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Chemical Degradation of TMR Multilure Dispensers for Fruit Fly Detection Weathered Under California Climatic Conditions

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Abstract

Degradation models for multilure fruit fly trap dispensers were analyzed to determine their potential for use in large California detection programs. Solid three-component male lure TMR (trimedlure [TML], methyl eugenol [ME], raspberry ketone [RK]) dispensers impregnated with DDVP (2, 2-dichlorovinyl dimethyl phosphate) insecticide placed inside Jackson traps were weathered during summer (8 wk) and winter (12 wk) in five citrus-growing areas. Additionally, TMR wafers without DDVP, but with an insecticidal strip, were compared to TMR dispensers with DDVP. Weathered dispensers were sampled weekly and chemically analyzed. Percent loss of TML, the male lure for *Ceratitis capitata* (Wiedemann) Mediterranean fruit fly; ME, the male lure for *Bactrocera dorsalis* (Hendel), oriental fruit fly; RK, the male lure for *Bactrocera cucurbitae* (Coquillett), melon fly; and DDVP was measured. Based on regression analyses for the male lures, TML degraded the fastest followed by ME. Degradation of the more chemically stable RK was discontinuous, did not fit a regression model, but followed similar seasonal patterns. There were few location differences for all three male lures and DDVP. Dispensers degraded faster during summer than winter. An asymptotic regression model provided a good fit for % loss (ME, TML, and DDVP) for summer data. Degradation of DDVP in TMR dispensers was similar to degradation of DDVP in insecticidal strips. Based on these chemical analyses and prior bioassay results with wild flies, TMR dispensers could potentially be used in place of three individual male lure traps, reducing costs of fruit fly survey programs. Use of an insecticidal tape would not require TMR dispensers without DDVP to be registered with US-EPA.

Key words: Tephritidae, fruit fly trap, survey, eradication program

Fruit flies (Diptera: Tephritidae) are composed of >4,000 species and include some of the most economically important pests attacking soft fruits worldwide (e.g., Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *Bactrocera cucurbitae* (Coquillett) (White and Elson-Harris 1992); White and Elson-Harris 1992). To detect and eradicate persistent invasions by these fruit flies, the state of California maintains arrays of fruit fly traps baited with the male lures trimedlure (TML), cue-lure (C-L), and methyl eugenol (ME) at ca. 30,000 sites (Vargas et al. 2010, CDFA 2013). At each site, three separate male lure traps are serviced. Mixed with the liquid attractants (i.e., ME and C-L) are restricted-use organophosphate insecticides which require special environmental and worker safety precautions (Vargas et al. 2008). Approximately 90% of *Bactrocera* species respond to either ME or C-L/RK (raspberry ketone)(Vargas et al. 2008), and three species of *Ceratitis* are known to respond to TML (Vargas et al. 2012). During the Hawaii AWPM Fruit Fly Program, solid lure dispensers were critically evaluated over a 10-yr period (Vargas et al. 2008, 2010; Leblanc et al. 2011). Workers in the program found solid lure dispensers and insecticidal tape to be more convenient and safer than traps with cotton wicks containing a liquid lure mixed with a restricted-use insecticide such as naled or malathion. Although TML solid plugs with sticky panels have become a standard replacement for liquid TML mixed with insecticides in Jackson traps for *C. capitata* detection (FDACS 2004, CDFA 2013), for unknown reasons, there has been a hesitation to replace liquid ME and C-L lures with solid formulations of *Bactrocera* species.

In previous studies, we evaluated and published bioassays with wild fruit flies for three-component solid dispensers installed inside standard Jackson traps and weathered in five citrus-growing regions in California (Vargas et al. 2016a). In addition, we published chemical analyses of two components ME and C-L lures with DDVP (2, 2-dichlorovinyl dimethyl phosphate) weathered in Hawaii (TML, ME, C-L/RK)(Vargas et al. 2016b). The objective of the present study was to determine chemical degradation, chemical dynamics, and longevity data for the three male lure component Mallet TMR dispensers by analyzing the amounts of lure (TML, ME, and RK) and insecticide (DDVP) lost over time on a second set of wafers sampled during the same time period as the published bioassay tests for California weathered dispensers (Vargas et al. 2016a). To avoid costly registration of dispensers impregnated with DDVP, evaluations of chemical degradation also included comparisons of weathered treatments of Mallet TMR wafers impregnated with DDVP versus Mallet TMR wafers without DDVP, but instead with a separate insecticidal strip placed inside a Jackson trap. This use is already registered for fruit fly surveys.

