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Characterization of spatial distribution of *Tetranychus urticae* in peppermint in California and implication for improving sampling plan

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Abstract. Twospotted spider mite, *Tetranychus urticae* Koch is an important pest of peppermint in California. Spider mite feeding on peppermint leaves causes physiological changes in the plant, which coupling with the favorable environmental condition can lead to increased mite infestations. Significant yield loss can occur in absence of pest monitoring and timely management. Understating the within-field spatial distribution of *T. urticae* is critical for the development of reliable sampling plan. The study reported here aims to characterize the spatial distribution of mite infestation in four commercial peppermint fields in northern California using spatial techniques, variogram and **S**patial **A**nalysis by **D**istance **I**ndic**E**s (SADIE). Variogram analysis revealed that there was a strong evidence for spatially dependent (aggregated) mite population in 13 of 17 sampling dates and the physical distance of the aggregation reached maximum to 7 m in peppermint fields. Using SADIE, 11 of 17 sampling dates showed aggregated distribution pattern of mite infestation. Combining results from variogram and SADIE analysis, the spatial aggregation of *T. urticae* was evident in all four fields for all 17 sampling dates evaluated. Comparing spatial association using SADIE, ~62% of the total sampling pairs showed a positive association of mite spatial distribution patterns between two consecutive sampling dates, which indicates a strong spatial and temporal stability of mite infestation in peppermint fields. These results are discussed in relation to behavior of spider mite distribution within field, and its implications for improving sampling guidelines that are essential for effective pest monitoring and management.

Key words: *Tetranychus urticae*, peppermint, sampling, semivariogram, SADIE, clustering indices

Introduction

Twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an economically significant pest of peppermint in the Pacific Northwest region including California (Frick 1961; Hollingsworth 1980; Marcum and Hanson 2006; Tollerup et al. 2013). All motile stages of *T. urticae* colonize the abaxial surface of foliage by constructing webs that has several functions including dispersal, reproduction, and protection from natural enemies (Gerson 1985; Kennedy and Smitley 1985). Spider mite feeding on leaves causes water stress in plants by imbalancing the water transpiration system (DeAngelis et al. 1982; 1983a) and reducing carbohydrate accumulation by interfering with the photosynthetic process (DeAngelis et al. 1983b). These physiological changes in the leaves and prolonged hot weather conditions ultimately favors spider mite reproduction and subsequently increases mite infestations in peppermint (Fuchs and Hirnyck 2000). Since peppermint oil contains a complex blend of several volatile compounds mainly monoterpenes (Gershenzon et al. 2000; Mahmoud and Croteau 2003; Kumar et al. 2014), severe mite infestation on leaves may likely influence oil quality by altering the biosynthetic pathways of these compounds (DeAngelis et al. 1983c). Severe *T. urticae* infestation in peppermint can cause crop loss of up to 70% in the absence of management intervention (Fuchs and Hirnyck 2000).

Success in implementing integrated pest management (IPM) principles (Stern et al. 1959) can be hindered by the lack of science-based action thresholds and reliable pest monitoring tools such as sampling plans to guide decision-making for pest control. On peppermint, Hollingsworth and Berry (1982a) developed a sampling plan for *T. urticae* in Oregon using a tally threshold of five mites per leaf and the action threshold of five mites per leaf based on 45 sample leaves. Recently, Tollerup et al. (2013) developed a separate sampling plan using a tally threshold of zero mites per leaf with an action threshold of five mites per leaf based on 20 sample leaves; this recommendation was based on studies conducted in California. However, these sampling plans were developed based on the models using mean-variance relationship (Taylor 1984; Kuno 1991; Young and Young 1990) without considering the true spatial distribution pattern of the pest population. Sampling plans that fail to account for the spatial distribution patterns of pest populations in the field can produce unreliable infestation assessments and lead to incorrect treatment decisions. In addition,

