Comparison of Two Traps for Monitoring California Red Scale (Hemiptera: Diaspididae)

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Comparison of two traps for monitoring California red scale (Hemiptera: Diaspididae)

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Abstract
California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is an economically important pest of citrus. In many cases, California red scale pest management is based on pheromone trap captures of males during their flight period. In California, where this study was conducted, California red scale pheromone traps are deployed from Mar until the end of Oct, at densities of 2 to 4 traps per 4 ha. Therefore, monitoring for California red scale represents a significant time expenditure for orange growers, and improved monitoring tools would be beneficial. This study was conducted to compare the efficacy of 2 California red scale trap designs during a 7-mo-long field study conducted in a commercial navel orange grove. In particular, we compare trap captures, and the occurrence of management thresholds, between a double-sided California red scale trap and a single-sided California red scale trap, both baited with commercial California red scale lures. The single-sided California red scale trap was incorporated into an internet-of-things platform designed for automated or remote monitoring. Mean trap captures, the occurrences of management thresholds, and the seasonal phenology of capture were similar between both trap types. Importantly, the proportion of traps reaching management thresholds were not significantly different between trap types. These results suggest that both trap types can be used in a similar fashion to monitor California red scale in commercial settings using currently recommended monitoring guidelines.

Key Words: citrus; integrated pest management; internet of things; pheromone; wireless

Resumen
La escama roja de California, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) es una plaga de los cítricos de importancia económica. En muchos casos, el manejo de escama roja de California se basa en la captura de machos con trampas de feromonas durante su periodo de vuelo. En California, donde se realizó este estudio, las trampas de feromonas de escama roja de California se implementan desde marzo hasta el final de octubre, con densidades de 2 a 4 trampas por 4 ha. Por lo tanto, el monitoreo de la escama roja de California representa un gasto de tiempo significativo para los productores de naranjas, y una mejor herramienta de monitoreo sería beneficiosa. Este estudio se realizó para comparar la eficacia de 2 diseños de trampa de la escama roja de California durante un estudio de campo de 7 meses de duración realizado en un huerto comercial de naranja navel. En particular, comparamos la captura de las trampas y la ocurrencia de umbrales de manejo, entre una trampa de dos lados y una trampa de un solo lado para la escama roja de California, ambas cebadas con cebos comerciales para la escama roja de California. La trampa de escama roja de California de un solo lado se incorporó a una plataforma de “Internet de Cosas” diseñada para el monitoreo automático o remoto. El promedio del número de las capturas, las ocurrencias de umbrales de manejo y la fenología estacional de captura fueron similares entre ambas trampas de captura. Es importante destacar que la proporción de trampas que alcanzan el umbral de manejo no fue significativamente diferente entre las clases de trampas. Estos resultados sugieren que ambas clases de trampas pueden usarse de manera similar para monitorear la escama roja de California en entornos comerciales utilizando las pautas de monitoreo actualmente recomendadas.

Palabras Clave: cítricos; manejo integrado de plagas; Internet de Cosas; feromona; inalámbrico

California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), is an economically important pest of citrus now found in most citrus growing regions (CABI 2018). California red scale damage is 2-fold, the presence of scales on fruit may result in rejection or downgrading of the commodity by wholesalers, whereas severe infestations may cause defoliation, branch die-back, and eventually tree death (Grafton-Cardwell & Reagan 1995). California red scale has a complex temperature dependent life cycle consisting of 3 to 5 generations per yr (Grout et al. 1989; Forster et al. 1995). Female California red scale will produce > 100 offspring known as crawlers, which is the only mobile immature life-stage (Tashiro & Beavers 1968). Crawlers travel short distances either by walking or wind-mediated dispersal, and settle on twigs, leaves, or fruit to develop (Willard 1974). Male California red scale will complete 2 larval instars and 2 pupal stages, and then emerge as short-lived winged adults (Tashiyo & Beavers 1968). The emergence of adult male California red scale corresponds with the development of third instar females; males are attracted to these females by the release of a sex pheromones (Tashiyo & Chambers 1967; Roelofs et al. 1977).