Materials and Methods

California Evaluation Sites

Study locations were selected from counties where fruit flies had either previously been detected or areas susceptible to fruit fly infestation. Jackson traps with Mallet TMR dispensers were placed in *Citrus* spp. trees at all sites. Summer weathering of wafer treatments (8 wk) took place from July 24–September 18, 2012 while winter weathering (12 wk) took place from January 8–April 2, 2013. The five locations (mean ± SEM summer and winter temperature °C) were Exeter (Tulare County; 27.57 ± 0.12; 11.18 ± 0.14), Riverside (Riverside County; 25.69 ± 0.12; 12.56 ± 0.14), Bakersfield (Kern County; 27.98 ± 0.12; 10.92 ± 0.15), Irvine (Orange County; 23.44 ± 0.10; 13.21 ± 0.13), and Ventura (Ventura County; 20.14 ± 0.14; 12.21 ± 0.13). A map of the study area is illustrated in Vargas et al. (2013). GPS coordinates and additional climate data are summarized in Vargas et al. (2016a).

Two treatments were weathered. In the first, Mallet TMR 6M (10.4% TML, 27.1% ME, 10.4% RK, 3.6% DDVP, one lure contained ∼10.6 g AI; 5.0 by 8.0 cm; Farma Tech International, North Bend, WA) was deployed, while in the second Mallet-TMR 6M without DDVP (10.4% TML, 27.1% ME, 10.4% RK) combined with an insecticidal strip, Hercon VAPORTAPE II (2, 2-dichlorovinyl dimethyl phosphate, 10% by weight, 2.5 by 10 cm; Emigsville, PA), was used. Raspberry ketone (RK) was used in these trials because it is thought to be the attractive molecule and because it volatilizes at a lower rate than C-L (Metcalf and Metcalf 1992), making it more persistent. Mallet TMR treatments were deployed simultaneously at each location and trial inside a standard Jackson trap with a sticky insert. Only the Exeter and Riverside locations included weathered Mallet TMR dispensers without DDVP combined with an insecticidal strip, which were handled the same as the other samples. The experimental design, sampling methodology, and shipping techniques are summarized in detail in Vargas et al. (2016a). Briefly, for each treatment and location, four replicate samples were sent by overnight delivery to North Bend, WA, for chemical analysis.

Chemical Analysis

Dispensers from Jackson traps were sent to Farmatech International Corporation (North Bend, WA) for chemical analyses. Each week four replicate samples for each lure treatment from each location were analyzed, and the amounts of ME, RK, TML, and DDVP remaining in the weathered dispensers and VAPORTAPE II were determined using a Shimadzu GC 2010 Gas Chromatograph (GC) and Shimadzu GC Solution Software. Chemical analysis methodology is outlined in detail by Vargas et al (2015).

Data Analysis

Mean percent loss (± SEM) of ME, TML, RK, and DDVP were analyzed using regression analysis. An asymptotic model provided a very good fit (based on R^2 values) for % loss (ME, TML, and DDVP) for the summer 2012 data. The model used was $y = a - cb^x$, where x = (date − 19207) (SAS represents 08/02/2012 with the value, 19207, so this scales the date axis to start at zero). Percent RK loss was discontinuous and could not be fitted to a regression model. An asymptotic or a linear model ($y = a + bx$, where x = week) was used for the winter 2013 data (ME, TML, and DDVP) on a "best fit" basis. Again % RK loss was discontinuous and could not be fitted to a regression model. Equations, R^2 values, and parameter estimates (a, b, and c) and 95% confidence limits (CL) are provided (SAS Institute 2013).