67 non-spatial techniques are unable to quantify and develop the distribution-based density maps that provide
68 visual representation of the infestation and can be useful in site-specific pest management.
69
70 Spatial patterns of insect densities can be characterized using variograms (Isaaks and Srivastava 1989;
71 Rossi et al 1992; Liebhold et al. 1993) that analyze and model the spatial dependence among individuals in
72 a population (Schotzko and O'Keeffe 1990; Williams et al. 1992). The spatial dependence (autocorrelation)
73 can be used to define sampling scales for independent samples, and to quantify the spatial pattern of insect
74 species (Williams et al. 1992). Spatial Analysis by Distance Indices, SADIE, is another method that has
75 been used to quantify the spatial distribution patterns of insect species based on ecological count data
76 (Perry 1995; Perry et al. 1999; Reay-Jones 2012; 2014). Within-field spatial distribution of *T. urticae*
77 infestations in peppermint has not been studied, and the sampling plan for monitoring and damage
78 assessment lacks this basic information. Since use of more than one method provides robust assessment
79 (Midgarden et al. 1993; Perry et al. 2002; Queiroz et al. 2010), we used two methods, variogram and
80 SADIE to characterize the spatial distribution of *T. urticae* in this study. The objective of this study was to
81 characterize the within-field spatial distribution pattern of *T. urticae* infestation using spatial techniques,
82 and to assist in improving sampling plan.
83
84 **Materials and Methods**
85 **Study sites and crop production practice**
86 The study reported here was conducted in 2014. Commercial peppermint fields in northern California with
87 a history of *T. urticae* infestations were selected from Tulelake of Siskiyou County (Field A & B; ~1.0 km
88 apart) and in McArthur of Shasta County (Field C & D, ~7.0 km apart), California. These sites are located
89 in the northeastern mountain region of California. Field-age ranged from 3-5 years and the area-size of
90 fields A, B, C and D was 28.3, 21.4, 20.2, 4.05 hectare, respectively. At these sites, peppermint was grown
91 according to recommended industry standards. Irrigation occurred at 7-10 day intervals using flooding
92 (Fields C & D) or the wheel line (Fields A and B) systems of irrigation depending on grower's existing
93 practices. All four fields were bordered by dirt roads at least from one side of the field. None of the fields
94 received any insecticides and acaricides during the growing period of the sampling year. The normal

95 growing season for peppermint is May-September. However, mid-June through August is the critical period
96 for pest management perspective. The average seasonal (June – September) temperature and humidity
97 ranges for Tulelake fields were 15.4 – 21.3^{0}C and 48.5 – 53.1%, respectively while those of McArthur
98 fields were 16.9 – 22.5^{0}C and 43.5 – 49.5%, respectively (CIMIS 2014). The rainfall accumulations during
99 the active mint growing period (June – September) of Tulelake and McArthur were 32.0 mm and 30.4 mm,
100 respectively.
101
102 **Sampling**
103 The mite sampling was conducted in an area of each field (16 m x 18 m) at least 6 m from the field edge.
104 The sampling area was comprised of 90 sample points distributed at every 2-m distance in a square grid
105 (i.e., 10 sample points across X-Coordinates, and 9 sample points across Y-Coordinates) demarcated by
106 using 91.5 cm tall marking flags (Blackburn Mfg. Co., Paso Robles, CA). From each sample point, one
107 fully-expanded top leaf of each of five adjacent stems around the marking flag was detached from the plant
108 and observed for the eggs and motile stages of spider mites present on those leaves *in situ* using a hand-
109 held lens (10X). Sampling was conducted weekly from Julian week (JW) 25 (= 3rd week of June) through
110 JW 31 (= 4th week of July) in McArthur and biweekly from JW 27 - JW 31 in Tulelake.
111
112 **Within-field spatial distribution**
113 Geostatistical method and Spatial Analysis by Distance IndicEs (SADIE) were used to characterize the
114 spatial distribution of motile *T. urticae* infestation in commercial peppermint fields. Data from the
115 sampling dates in which the *T. urticae* population ≥ 0.10 per five leaves were included in the analysis.
116
117 *Spatial aggregation using variogram analyses.* Variogram is a plot depicting the spatial
118 dependency of the sample points, and is one of the commonly used geostatistical techniques to assess
119 spatial autocorrelation (or spatial dependence). The spatial dependence is determined by developing an
120 experimental variogram which describes the relationship between sample values with distance and/or
121 direction within the sampling space. Mathematically, the semivariogram (γ) can be represented by (Davis
122 1994),