California red scale management regimes vary widely, and may rely on several tactics including augmentative biological control (Moreno & Luck 1992), mating disruption (Vacas et al. 2009, 2010), natural enemies (Vacas et al. 2012; Anonymous 2017), and various insecticides (Grout & Richards 1991; Grafton-Cardwell & Reagan 1995; Grafton-
Cardwell et al. 2006). Importantly, California red scale has demonstrated a capacity to develop resistance to conventional insecticides (Neil et al. 1979; Grafton-Cardwell & Vehrs 1995; Levitin & Cohen 1998), which has led to the development of integrated pest management programs using combinations of insect growth regulators, horticultural oils, biological control, and mating disruption (Moreno & Luck 1992; Vacas et al. 2009, 2010; Anonymous 2017).

In the context of integrated pest management, California red scale is monitored by counting immobile scales on twigs or fruit, or by using pheromone-baited traps to capture males during their flight period (Moreno & Kennett 1985; Anonymous 2017). Importantly, the number of males caught during a flight can be used to predict fruit infestations at harvest (Moreno & Kennett 1985). Furthermore, pheromone trap counts can be used to target pesticide applications and implement mating disruption programs (Vacas et al. 2012, 2015). Particularly important are pheromone trap count results for the final annual generation, because these counts help inform the California red scale management program in the spring. Pheromone traps for scale insects typically consist of a pheromone-baited sticky card rather than a closed trap (Hoyt et al. 1983; Gieselmann & Rice 1990). A typical California red scale trap consists of a double-sided sticky card (Anonymous 2017) baited with a California red scale pheromone lure containing (3S, 6R)-3-Methyl-6-isopropenyl-9-decen-1-yl acetate, and (3S, 6S)-3-Methyl-6-isopropenyl-9-decen-1-yl acetate (Tashiro & Chambers 1967; Roelofs et al. 1977). In California, where this research was conducted, it is recommended that California red scale traps be deployed early in the yr (e.g., Feb or Mar) before the first male flight, and monitored every wk until the end of Oct (Anonymous 2017). Traps are deployed at density of 2 to 4 per 4 ha (Anonymous 2017). In this region, 4 to 5 generations are expected with an accumulation of about 611 degree-d between the first and second male flight, and 1,833 degree-d between the first and fourth male flight, both calculated using a lower threshold of 11.7 °C (Anonymous 2017).

Recently there has been growing interest in using digital sensors to monitor agricultural pest insects (Potamitis et al. 2017; Zhu et al. 2017; Ahmad et al. 2018). One approach is to use digital sensors to create self-counting insect traps (Doitsidis et al. 2017). These traps (now referred to as “internet of things” traps or IOT traps) can then be wirelessly networked, integrated across large areas, and accessed remotely (Potamitis et al. 2017). This approach could provide users with pest data in near real-time, thereby expediting pest management decisions and implementation. Furthermore, self-counting traps would be beneficial additionally in situations where trap densities are high, and monitoring is frequent as is with California red scale. In this paper, we examine the efficacy of 2 California red scale traps during a 7-mo-long field study conducted in a commercial navel orange grove. Specifically, we compare trap captures, and occurrence of management thresholds, between a single-sided California red scale trap incorporated into an internet-of-things platform, and double-sided California red scale trap.

### Materials and Methods

#### FIELD EXPERIMENT

This study was conducted in a commercial navel orange grove in Tulare County, California, USA. An approximately 30-ha-plot was divided into 30 trap locations. Each trap location was separated by at least 70 m. A single-sided California red scale trap (Fig. 1A), and a commercial double-sided California red scale trap (Trécé, Adair, Oklahoma, USA) (Fig. 1B) were installed at each trap location, separated by 25 m. The single-sided California red scale trap consisted of a scale card (Alpha Scents, Inc., West Linn, Oregon, USA), mounted on a 36 × 38 cm green polypropylene corrugated plastic platform attached to a wireless networked digital camera (Fig. 1A). All traps were installed in the northeast quadrant of a tree just inside the canopy at a height of 1.8 to 2.4 m. The single-sided traps were oriented such that the sticky surface was facing into the canopy. All traps were baited with a commercial California red scale lure (Trécé, Adair, Oklahoma, USA). Lures were replaced approximately every 4 wk (Supplementary Table 1). All traps were installed on 15 Feb and monitored every 7 to 29 d as weather and grove management permitted (Supplementary Table 1).