Results and Discussion

During the summer, mean temperatures and relative humidity varied by location, with highest temperatures recorded at the inland Bakersfield and lowest temperatures recorded at the coastal Ventura. Conversely, the highest mean relative humidity was recorded at Ventura and the lowest at Bakersfield (Vargas et al. 2016a). The loss of ME, TML, DDVP (in TMR), and DDVP (in VAPORTAPE II) in weathered dispensers are summarized for summer (Fig. 1) and winter (Fig. 2). In Table 1, nonlinear ($y = a - cb^x$, where x = week) regression equations for 1) % ME loss, 2) % TML loss, and 3) % DDVP loss of solid lure dispensers weathered under California summer climatic conditions at the five locations from 24 July to September 18, 2012 (8 wk) are presented along with R^2 values and 95% CL for a, b, and c. In Fig. 2 and Table 2, linear ($y = a + bx$, where x = week) or nonlinear ($y = a - cb^x$, where x = week) regression equations are presented for 1) % ME loss, 2) % TML loss, and 3) % DDVP loss in solid lure dispensers weathered under California winter climatic conditions at the five locations from 8 January to 2 April 2013 (12 wk) based on the best fit along with R^2 values and 95% CL for a, b, or c. Percent loss of lures was faster during summer than winter, suggesting that traps should be serviced more frequently during summer. Within-season percent loss of the male lures did not differ much by location. For the male lure RK, the loss of lure (Tables 3 and 4) was much more discontinuous than for TML and ME and did not fit a regression equation.

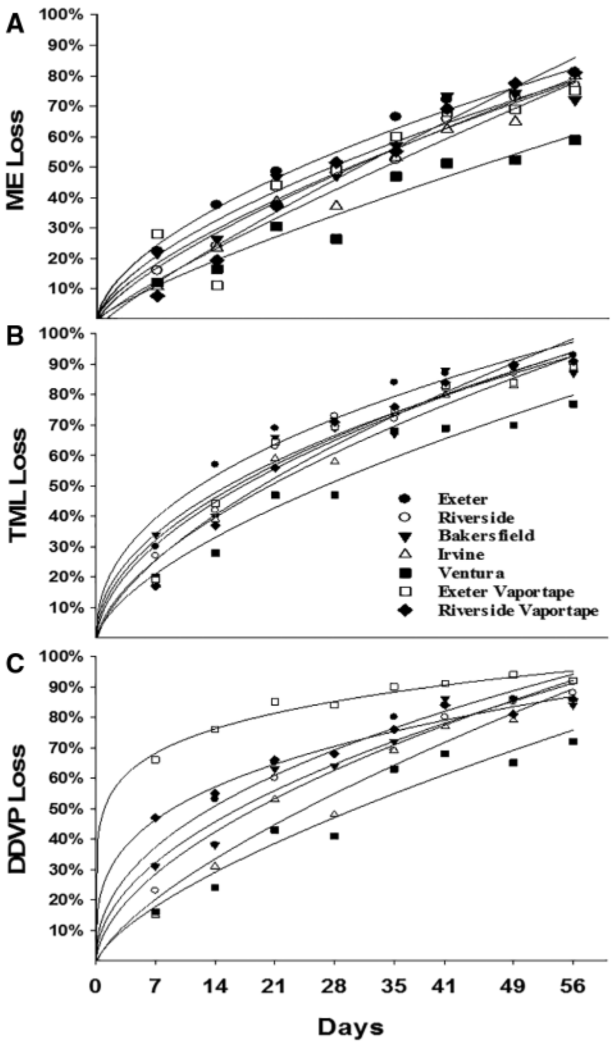

Fig 1. Regression plots for (**a**) % ME loss, (**b**) % TML loss, and (**c**) % DDVP loss in solid lure dispensers weathered under California summer climatic conditions at five locations from 24 July to 18 September 2012.