$$\hat{\gamma}(h) = \frac{1}{2}\, n(h)\, \sum_{i=1}^{n(h)} \lfloor z(x_i) - z(x_{i+h}) \rfloor^2 \ldots\ldots\ldots\ldots 1$$

where $\hat{\gamma}(h)$ is the estimated semivariance for the entity of interest (z) at all points (x_i) separated by lag distance (h), and *n(h)* is the number of pairs of samples separated by lag distance *h*.

T. urticae count datasets that did not meet the normality assumption were transformed (n = 16) using $\log(x + 1)$. All variograms were developed using the geostatistical software, GS$^+$ (Gamma Design Software 2008).

The variogram model has three parameters, range, sill ($C_0 + C$), and nugget (C_0) that determine the shape of the variogram. The maximum distance within which the spatial autocorrelation exists is the range (Liebhold et al. 1993; Fortin and Dale 2005). The semivariance value at which the variogram plot of $\hat{\gamma}(h)$ reaches a saturation point is the sill, and semivariance at zero lag distance is the nugget (Liebhold et al. 1993).

Nugget or linear variogram models do not have a definite sill and this signifies no discernible spatial dependence for the collected infestation data, i.e., the pest is randomly distributed within the field (Liebhold et al. 1991; Rossi et al. 1992). An aggregated distribution pattern is represented by the variograms with a definite sill and are an indicator of the existence of spatial dependence (Schotzko and O'Keeffe 1989; 1990). The empirical variogram is determined from collected spatial data and its attributes (i.e. sill, range, and nugget) can be estimated by fitting several theoretical variogram models such as spherical, exponential, and Gaussian. Spherical model reaches the specified sill at the specified range, while Gaussian and exponential models reaches the sill asymptotically at the practical range (i.e. the lag distance at which semivariance reaches to 95% of the specified sill). Spatial autocorrelation is practically zero beyond the range (Bohling 2005). These variogram models are best fitted on one of the three models, exponential, spherical, or Gaussian (Journel and Huijbregts 1978; Isaaks and Srivastava 1989). Based on directional component, there are two types of variograms. Variograms can account for spatial dependence that varies with direction in space (anisotropic) or is independent of direction (isotropic) (Isaaks and Srivastava 1989; Liebhold et al. 1993). The isotropic variograms contain more sample pairs than any other

151 directional variograms and therefore, produced more accurate and discernible semivariograms (Isaaks and
152 Srivastava 1989). Because of insufficient sample points to detect anisotropy (Liebhold et al. 1991;
153 Robinson and Metternicht 2006), and also no significant ecological relevancy of using the directional
154 variograms to study mite distributions, we used all isotropic variograms for the study reported here. The
155 best fitted isotrophic variogram models of *T. urticae* infestation in different sampling dates were selected
156 based on the greatest r^2 value (Park and Tollefson 2005; Frank et al. 2011; Rijal et al. 2014), although the
157 lowest residual sum of square is another criteria to select fitted variograms (Robertson 2008) The lag
158 distance used to generate the best fitted variograms ranged from 11.00 to 13.50 m with uniform lag interval
159 of 2 m. Nugget-to-sill ratio ($C_0/ C_0 + C$) was used to determine the extent of aggregation (Trangmar et al.
160 1986), where ratio < 0.25, 0.25 – 0.75, and > 0.75 indicate strong, moderate, and weak aggregation,
161 respectively (Farias et al. 2002; Frank et al. 2011; Rijal et al. 2014).
162
163 *Spatial aggregation using SADIE.* Spatial Analyses by Distance Indices (SADIE) has been used to
164 characterize the spatial distribution pattern of the insects and other arthropods and to test the statistical
165 significance of the aggregation. This method of spatial analysis is useful for ecological count data
166 generated from spatially-referenced sample points with *x* and *y* coordinates (Perry 1995; Perry et al. 1999).
167 Characterizing the spatial distribution using SADIE has the advantage that it is appropriate to determine
168 spatial distribution of ecological count data where many samples points in the grid likely have no counts
169 (Madden and Hughes 1995, Perry 1998, Perry et al. 2002). However, semivariogram is sensitive to low
170 population and may not detect the spatial structure with light pest infestation in which many sample points
171 have zero counts (Blom and Fleischer 2001). SADIE measures the overall aggregation based on the
172 distance to regularity (*D*), which represents the minimum total distance that individuals would need to
173 move in order to achieve the same number (i.e. mean) for each sample point within a sampling area. Higher
174 *D* value indicates stronger aggregation. The magnitude of *D* is assessed by a randomization test in which
175 permutations of all observed counts among sample points are performed (Perry and Dixon 2002). The
176 assessment provides an index of aggregation, I_a with an associated probability, P_a. Aggregated, uniform,
177 and random distribution patterns are indicated by $I_a > 1$, $I_a = 1$, and $I_a < 1$, respectively (Perry 1995). The

