treatment assessment in the 2015–16 trial, 50 fruit per plot in the post-treatment assessment in the 2018 trial and 50 fruit and 50 leaves per plot in all the other assessments. When both young and mature fruit were present, the fruit sample was split into 25 young fruit and 25 mature fruit. Infestation level was recorded as either the presence or absence of red scales in each sample unit or the numbers of sample units infested by 0, 1–10 and > 10 scales.

Proportions of red scale infested fruit and leaves were analysed with respect to replication and spray timing by general linear model (GLM) followed by analysis of variance (ANOVA) (Venables and Ripley 2002). Proportional data were 'arcsine' transformed before being analysed. Where a significant timing effect was detected by ANOVA, mean infested proportions in different timing treatments were separated by Fisher's LSD test (Steel et al*.* 1997). The analyses were conducted in R (R Core Team 2012).

Results

2015/16 trial

This trial compared mixed sprays of chlorpyrifos (50 mL/100 L) and Biopest oil (0.5%) applied on 10, 16 and 23 November and 2 December 2015. Prior to the sprays, 60–80% of mature fruit and 10–30% of leaves were infested with red scale. There were no significant differences in the proportions of infested mature fruit ($F = 0.20$, $D.F. = 4, 16$, $P = 0.9353$) or leaves ($F = 0.92$, $D.F. = 4, 16$, $P = 0.4763$) among the four timing treatments and control. In the following March, similar proportions of mature fruit was infested by red scale in all plots ($F = 2.49$, $D.F. = 4, 16$, $P = 0.0846$). In the following June, 26–35% of young fruit in treated trees was infested with red scale and 59% in the control. The difference was significant (Figure A4-1). The proportion of young fruit infested by over 10 scale/fruit was significantly lower in plots sprayed on 10 November than in plots sprayed on 2 December (Figure A4-1). Infestation levels in mature fruit were similar in all timing treatments and control ($F = 1.89$, $D.F. = 4, 15$, $P = 0.1648$).

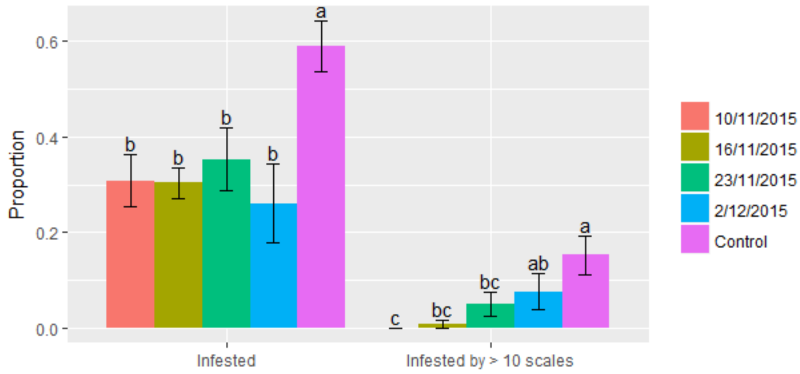

Figure A4-1. Mean proportions of young fruit infested by any number of red scales and by > 10 scale/fruit in the four timing treatments and control at the second post-spray assessment in the 2015–16 trial. Wire bars show the standard errors (SE). Columns in the same group with different letters are significantly different (*P < 0.05*).

2016/17 trial

This trial compared mixed sprays of chlorpyrifos (50 mL/100 L) and Biopest oil (0.5%) applied on 3, 17 and 31 October and 14 November 2016. Prior to the sprays, 30–44% of mature fruit and 6–16% of leaves were infested with red scale. There were no significant differences in the proportion of infested mature fruit (*F = 0.55, D.F. = 4, 16, P = 0.7056*) or leaves (*F = 1.23, D.F. = 4, 16, P = 0.3384*) among the four timing treatments and control. In the following May, significantly higher proportions of mature fruit were infested by red scale in the control than in any of the four timing treatments but the differences were not significant between any of the four timing treatments (Figure A4-2). Among those infested by over 10 scale/fruit, a significantly lower proportion was found in plots sprayed on 14 November than in the plots spayed on 3 and 17 October. In the following July, 24% of young fruit were infested by red scale in the plots sprayed on 14 November, which was significantly lower than those in plots sprayed on the other three dates (43–61%) and in the control plots (70%) (Figure A4-3). Plots sprayed on 31 October also had a significantly lower proportion of infested young fruit (43%) than those sprayed on 17 October (61%). Among those infested by over 10 scale/fruit, a significantly lower proportion was found in the plot sprayed on 14 November than in plots spayed on 3 and 17 October (Figure A4-3).