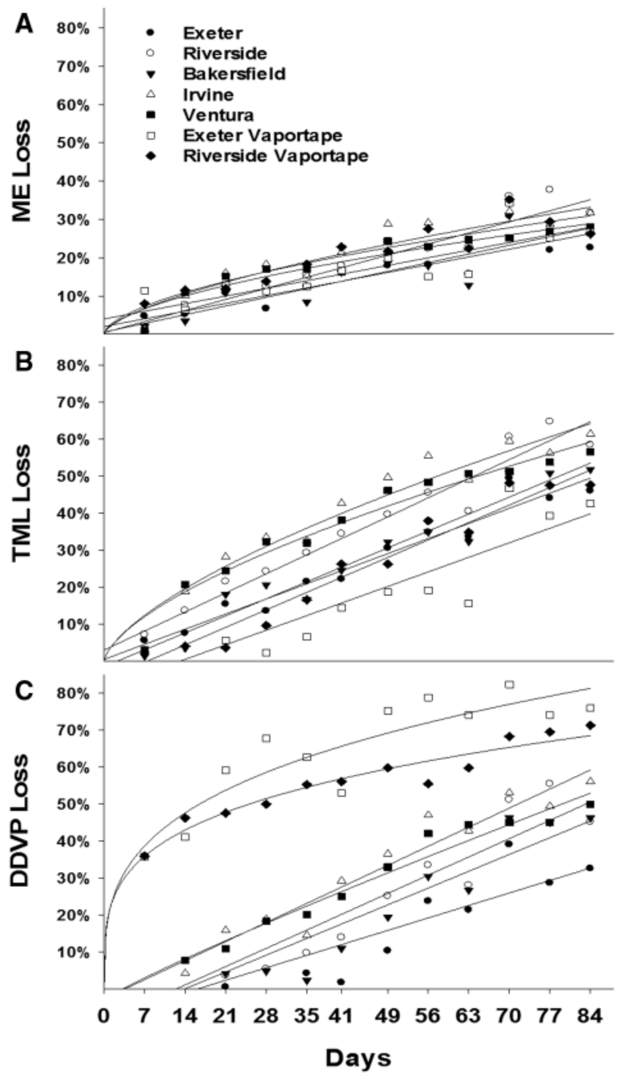

Fig. 2. Regression plots for (**a**) % ME loss, (**b**) % TML loss, and (**c**) % DDVP loss in solid lure dispensers weathered under California winter climatic conditions at five locations from 8 January to 2 April 2013.

However, loss was again greater during summer than winter. Overall, the rate of loss was TML > ME > RK.

In earlier bioassay studies at the same California weathering sites, captures of *C. capitata*, *B. cucurbitae*, and *B. dorsalis* in traps baited with Mallet TMR treatments impregnated with DDVP did not significantly differ among lures set at different locations throughout winter or summer trials (Vargas et al. 2016a). This current study provides additional chemical information on the stability of TML, ME, R-K, and DDVP weathered at the same California locations representing a range of environmental conditions. One new finding from this study is the rapid breakdown of TML, which degraded even faster than ME, which itself had been documented to be more volatile than C-L/RK as a lure in fruit fly traps (Vargas et al. 2015). Both ME and DDVP had previously been fitted to asymptotic exponential curves, while the C-L levels remained nearly constant (Vargas et al. 2015). Using chemical analysis to understand how male specific lures degrade inside traps in the field over time in different dispenser matrices, when coupled with information on fly attraction, will allow us to better predict the duration of lure effectiveness, effects of climate, and when traps should be re-baited. From a formulation perspective, on the basis of such an understanding of chemical degradation, calibrated amounts of TML, ME, and RK/C-L could be deployed in a single multilure dispenser. Besides the environmental benefits of more effective trapping, consolidating traps in this way would reduce the labor and material costs of numerous traps, two of the biggest expenses of large fruit fly survey programs (USDA-APHIS 2006).

With respect to solid insecticides, Vargas et al. (2009) found no difference between numbers of *B. dorsalis* and *B. cucurbitae* caught with DDVP strips and liquid naled in monitoring programs in California and Florida, and Vargas et al. (2015) also found the degradation of DDVP was effectively described by asymptotic nonlinear regression curves, in which the amount of DDVP in the dispenser decreased rapidly for the first 3 wk and decreased more slowly thereafter. This early volatility has been associated with repellency or even death to fruit flies approaching traps. The effect such high volatility of DDVP has on the number of flies reaching traps with vaportapes has been documented with cameras recording trap captures (Manoukis 2016). However, this repellency can be avoided by aging the lures 1–2 d (Vargas et al. unpublished data). Our present study found similar levels of degradation of DDVP in TMR + Vaportape

Table 1. (for Fig. 1) Nonlinear ($y = a - cb^x$, where x = week) regression equations for (a) % ME loss, (b) % TML loss, and (c) % DDVP loss of solid lure dispensers weathered under California summer climatic conditions at five locations from 24 July to 18 September 2012 (8 wk) are presented along with R^2 values and 95% confidence limits (CL) for a, b, and c