178 associated probability (i.e. $P_a < 0.025$) determines whether or not the resultant distribution pattern is
179 significantly different from randomness (Perry 1995; Reay-Jones 2012; Rijal et al. 2014).
180 Another advantage of using SADIE is the ability to generate distribution maps that predict the spatial
181 ecology of pest infestations such as the location, size, and dimension of aggregation clusters. Clustering
182 index is a unique value calculated for every sample point based on the nature of the cluster. A cluster is
183 defined as a selection of neighboring sample units whose mean is greater (a patch) or smaller (a gap) than
184 the overall sample mean of the experimental area. The clustering indices were used to generate infestation
185 distribution maps for individual sampling date and field combinations. Furthermore, mean clustering
186 indices representing all units in a patch are denoted by $\overline{v}_i$ with associated *P*-value, $P\overline{v}_i$, while mean cluster
187 indices representing all units in a gap are denoted by $\overline{v}_j$ with associated *P*-value, $P\overline{v}_j$. The presence of
188 statistically significant patches and gaps are indicated by $P\overline{v}_i < 0.025$ and $P\overline{v}_j < 0.025$, respectively. In this
189 study, cluster index-based distribution maps were used to characterize the spatial distribution of *T. urticae*
190 infestation counts on peppermint fields based on 90-point sample grids. On these maps, patches refer to
191 areas whose densities exceed the mean threshold of 1.5, whereas a gap refers to areas whose densities fall
192 below an index threshold of -1.5 (Perry et al. 1999).
193
194 The calculation of the index of aggregation and index of clustering in SADIE was carried out using
195 SADIEShell (Rothamsted Experimental Station 2008). In total, 150 permutations and 12345
196 randomizations with a non-parametric option were used for SADIE analyses. Estimated infestation
197 distribution maps showing patches and gaps of the peppermint fields for different sampling dates were
198 developed using JMP (SAS Institute 2010).
199
200 Spatial association, indicated by index of spatial association (*X),* can be determined to establish relationship
201 between two datasets measured in two different occasions from the same spatially referenced location
202 (Perry 1998). The datasets can include two temporally separated samplings for one species, two pest
203 species measured during one occasion, two variables (i.e. insect population and injury) measured in one
204 occasion (Reay-Jones 2012). This information may be helpful to better explain the ecological roles of
205 different factors in spatial distribution and in sampling. In our study, an overall index of spatial association