Trap counts were assessed in 2 ways. First, the number of California red scale on the entire trap was counted to a maximum of 1,000, subsequently referred to as actual California red scale counts. A 1,000 California red scale cut-off was chosen because it represents management threshold for California citrus producers during the fourth male flight (Anonymous 2017). Second, all California red scale within bounding boxes on the trap were counted. The bounding boxes are used to expedite manual trap counts; the boxes represented 13% and 20% of the total sticky area of the single-sided and double-sided California red scale traps, respectively (Table 1). To compare, box counts between trap type counts were standardized to 193.50 cm^2 such that counts could be expressed in terms of the management threshold of 1,000 California red scale per 193.50 cm^2 (Anonymous 2017). To achieve this, box counts were multiplied by a factor of 10 and 5 for the single-sided and double-sided California red scale traps, respectively (Table 1). Box counts are subsequently referred to as estimated California red scale counts.

Degree-d accumulation was calculated from hourly temperature recordings retrieved from an onsite weather station (ATMOS 14, METER Group, Inc., Pullman, Washington, USA) located near the center of the experimental plot. Degree-d were calculated with the single sine horizontal method, with a lower threshold of 11.7 °C (Grout et al. 1989; Anonymous 2017). Degree-d based on regional temperature records were retrieved from http://ipm.ucanr.edu/weather/; degree-d were calculated with the same parameters as above.

#### STATISTICAL ANALYSIS

The estimated California red scale counts during male flights were analyzed with a generalized linear model, with negative binomial errors and a log link function. Variance was partitioned into the main effects, trap type, trap location (replicate), flight number, and all interactions. Akaike information criteria was used to obtain a minimal adequate model. Significant effects were assessed with likelihood-ratio tests.

The proportion of all traps reaching management thresholds of 1,000 California red scale were analyzed using a generalized linear model with binomial errors and log link function. Variance was partitioned into the effects of trap type, sampling date, and a trap type × sampling date interaction. To determine if data set (e.g., actual California red scale count, estimated California red scale count), influenced the probability of > 1,000 California red scale trap captures, a second analysis was conducted as above with the effect of trap type, data set, sampling date, and all 2-way interactions. All analyses were performed in R vers. 3.5.1 at a significance level of α = 0.05 (R Development Core Team 2015).

#### Results

The California red scale trap captures varied across time, and 3 distinct male flights were evident during the sampling period (Fig. 2A). The first male captures occurred on 13 Apr, and this date was used as a biofix. Predicted flight times based on accumulation of degree-d from onsite and regional weather stations were similar for both the second
(on-site 19 Jun; regional 18 Jun) and fourth flights (on-site 5 Sep; regional 2 Sep) (Fig. 2B). The occurrence of male flights conformed with the phenological growth model for California red scale (Anonymous 2017) (Fig. 2B). Estimated California red scale trap captures ranged from 0 to 8,350.

**CALIFORNIA RED SCALE CAPTURES DURING MALE FLIGHT PERIODS**

The saturated model used to describe estimated California red scale counts during male flights contained the effects of trap location, trap type, flight number, and all interaction terms. The minimal adequate model derived from Akaike information criteria contained only the main effects of trap location ($\chi^2 = 622.55; df = 29; P < 0.001$) and flight number ($\chi^2 = 585.11; df = 2; P < 0.001$). Importantly, there was no effect of trap type ($\chi^2 = 0.43; df = 1; P = 0.51$). Estimated California red scale trap counts varied widely between trap locations (Fig. 3A), and there was no obvious pattern in their spatial distribution (Fig. 3B).

Finally, mean estimated California red scale trap counts increased in each successive male flight period (Fig. 3C).