Location	Equation	R^2	a (95% CL)	b (95% CL)	c (95% CL)
(a) ME					
Exeter	$y = 0.80–(0.56)(0.97)^x$	0.96	(0.62, 0.98)	(0.93, 1.0)	(0.39, 0.72)
Riverside	$y = 0.71–(0.57)(0.96)^x$	0.89	(0.49, 0.93)	(0.90, 1.01)	(0.33, 0.80)
Bakersfield	$y = 0.76–(0.56)(0.97)^x$	0.92	(0.48, 1.05)	(0.92, 1.02)	(0.31, 0.81)
Irvine	$y = 0.73–(0.61)(0.97)^x$	0.93	(0.39, 1.07)	(0.93, 1.01)	(0.32, 0.90)
Ventura	$y = 0.95–(0.82)(0.99)^x$	0.96	(−1.25, 3.15)	(0.95, 1.03)	(−1.31, 2.9)
Exeter Vaportape	$y = 0.78–(0.58)(0.97)^x$	0.78	(−0.50, 2.06)	(0.85, 1.10)	(−0.55, 1.71)
Riverside Vaportape	$y = 0.68–(0.62)(0.95)^x$	0.92	(0.39, 0.97)	(0.88, 1.02)	(0.28, 0.96)
(b) TML					
Exeter	$y = 0.88–(0.57)(0.93)^x$	0.98	(0.83, 0.94)	(0.90, 0.96)	(0.47, 0.67)
Riverside	$y = 0.84–(0.60)(0.94)^x$	0.94	(0.74, 0.94)	(0.89, 0.98)	(0.42, 0.78)
Bakersfield	$y = 0.87–(0.55)(0.95)^x$	0.93	(0.75, 0.99)	(0.90, 0.99)	(0.38, 0.73)
Irvine	$y = 0.84–(0.65)(0.95)^x$	0.96	(0.74, 0.94)	(0.92, 0.98)	(0.50, 0.79)
Ventura	$y = 0.78–(0.59)(0.97)^x$	0.98	(0.63, 0.92)	(0.94, 0.99)	(0.46, 0.71)
Exeter Vaportape	$y = 0.88–(0.55)(0.97)^x$	0.66	(−0.10, 1.86)	(0.80, 1.13)	(−0.36, 1.46)
Riverside Vaportape	$y = 0.83–(0.68)(0.93)^x$	0.97	(0.69, 0.98)	(0.89, 0.98)	(0.47, 0.89)
(c) DDVP					
Exeter	$y = 0.86–(0.54)(0.94)^x$	0.97	(0.80, 0.93)	(0.91, 0.97)	(0.43, 0.66)
Riverside	$y = 0.82–(0.61)(0.94)^x$	0.93	(0.71, 0.94)	(0.89, 0.97)	(0.41, 0.80)
Bakersfield	$y = 0.85–(0.55)(0.95)^x$	0.93	(0.71, 0.98)	(0.91, 1.0)	(0.38, 0.73)
Irvine	$y = 0.83–(0.66)(0.96)^x$	0.95	(0.64, 1.01)	(0.92, 1.0)	(0.47, 0.86)
Ventura	$y = 0.75–(0.59)(0.97)^x$	0.97	(0.54, 0.97)	(0.94, 1.0)	(0.41, 0.78)
Exeter Vaportape	$y = 0.91–(0.25)(0.93)^x$	0.97	(0.86, 0.96)	(0.88, 0.98)	(0.17, 0.33)
Riverside Vaportape	$y = 0.83–(0.34)(0.96)^x$	0.95	(0.68, 0.98)	(0.90, 1.01)	(0.18, 0.50)

Table 2. (for Fig. 2) Linear ($y = a + bx$, where x = week) or nonlinear ($y = a - cb^x$, where x = week) regression equations for (a) % ME loss, (b) % TML loss, and (c) % DDVP loss in solid lure dispensers weathered under California winter climatic conditions at five locations from 8 January to 2 April 2013 (12 wk) are presented based on the best fit along with R^2 values and 95% confidence limits (CL) for a, b, or c