206 of mite distributions between two consecutive sampling was calculated. Significant positive association (X
207 > 0; $P < 0.025$) indicates presence of either a gap or a patch for both variables (i.e. mite counts in two
208 consecutive sampling dates) while significant negative association ($X < 0$; $P > 0.975$) indicates association
209 of a patch of one variable with a gap of the other variable or vise-versa (Reay-Jones 2014). The spatial
210 association between two datasets was conducted using N_AShell which is also a part of the SADIE
211 (Rothamsted Experimental Station 2008).
212
213 **Results**
214 **Temporal distribution of *T. urticae* infestation in peppermint.** The infestation of mites varied based on
215 sampling date and the site in Tulelake. In Field A, there was low infestation (0.29 mites per five leaves) on
216 JW 27 and which increased to 3.36 and 3.88 mites per five leaves on JW 29 and JW 31, respectively (Fig.
217 1a). Mean number of mite eggs per five leaves was the highest at JW 31 (Fig. 1b). In Field B, mean
218 numbers of mites were 0.88, 1.9 and 1.57 on JWs 27, 29, and 31, respectively (Fig. 1a). The presence of
219 eggs per five leaves in JWs 29 and 31 was lower in Field B, compared to Field A (Fig. 1b). In McArthur,
220 there was a wide gap in the degrees of mite infestation (both eggs and mites) between two fields (Fig. 2a,
221 b). In Field C, mean number of mites per five leaves was 0, 2.46, 3.46, 5.94, 3.93, 4.58, and 3.96 on JWs
222 25, 26, 27, 28, 29, 30, and 31, respectively (Fig. 2a). In Field D, no mite and egg infestations were recorded
223 during JWs 25 and 26. The mean spider mite counts were extremely low from 0.09 (JW 27), 0.47 (JW 28),
224 0.23 (JW 29), 0.47 (JW 30), and 0.21 (JW 31) mites per five leaves (Fig. 2a). The number of eggs per five
225 leaves in Field D remained low throughout the season (Fig. 2b).
226
227 **Within-field distribution of *T. urticae* in peppermint.** Variogram and SADIE analyses detected
228 significant levels of spatial dependence in *T. urticae* infestations from 16 sample dates (Table 1 and 2).
229
230 *Spatial aggregation using variogram analysis.* Omnidirectional variograms were used to
231 characterize the spatial dependence of *T. urticae* distribution in commercial peppermint fields in northern
232 California. Based on the fitted models, r^2 and RSS values, and nugget-to-sill ratio (C_0/C_0+C), variograms
233 showed aggregation distribution pattern of spider mite infestation in all sampling dates of Field A (JWs 27,

29, 31; r^2 = 0.09, 0.84, 0.85, respectively) and Field B (JWs 27, 29, and 31; r^2 = 0.13, 0.15, and 0.36, respectively) and Field C (JWs 26, 27, 28, 29, 30, and 31; r^2 = 0.17, 0.18, 0.59, 0.42, 0.92, and 0.93, respectively) (Table 1). In Field D, aggregated distribution pattern of spider mite infestation was detected on JW 29 (r^2 = 0.48), while aggregation was not evident for rest of the weeks (JWs 27, 28, 30, and 31; r^2 = 0.78, 0.92, 0.96, and 0.39, respectively) (Table 1). In 17 sampling dates across four fields, Gaussian (Field A, JW 27; Field C, JW 27), spherical (Field C; JWs 29, 31), exponential (Field A, JWs 29, 31; Field B, JWs 27, 29, 31; Field C, JWs 27, 28, 30; Field D, JW 29), linear (Field D, JWs 27, 28, 30) , and nugget (Field D, JW 31) models were fitted (Table 1). The nugget-to-sill ratio (C_0/C_0+C), measures of degree of aggregation, was <0.25 in 13 sampling dates with evidence of aggregated distribution patterns of spider mite infestation (Table 1). The range value of variograms were between 2.14 to 6.51 (Table 1).

Spatial aggregation using SADIE. Aggregation distribution pattern of spider mite infestation as indicated by the SADIE-based aggregation index (I_a) >1 was detected in 11 of 1 sampling dates analyzed. However, the aggregation was significant ($P < 0.025$) in 7 of 17 sampling dates evaluated (Table 2). Mite within-field aggregation distribution patterns at JW 31 for Field A (I_a = 1.378; P = 0.023), and Field B (I_a = 1.675; P = 0.001) were significant. The spatial distribution at JW 29 was significantly aggregated in Field C (I_a = 1.569; P = 0.002), while 4 of 5 sampling dates from Field D showed statistically a significant aggregation (JW 27, I_a = 1.908, P = <0.001; JW 28, I_a = 2.133, P = <0.001; JW 29, I_a = 1.908, P = <0.001; JW 30, I_a = 2.024, P = <0.001) of mites (Table 2).