**PROPORTION OF TRAPS WITH > 1,000 CALIFORNIA RED SCALE PER SAMPLING PERIOD**

The probability of a trap capturing > 1,000 California red scale was affected only by sampling date for both actual California red scale counts ($\chi^2 = 169.36; df = 14; P < 0.001$) (Fig. 4A), and estimated California red scale counts ($\chi^2 = 237.24; df = 13; P < 0.001$) (Fig. 4B), whereas the effect of trap type (actual California red scale count: $\chi^2 = 1.11; df = 1; P = 0.29$; estimated California red scale count: $\chi^2 = 0.16; df = 1; P = 0.69$) and the interaction between trap type and sampling date (actual California red scale count: $\chi^2 = 7.10; df = 14; P = 0.93$, estimated California red scale count: $\chi^2 = 3.52; df = 14; P = 0.99$) did not have an effect on the probability of capturing > 1,000 California red scale. Furthermore, there was no difference between data sets in the probability of a > 1,000 California red scale trap capture (data set: $\chi^2 = 3.04; df = 1; P = 0.081$, data set × trap: $\chi^2 = 1.08; df = 1; P = 0.30$, data set × date: $\chi^2 = 8.21; df = 14; P = 0.88$).

**Discussion**

Literature comparing the efficacy of different pheromone baited traps designs for monitoring armored scales is scarce (Hoyt et al. 2018).
Hoyt et al. (1983) examined various sizes of pheromone baited tent traps and closed traps for monitoring male San Jose scale, *Quadraspidiotus perniciosus* (Comstock) (Hemiptera: Diaspididae). In that study, all traps regardless of design and size caught similar numbers of males per cm$^2$ of sticky surface; however, the authors ultimately endorsed the use of the tent traps due to concerns with by-catch. Similar results were observed here because both trap designs caught equal numbers of male California red scale per cm$^2$ of sticky surface during male flights. Furthermore, in our study the temporal pattern of California red scale captures and the probability of > 1,000 California red scale captures between traps were equivalent. Together this suggests that both of these traps can be used in a similar fashion to monitor California red scale. There was no clear pattern in the spatial distribution of California red scale infestation observed in this study; the infestation could be described as patchy (Fig. 3), and this highlights the importance of pheromone-based monitoring for this pest.

Trap saturation, the decrease in trap capture due to the accumulation of a target pest in a trap, can affect capture rates and influence the reliability of trap counts (Brown 1984; Sanders 1986; Kuenen & Siegel 2016). In this study, there is no evidence that trap saturation was affecting captures because mean California red scale count per cm$^2$ (in this case standardized 193.50 cm$^2$) were not significantly different between the 2 traps (Table 1). If trap saturation was occurring, we would expect to observe higher mean California red scale count per cm$^2$ for the single-sided California red scale trap due to its smaller surface area. Furthermore, mean California red scale counts for both trap designs (Figs. 2 & 3) frequently exceeded the management threshold of 1,000 California red scale per 193.50 cm$^2$ of trap surface. Therefore, it seems implausible that trap saturation with California red scale would influence management decisions using either of these traps.

The design of the single-sided California red scale trap used in this study incorporated a mounting platform to which the trap’s sticky surface was attached (Fig. 1A). This mounting platform was designed to
stabilize (e.g., prevent rotation) and protect the trap’s sticky surface from leaves and branches (e.g., trap saturation by debris), such that it could be more easily photographed by the imbedded digital camera. It seems reasonable that the mounting platform could act as funnel, like a barrier pit-fall trap (Boetzl et al. 2018), such that California red scale encountering the platform would be directed to the sticky surface, potentially increasing trap captures. Alternatively, the mounting platform could impede California red scale from being captured, depending on the direction in which an individual California red scale approaches the trap. Given that male California red scale have relatively poor upwind flight ability (Rice & Moreno 1970), the trap orientation in relation to the prevailing winds may have some effect on trap captures. However, since all the single-sided traps in this study were installed in the same orientation, in the northeast quadrant of the tree with the sticky surface facing inwards, we cannot assess the effect of the trap orientation on trap captures.

The objective of this research was to compare the efficacy of a single-sided California red scale trap incorporated into an internet-of-things platform with a commercial double-sided California red scale trap. In this field study, both California red scale traps performed similarly and, importantly, the information content in terms of male flight phenology and mean trap captures were equivalent. These results sug-

![Graph A](image1.png)

**Fig. 3.** (A) Mean (± SE) California red scale counts (based on estimated California red scale counts) for all trap locations in this study. (B) Schematic of relative position of trap locations in our field plot; shape indicates estimated marginal mean California red scale counts. In (A) and (B) circles are < 500 California red scale, triangle is 501 to 1,000 California red scale, and squares > 1,000 California red scale. (C) Mean (± SE) California red scale trap counts by flight number. Bars with different letters are significantly different (α = 0.05).