Location	Equation	R^2	a (95% CL)	b (95% CL)	c (95% CL)
(a) ME					
Exeter	$y = 0.01 + 0.02x$	0.83	(−0.02, 0.06)	(0.01, 0.02)	
Riverside	$y = 0.01 + 0.02x$	0.87	(−0.04, 0.05)	(0.02, 0.03)	
Bakersfield	$y = 0.01 + 0.02x$	0.85	(−0.03, 0.05)	(0.01, 0.02)	
Irvine	$y = 0.36 - (0.37)(0.84)^x$	0.93	(0.23, 0.49)	(0.73, 0.96)	(0.26, 0.49)
Ventura	$y = 0.30 - (0.31)(0.83)^x$	0.95	(0.23, 0.37)	(0.73, 0.91)	(0.24, 0.37)
Exeter Vaportape	$y = 0.04 + 0.02x$	0.76	(−0.13, 0.09)	(0.01, 0.02)	
Riverside Vaportape	$y = 0.37 - (0.36)(0.87)^x$	0.90	(0.17, 0.56)	(0.74, 1.0)	(0.19, 0.52)
(b) TML					
Exeter	$y = 0.01 + 0.04x$	0.96	(−0.03, 0.04)	(0.03, 0.04)	
Riverside	$y = 0.03 + 0.05x$	0.96	(−0.02, 0.08)	(0.04, 0.06)	
Bakersfield	$y = -0.01 + 0.05x$	0.95	(−0.06, 0.03)	(0.04, 0.05)	
Irvine	$y = 0.73 - (0.75)(0.86)^x$	0.97	(0.55, 0.90)	(0.79, 0.92)	(0.60, 0.90)
Ventura	$y = 0.67 - (0.68)(0.85)^x$	0.98	(0.54, 0.78)	(0.80, 0.91)	(0.57, 0.79)
Exeter Vaportape	$y = -0.07 + 0.04x$	0.83	(−0.16, 0.01)	(0.03, 0.05)	
Riverside Vaportape	$y = -0.05 + 0.05x$	0.96	(−0.10, −0.01)	(0.04, 0.05)	
(c) DDVP					
Exeter	$y = 0.54 - (1.15)(0.86)^x$	0.83	(−0.63, 1.70)	(0.39, 1.3)	(0.11, 2.20)
Riverside	$y = -0.08 + 0.05x$	0.90	(−0.17, −0.01)	(0.04, 0.06)	
Bakersfield	$y = 0.75 - (1.51)(0.86)^x$	0.94	(−0.16, 1.66)	(0.61, 1.11)	(0.87, 2.16)
Irvine	$y = -0.02 + 0.05x$	*0.95*	(−0.08, 0.03)	(0.04, 0.06)	
Ventura	$y = 1.35 - (1.39)(0.96)^x$	0.98	(−0.42, 3.12)	(0.90, 1.02)	(−0.35, 3.12)
Exeter Vaportape	$y = 0.76 - (0.73)(0.65)^x$	0.92	(0.68, 0.84)	(0.51, 0.79)	(0.57, 0.88)
Riverside Vaportape	$y = 0.63 - (0.59)(0.61)^x$	0.91	(0.57, 0.69)	(0.45, 0.76)	(0.46, 0.72)

Table 3. Percent loss of RK (means ± SEM) for dispensers weathered under California summer climatic conditions at five locations from 24 July to 18 September 2012 (8 wk)

Location	Week							
	1	2	3	4	5	6	7	8
Exeter	4.2 ± 5.6	8.0 ± 2.9	14.4 ± 4.4	7.1 ± 5.7	28.7 ± 1.4	30.8 ± 1.1	33.3 ± 1.1	39.6 ± 2.0
Riverside	5.0 ± 3.3	3.3 ± 1.2	14.5 ± 2.8	4.9 ± 1.3	20.8 ± 3.9	32.6 ± 2.3	31.6 ± 2.7	32.5 ± 2.3
Bakersfield	7.9 ± 5.0	8.2 ± 3.8	17.3 ± 1.5	12.4 ± 2.5	27.9 ± 5.8	28.0 ± 2.6	34.0 ± 2.5	33.1 ± 5.1
Irvine	1.0 ± 2.1	12.3 ± 1.9	15.8 ± 1.6	3.6 ± 2.8	16.6 ± 5.5	38.1 ± 2.2	31.7 ± 2.4	34.9 ± 1.8
Ventura	4.2 ± 1.2	7.3 ± 2.6	15.4 ± 2.0	4.8 ± 3.0	22.3 ± 2.4	30.5 ± 3.3	32.3 ± 2.1	36.1 ± 1.6
Exeter Vaportape	0 ± 0	0 ± 0	3.1 ± 1.3	1.7 ± 1.7	13.3 ± 4.5	20.5 ± 2.0	33.8 ± 5.2	26.6 ± 1.8
Riverside Vaportape	0 ± 0	0 ± 0	0 ± 0	8.4 ± 2.4	0 ± 0	23.0 ± 1.5	27.9 ± 1.7	29.8 ± 1.7