The clustering indices of individual sample points were calculated based on the mite counts in several sampling dates for four fields (Table 2). The statistically significant ($P < 0.025$) patches and gaps were detected in Field B (JW 31), Field C (JW 29) and Field D (JWs 27-30) (Table 2). Estimated infestation density maps developed by using clustering indices showed mite infestation distribution pattern within the sampled area visually (Fig 3, Fig. 4.).

Spatial association using SADIE. In Field A and B, there was not a significant association of *T. urticae* infestations between two consecutive samplings conducted at 2-wk interval (Table 3). In Field C,

significant positive associations between JW 26 and 27 ($X = 0.931$; $P = <0.001$), JW 27 and 28 ($X = 0.935$; $P = <0.001$), JW 28 and 29 ($X = 0.862$; $P = <0.001$), JW 29 and 30 ($X = 0.574$; $P = <0.001$), and JW 30 and 31 ($X = 0.650$; $P = <0.001$) were detected. Similarly, significant positive associations between JW 27 and 28 ($X = 0.421$; $P = 0.006$), JW 28 and 29 ($X = 0.336$; $P = 0.009$), and JW 29 and 30 ($X = 0.330$; $P = 0.013$) were detected, while no significant spatial association was observed in mite counts between JW 30 and 31 (Table 3).

Discussion

This study provides the first evidence of characterizing and developing the spatial distribution maps of *T. urticae* in peppermint. The characterization of spatial distribution of *T. urticae* in peppermint has provided crucial information for development of an effective sampling plan which will ultimately improve the monitoring of this pest and assist in effective pest management targeting this economically important pest. Combining variogram and SADIE-based analysis results, *T. urticae* infestations in all four fields showed a true spatial dependency (i.e., spatial aggregation) regardless of the sampling date. A positive spatial association of mite infestation was detected in ~62% of the total sampling pairs (n = 13) used, indicating a strong spatial and temporal stability of mite infestation distribution. The physical distance of peppermint field in which spider mite population were aggregated ranged from 2.14 to 6.51 m, based on the range value of the variograms.

Infestation density maps showed the extent of aggregation in the fields with infestation clusters. The 'edge effect' has been reported for spider mite infestations in several crops (Margolies and Kennedy 1985). In this study, a high density aggregation area (patch) in one side and low density aggregation area (gap) on rest of the sampled area was evident in Field D (Fig. 4), which was strongly supported by the spatial association analysis results showing a positive association among four consecutive samplings. Spatial dependency was not evident in 80% of the sampling dates (Table 1) when variogram was used, and it might have been due to little to no sensitivity of this technique for extremely low degree of infestations (Blom and Fleischer 2001). Similar variability in detecting spatial distribution using variograms were reported previously for other insects (Farias et al. 2002; 2003; Wright et al. 2002), but this issue was addressed by

290 coupling variograms with the SADIE (Kamdem et al. 2012; Perry and Dixon 2002; Rijal et al. 2014) in this
291 study. The different results from the two types of spatial analyses could possibly be due to the different
292 ways of calculating the spatial weights for individual sample points (Kamdem et al. 2012), and results from
293 one method should not negate the results from other methods (Perry et al. 2002, Queiroz et al. 2010).
294
295 Presence of patch or gap comparing two consecutive samplings was consistent for all sampling dates in
296 Field C (Table 3). The results indicate that *T. urticae* population concentrated into certain areas for the
297 major part of the growing season, likely due to variation in within-field environmental conditions for
298 survival. Since aggregation distribution pattern of spider mites in peppermint renders more or less stable
299 throughout the growing season, the distribution maps for site-specific management remain consistent
300 regardless of the sampling date. This is the first report of the spatial association between two different
301 sample times in two spotted spider mites and has an important implication in site-specific pest
302 management.
303
304 An understanding of the spatial distribution of *T. urticae* in peppermint is an important first step for the
305 development of accurate sampling plan and for selecting suitable experimental designs (Williams et al.
306 1992). Based on the range value of variograms, the farthest distance at which the samples are remained
307 spatially dependent was ~7 m. If the purpose of the sampling is to develop estimated density maps, the
308 sampling distance should be within the distance of spatial dependence (<7 m) to identify the aggregation
309 hot-spots (Weisz et al. 1995, Fleischer et al. 1999, Frank et al. 2011) and apply targeted control measures
310 (Weisz et al. 1996, Blom et al. 2002). Although producing density maps every year may not be pragmatic
311 for mint growers, the maps produced every in few years should still provide a good reference about mite
312 infestation patterns in a particular field and assist pest management planning. For regular pest monitoring to
313 decide treatment thresholds, the sampling distance should be higher than 7 m (i.e., the diameter of the
314 aggregated area) to obtain independent samples. This crucial information had never been quantified and
315 used previously in developing sampling plans for spider mites in peppermint. Since spatial dependence
316 occurs at every 7m or less, the sampling distance for unbiased sampling should be 7.1 m at the minimum or
317 multiple of that distance depending on the purpose of the sampling and practicality. Given the strong