![Graph B](image2.png)

**Fig. 4.** The probability of a trap capturing >1,000 California red scale in a sampling period, based on (A) actual California red scale counts, and (B) estimated California red scale counts. Male flight periods are delimited with vertical dashed lines.
suggest that single-sided internet-of-things traps can be used in a similar fashion to double-sided traps for monitoring California red scale in orange groves. The next step of this research is to develop an image processing algorithm for California red scale detection, which can then be used to automate California red scale trap counts. In general, this study illustrates the potential for internet-of-things insect traps to contribute to pest monitoring. (Potamitis et al. 2017), by demonstrating that traps modified to incorporate internet-of-things components can function like those currently used for pest monitoring. Future work will be required to determine if the potential benefits of internet-of-things traps, such as automated pest counts, and real-time pest reporting, are capable of meaningful improvements in California red scale pest management programs.

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References Cited

Ahmad MN, Shariff ARM, Moslim R. 2018. Monitoring insect pest infestation via different spectroscopic techniques. Applied Spectroscopy Reviews 53: 836–853.

Anonymous. 2017. University of California pest management guidelines for California red scale and yellow scale on citrus. UC ANR Publication 3441. University of California, Agriculture & Natural Resources, Davis, California, USA. http://ipm.ucanr.edu/PMG/r107301111.html (last accessed 13 May 2019).

Boetzel FA, Ries E, Schneider G, Krauss J. 2018. It’s a matter of design—how pitfall trap design affects trap samples and possible predictions. PeerJ 6: e5078. https://doi.org/10.7717/peerj.5078

Brown MW. 1984. Saturation of pheromone sticky traps by Platynota-ideaesa(lis) (Walker) (Lepidoptera, Tortricidae). Journal of Economic Entomology 77: 915–918.

CABI. 2018. Aonidiella aurantii (red scale). CABI Invasive Species Compendium, CABI, Wallingford, Oxon, United Kingdom. www.cabi.org/isc (last accessed 13 May 2019).

Doitsu L, Fouskitakis GN, Varikou KN, Rigakis IL, Chatzichristofos SA, Papafilippaki AK, Biouraki AE. 2017. Remote monitoring of the Bactrocera oleae (Gmelin) (Diptera: Tephritidae) population using an automated McPhail trap. Computers and Electronics in Agriculture 137: 69–78.

Forster LD, Luck RF, Grafton-Cardwell EE. 1995. Life stages of California red scale and its parasitoids. Publication #21529. University of California, Division of Agriculture and Natural Resources, Oakland, California, USA.

Gieselmann MJ, Rice RE. 1990. Use of pheromone traps, pp. 349–352 in Rosen D (eds), Armored Scale Insects: Their Biology, Natural Enemies and Control. Volume B, Elsevier, New York, USA.

Grafton-Cardwell EE, Reagan CA. 1995. Selective use of insecticides for control of armored scale (Homoptera, Diaspididae) in San-Joquin Valley California citrus. Journal of Economic Entomology 88: 1717–1725.

Grafton-Cardwell EE, Vanek SLC. 1995. Monitoring for organophosphate-resistant and carbamate-resistant armored scale (Homoptera, Diaspididae) in San-Joquin Valley citrus. Journal of Economic Entomology 88: 495–504.

Grafton-Cardwell EE, Lee JE, Stewart JR, Olsen KD. 2006. Role of two insect growth regulators in integrated pest management of citrus scales. Journal of Economic Entomology 99: 733–744.

Grout TG, Richards GI. 1991. Effect of bugpofezin applications at different physiological times on California red scale (Homoptera, Diaspididae). Journal of Economic Entomology 84: 1802–1805.

Grout TG, Duttoff WJ, Hofmeyer JR, Richards GI. 1989. California red scale (Homoptera, Diaspididae) phenology on citrus in South-Africa. Journal of Economic Entomology 82: 793–798.