Table 4. Percent loss of RK (means ± SEM) for dispensers weathered under California winter climatic conditions at five locations from 8 January to 2 April 2013

Location	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
Exeter	7.9 ± 1.4	5.8 ± 2.6	8.2 ± 2.4	2.7 ± 2.6	6.3 ± 3.4	8.0 ± 1.1	9.5 ± 1.6	4.9 ± 0.7	1.1 ± 1.1	15.2 ± 3.3	2.4 ± 1.1	3.8 ± 3.2
Riverside	6.9 ± 2.4	6.1 ± 1.2	7.7 ± 0.8	12.1 ± 1.0	8.6 ± 2.1	5.1 ± 2.6	6.1 ± 1.3	0.8 ± 0.5	1.1 ± 1.1	12.0 ± 1.0	2.8 ± 1.4	2.7 ± 1.0
Bakersfield	4.3 ± 2.2	3.9 ± 2.2	9.2 ± 3.6	8.2 ± 2.1	3.1 ± 1.0	5.6 ± 1.8	11.0 ± 0.5	6.7 ± 3.5	0.2 ± 0.2	14.2 ± 3.1	2.7 ± 1.8	1.9 ± 1.1
Irvine	4.2 ± 0.7	4.2 ± 2.5	11.0 ± 2.6	8.2 ± 0.6	5.3 ± 2.0	2.5 ± 1.5	10.8 ± 0.6	1.9 ± 1.2	1.4 ± 1.4	6.2 ± 2.9	4.8 ± 2.0	2.8 ± 2.8
Ventura	2.6 ± 1.7	4.5 ± 2.3	9.0 ± 2.8	6.5 ± 3.4	6.2 ± 1.8	4.1 ± 1.5	7.1 ± 2.3	1.3 ± 0.8	2.0 ± 2.0	1.0 ± 0.7	3.7 ± 2.8	2.2 ± 1.5
Exeter Vaportape	14.4 ± 0.8	7.9 ± 0.7	10.7 ± 4.0	9.5 ± 1.5	6.7 ± 1.7	10.8 ± 3.1	13.8 ± 3.9	4.8 ± 2.1	8.7 ± 1.8	18.7 ± 1.5	5.7 ± 1.9	6.0 ± 1.6
Riverside Vaportape	11.1 ± 2.9	9.8 ± 3.1	8.9 ± 1.8	8.4 ± 2.0	10.9 ± 3.1	12.4 ± 2.4	9.8 ± 1.3	9.0 ± 3.3	4.4 ± 0.9	14.9 ± 2.6	2.2 ± 1.3	0 ± 0

and TMR impregnated with DDVP. The use of insecticidal strips instead of liquid organophosphates (e.g., naled and malathion) would represent an important improvement to the safety of workers and in their acceptance of the practice, as was demonstrated in the Hawaii AWPM program (Vargas et al. 2016b). Chemical degradation models based on data collected at Exeter and Riverside confirm that insecticide strips could be used as a replacement for DDVP-impregnated wafers under environmental conditions prevalent in California, thus not requiring TMR wafers without DDVP, but with a VaportapeII strip, to be registered for survey purposes

In conclusion, based on four previous studies (Vargas et al. 2012, 2015, 2016a; and Shelly et al. 2012) and the present study, we propose three possible applications for solid lure detection traps in large mainland survey programs (such as California and Florida where there are 25,000 to 30,000 survey sites) utilizing ME, C-L/RK, and TML traps: 1) three individual traps baited with three separate solid wafers (TML, ME, and C-L/RK); 2) two individual traps baited with two solid wafers (TML and ME + C-L/RK); or 3) one trap baited with Mallet TMR (TML, ME, and C-L/RK; Vargas et al. 2016b).

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