aggregation of spider mite population, and relatively low degree of infestation in peppermint fields in California, current sample size (i.e. 7 random locations per 16-ha) proposed by Tollerup et al. (2013) likely underestimate the actual mite population. Despite their findings about the sample size, Tollerup et al. (2013) also suggested to scale up the sample size based on local field conditions. Although tentative, here we propose a systematic sampling plan consisting of five random leaves from each of four sample locations per hectare basis, with the maximum sample locations being 40 for the average field size (~15-20 ha), and the sample locations should be separated by ~50 m. Despite the systematic sampling plan, early in the season when mite infestation is light, random sampling in the areas including that with relatively dry portion of the field, near to the dirt roads, and edges should still provide a reasonable estimation of the mite population. Further studies should focus on the validation of the systematic sampling plan in comparison to the existing plans and its effectiveness to monitor mite populations.

Overall, the infestation of *T. urticae* in northern California in 2014 remained low. In McArthur, the mite population was relatively high in Field C with peak counts during 2nd week of July (JW 28). In Tulelake, both fields (A and B) showed high spider mite pressure starting 3rd week of July (JW 29) until the harvest (JW 31). The degree of infestation of *T. urticae* population in peppermint crop depends on several biotic and abiotic factors such as weather condition, intraspecific competition, host plant condition, predators and other natural enemies, and agricultural practices (McMurtry et al. 1970; van de Vrie et al. 1972; Hollingsworth and Berry 1982b). Most of the growers tend to apply control measures targeting spider mites before reaching the recommended threshold level (i.e., 5 mites/leaf developed in Oregon) in California (L. D. Godfrey, personal communication). This might have been due to a potential risk of higher damage in peppermint by spider mites in California compared with Oregon because of differences in seasonal weather and natural enemy population. Morris et al. (1999) reported the occurrence of three predatory mite species [*Neoseiulus fallacis* (Garman)*, Typhlodromus pyri* Scheuten *Amblyseius andersoni* (Chant)] with 3.6 times higher abundance of *N. fallacis* in peppermint in Oregon compared with California, and only two species (*N. fallacius, A. andersoni*) were found in California. Future study should focus to assess the occurrence and abundance of predatory mite species in peppermint in California.

From this study, we were able to characterize the within-field distribution of the *T. urticae* in peppermint using spatial methods. In addition, we have provided a criterion for sampling distance for site-specific and traditional pest management practices based on the true spatial distribution of the mite population in the field. This information may be useful for growers and other stakeholders to design and improve the monitoring and infestation evaluation plan for *T. urticae* in peppermint.