Hoyt SC, Westgard PH, Rice RE. 1983. Development of pheromone trapping techniques for male San Jose scale (Homoptera, Diaspididae). Environmental Entomology 12: 371–375.

Kuenen LPS, Siegel JP. 2016. Sticky traps saturated with navel orangeworm in a nonlinear fashion. California Agriculture 70: 32–38.

Levitin E, Cohen E. 1998. The involvement of acetylcholinesterase in resistance of the California red scale Aonidiella aurantii to organophosphorus pesticides. Entomologia Experimentalis et Applicata 88: 115–121.

Moreno DS, Kennett CE. 1985. Predictive year-end California red scale (Homoptera, Diaspididae) orange fruit infestations based on catches of males in the San-Joqul Valley. Journal of Economic Entomology 78: 1–9.

Moreno DS, Luck RF. 1992. Augmentative releases of Aphytis-melinus (Hymenoptera, Aphelinidae) to suppress California red scale (Homoptera, Diaspididae) in southern California lemon orchards. Journal of Economic Entomology 85: 1112–1119.

Nel JC, Delange L, Vanark H. 1979. Resistance of citrus red scale, Aonidiella aurantii (Mask), to insecticides. Journal of the Entomological Society of Southem Africa 42: 275–281.

Potamitis I, Eliopoulos P, Rigakis IL. 2017. Automated remote insect surveillance at a global scale and the internet of things. Robotics 6. doi.org/10.3390/robotics6030019 (last accessed 13 May 2019).

R Development Core Team 2015. R: a language and environment for statistical computing. version 3.5.1. R Foundation for Statistical Computing, Vienna, Austria.

Rice Re, Moreno DS. 1970. Flight of male California red scale. Annals of the Entomological Society of America 63: 91–96.

Roelofs W, Gieselmann MJ, Carde AM, Tashiro H, Moreno DS, Hendrix CA, Anderson RJ. 1977. Sex-pheromone of California red scale, Aonidiella aurantii. Naturae. 267: 698–699.

Sanders CJ. 1986. Evaluation of high-capacity, non-saturating sex-pheromone traps for monitoring population-densities of spruce budworm (Lepidoptera, Tortricidae), Canadian Entomologist 118: 611–619.

Tashiro H, Beavers J. 1968. Growth and development of California red scale Aonidiella aurantii. Annals of the Entomological Society of America 61: 1009–1014.

Tashiro H, Chambers DL. 1967. Reproduction in California red scale Aonidiella aurantii (Homoptera - Diaspididae). I. Discovery and extraction of a female sex pheromone. Annals of the Entomological Society of America 60: 1166–1170.

Vacas S, Alfarco C, Navarro-Llopis V, Primo J. 2009. The first account of the mating disruption technique for the control of California red scale, Aonidiella aurantii (Maskell) (Homoptera: Diaspididae) using new biodegradable dispensers. Bulletin of Entomological Research 99: 415–423.

Vacas S, Alfarco C, Navarro-Llopis V, Primo J. 2010. Mating disruption of California red scale, Aonidiella aurantii (Maskell) (Homoptera: Diaspididae), using biodegradable mesoporous pheromone dispensers. Pest Management Science 66: 745–751.

Vacas S, Alfarco C, Primo J, Navarro-Llopis V. 2015. Deployment of mating disruption dispensers before and after first seasonal male flights for the control of Aonidiella aurantii in citrus. Journal of Pest Science 88: 321–329.

Vacas S, Vanaclocha P, Alfarco C, Primo J, Verdu MJ, Urbaneja A, Navarro-Llopis V. 2012. Mating disruption for the control of Aonidiella aurantii (Maskell) (Homoptera: Diaspididae) in field. Australian Journal of Zoology 52: 531–548.

Zhu SM, Malmqvist E, Li WS, Jansson S, Li YY, Duan Z, Svanberg K, Feng HQ, Song ZW, Zhao GY, Brydegaard M, Svanberg S. 2017. Insect abundance over Chinese rice fields in relation to environmental parameters, studied with a polarization-sensitive CW near-IR lidar system. Applied Physics B-Lasers and Optics 123. doi.org/10.1007/s00340-017-6784-x (last accessed 13 May 2019).