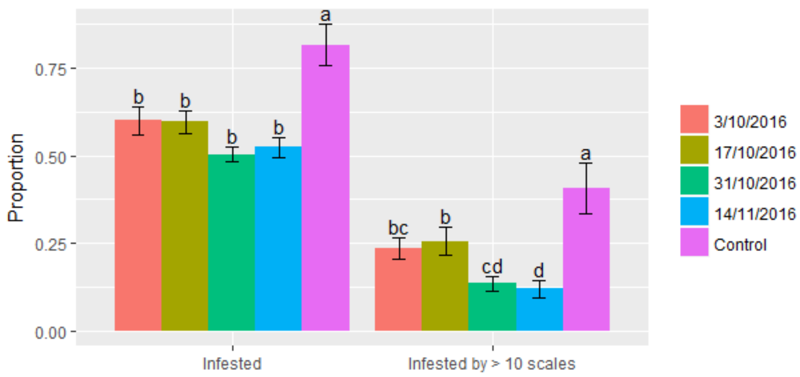

Figure A4-2. Mean proportions of mature fruit infested by any number of red scales and by > 10 scale/fruit in the four timing treatments and control at the first post-spray assessment in the 2016/17 trial. Wire bars show the standard errors (SE). Columns in the same group with different letters are significantly different (P < 0.05).

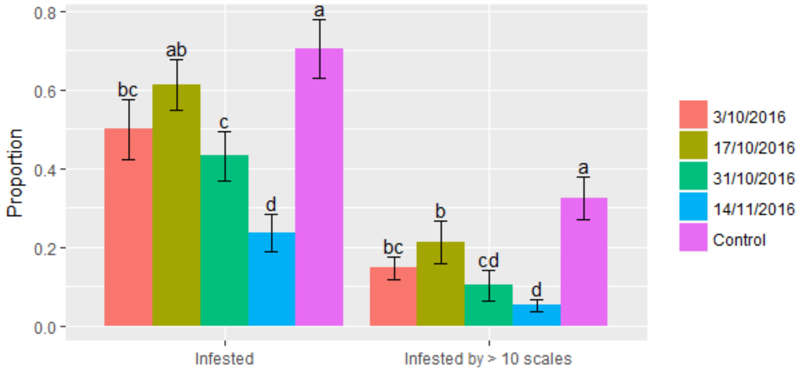

Figure A4-3. Mean proportions of young fruit infested by any number of red scales and by > 10 scale/fruit in the four timing treatments and control at the second post-spray assessment in the 2016/17 trial. Wire bars show the standard errors (SE). Columns in the same group with different letters are significantly different ($P < 0.05$).

2018 trial

This trial compared mixed sprays of chlorpyrifos (50 mL/100 L) and Biopest oil (0.5%) applied on 2 and 16 May and 1 and 13 June. Prior to spraying, 90–95% of mature fruit, 63–79% of young fruit and 66–82% of leaves were infested with red scale. There were no significant differences in the infested proportions (mature fruit: $F = 1.70$, $D.F. = 4, 16$, $P = 0.1991$; young fruit: $F = 1.62$, $D.F. = 4, 16$, $P = 0.2185$; leaf: $F = 1.62$, $D.F. = 4, 16$, $P = 0.2189$) among the four timing treatments and control. In mid-September, 50–68% of young fruit were infested with red scales. The infested proportions were not significantly different among the four timing treatments and control ($F = 2.62$, $D.F. = 4, 16$, $P = 0.0737$).

Discussion

Timing is important in red scale management as most chemical options for red scale control are only effective against young scales when their scale covers are not fully developed. Walker et al. (1990) compared a series of timings for chlorpyrifos sprays and noted 2-7 fold differences in proportions of infested fruit. To investigate possible timing effects in Australian conditions, three timing trials were conducted in this project using the industry standard chemical option of 'half rate' chlorpyrifos plus oil.

Spray timing showed a significant effect in red scale control in the 2015/16 trial and the 2016/17 trial. In both trials, mid-November sprays achieved better controls than sprays at other times in terms of red scale infestation in young fruit (current season fruit). In the 2015/16 trial, there was an over 8-fold difference in the proportion of young fruit infested by over 10 scale/fruit between the four timing treatments in June of the following year after the sprays. In the 2016/17 trial, there was a 4-fold difference in the proportion of young fruit infested by over 10 scale/fruit and a 2.5-fold difference in the proportion of young fruit infested by any number of scales between the four timing treatments in May of the following year after the sprays.

Monitoring results in the southern citrus production regions showed that red scale crawler numbers were higher in November than in other months (Appendix-1). Crawler density on the two mid-November spray dates in the 2015/16 trial was 11.8–15.2/tape, which was almost three times that on the other two spray dates (4.1–5.9/tape). Crawler density was relatively low on all four spray dates in the 2016/17 trial but was higher in mid-November (1.3/tape) than on the other three spray dates (< 0.0/tape). The results confirmed the benefit of targeting crawlers in red scale control. It is worth noting that while the mid-November sprays achieved the best control in both trials, optimal timing will differ from year to year due to different seasonal patterns of crawler numbers (Appendix-1). The optimal timing can be predicted with local temperature data (Appendix-2).

No timing effects were detected in the 2018 trial. Crawler data was not collected in the trial. Monitoring data collected in the region showed a trend for crawler peaks to become increasingly merged as the season progressed. This trial was conducted in autumn and it is likely crawler density was similar on all four spay dates in this trial.

Appendix-5 Timing guide

To help growers and pest consultants predict crawler and adult male peaks in spring, an online timing guide has been developed. The prediction tool is easy to use. Growers select the closest weather station and the prediction results will be shown (Figure A5-1). The prediction tool also provides monthly charts of crawler and adult male activity in the southern citrus production regions of Australia. The prediction tool can be found at https://redscale.shinyapps.io/predict/.

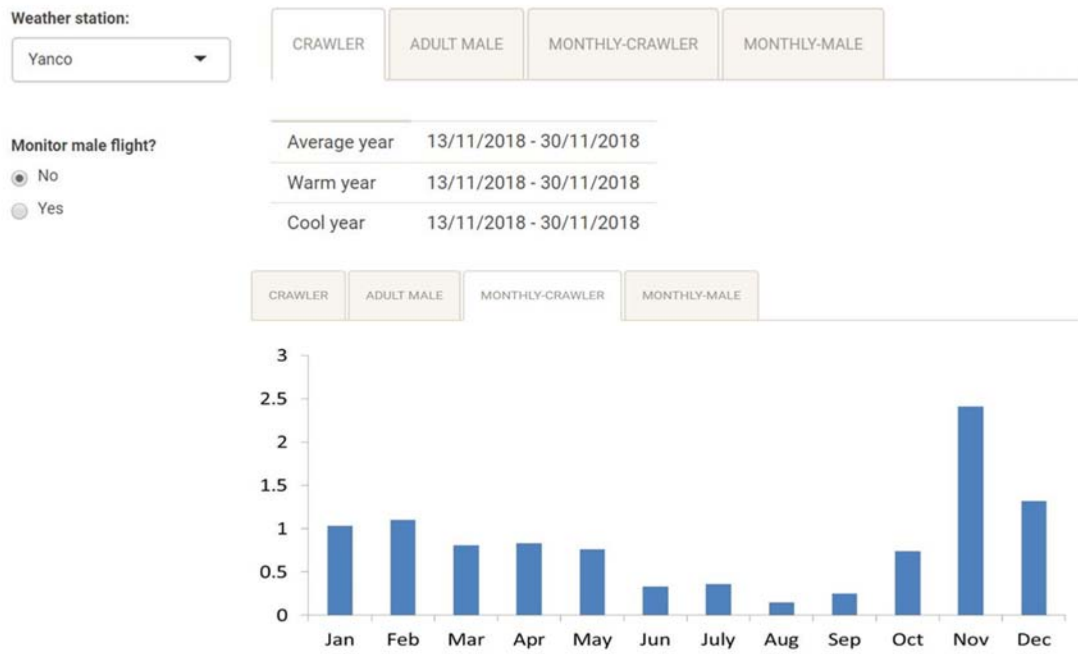

Figure A5-1. A screenshot of the red scale prediction tool.

Appendix-6 Communications

Grower meetings

Region	Place	Date	Organiser	Attendants	Presenter(s)[1]
Riverina	Griffith NSW	30/08/2016	NSW DPI	14	J Mo
Riverina	Griffith NSW[2]	16/10/2017	NSW DPI	110	J Mo and Rob Weppler
Sunraysia	Mildura[2]	18/10/2017	NSW DPI	72	J Mo
Riverland	Loxton SA[2]	19/10/2017	CASAR[3]	> 33	J Mo
WA	Perth[2]	12/09/2017	WA Citrus	20	J Mo and B Walsh
Riverina	Griffith NSW	28/09/2018	NSW DPI	34	J Mo
Riverina	Leeton NSW	28/09/2018	NSW DPI	28	J Mo

[1]Person(s) who presented red scale project findings; [2]NSW DPI 2017 Roadshow; [3]Citrus Australia SA Region.

The R&D roadshow toured Perth WA, Riverland, Sunraysia and Riverina during September–October 2017. Scientists and industry experts presented practical technical information on citrus production and protection to citrus growers and industries in a series of workshops during the roadshow. Jianhua Mo presented up findings of the CGW project (CT15006) and the red scale project (CT15008).

Industry publications

Towards better red scale control. *Australian Citrus News*, June 2015, P. 23.

Red scale spray timing prediction tool. *Citrus Connect*, December 2016. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=fb04ffc056#mctoc5.

A preliminary timing guide for red scale management. *Australian Citrus News*, Autumn 2018, p 28. www.citrusaustralia.com.au/wp-content/uploads/Citrus-News-Autumn-18_LR-FINAL.pdf.

New tool to target red scale. *Australian Citrus News*, Summer 2015–16, p 29.

Red scale spray timing prediction tool. *Citrus Connect*, December 2016. https://us11.campaign-archive.com/?u=59ba43482b8c913efe7355823&id=fb04ffc056#mctoc5.

Timing of red scale control in citrus. Citrus Technical Forum 2017, Mildura, p 33.

Towards better red scale control. *Australian Citrus News*, June 2015, p 23.