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Table 1. Best fitted Variogram models and parameters representing the spatial distribution of twospotted spider mites in peppermint in northern California

Field	Julian week	Mean number of TSM per five leaves (± SE)	Range (m)	Model	r^2	RSS	C_0	C_0+C	C_0/C_0+C
Field A	27	0.29 ± 0.10	2.14	Ga	0.09	0.00009	0.019	0.154	0.123
	29	3.36 ± 0.82	4.47	Ex	0.86	0.00330	0.001	0.785	0.001
	31	3.88 ± 0.41	5.04	Ex	0.85	0.00295	0.062	0.679	0.091
Field B	27	0.88 ± 0.25	3.24	Ex	0.13	0.78000	0.010	4.429	0.002
	29	1.90 ± 0.37	2.82	Ex	0.15	3.10000	0.010	11.540	0.001
	31	1.57 ± 0.24	3.15	Ex	0.36	0.00294	0.018	0.495	0.036
Field C	26	2.46 ± 0.31	2.17	Ga	0.17	0.00064	0.069	0.579	0.119
	27	3.46 ± 0.46	2.31	Ex	0.18	0.00251	0.073	0.708	0.103
	28	5.94 ± 0.58	2.52	Ex	0.59	0.00119	0.107	0.957	0.112
	29	3.93 ± 0.32	3.19	Sp	0.42	0.00320	0.007	0.642	0.011
	30	4.58 ± 0.31	4.26	Ex	0.92	0.00059	0.089	0.628	0.142
	31	3.96 ± 0.27	3.66	Sp	0.93	0.06060	0.320	6.737	0.047
Field D	27	0.10 ± 0.04	-	Li	0.78	0.00010	0.021	0.106	-
	28	0.47 ± 0.13	-	Li	0.92	0.00027	0.126	0.360	-
	29	0.23 ± 0.06	6.51	Ex	0.48	0.00052	0.009	0.106	0.084
	30	0.47 ± 0.12	-	Li	0.96	0.00040	0.126	0.255	-
	31	0.21 ± 0.06	-	Nu	0.39	0.00006	0.095	0.095	-

TSM, twospotted spider mite; RSS, residual sum of squares; C_0 , nugget; $C_0 + C$, sill; C_0/C_0+C , nugget-to-sill ratio; Nu, nugget model ($C_0 = C_0 + C$); Ga, Gaussian model; Ex, exponential model; Sp, spherical model; Li, linear model.

Table 2. SADIE parameters for *T. urticae* infestations in peppermint in northern California

Field	Julian week	Mean number of TSM per five leaves (± SE)	I_a	P_a	$\overline{v}_j$	$P\overline{v}_j$	$\overline{v}_i$	$P\overline{v}_i$
Field A	27	0.29 ± 0.10	0.903	0.706	-0.905	0.698	0.898	0.731
	29	3.36 ± 0.82	1.133	0.173	-1.039	0.319	1.090	0.226
	31	3.88 ± 0.41	1.378	0.024*	-1.256	0.059	1.234	0.079
Field B	27	0.88 ± 0.25	0.913	0.689	-0.913	0.686	0.926	0.648
	29	1.90 ± 0.37	0.786	0.978	-0.845	0.902	0.795	0.971
	31	1.57 ± 0.24	1.675	0.001**	-1.674	0.001**	1.652	0.001**
Field C	26	2.46 ± 0.31	0.875	0.806	-0.895	0.742	0.890	0.761
	27	3.46 ± 0.46	0.962	0.526	-0.973	0.507	0.952	0.573
	28	5.94 ± 0.58	1.017	0.379	-1.034	0.34	1.051	0.308
	29	3.93 ± 0.32	1.569	0.002**	-1.580	0.004**	1.530	0.005*
	30	4.58 ± 0.31	1.134	0.170	-1.061	0.277	1.016	0.361
	31	3.96 ± 0.27	0.971	0.504	-0.927	0.628	0.703	0.526
Field D	27	0.09 ± 0.04	1.908	<0.001**	-1.921	<0.001**	1.833	<0.001**
	28	0.47 ± 0.13	2.133	<0.001**	-2.138	<0.001**	2.150	<0.001**
	29	0.23 ± 0.06	1.908	<0.001**	-1.918	<0.001**	1.914	<0.001**
	30	0.47 ± 0.12	2.024	<0.001**	-2.026	<0.001**	2.006	<0.001**
	31	0.21 ± 0.06	1.249	0.070	-1.246	0.072	1.264	0.061

[§] Means were calculated based on the total of motile TSM counted per five leaves.

* Significant at $P < 0.025$, ** significant at $P < 0.001$

I_a: Index of aggregation; P_a: P value of I_a

$\overline{v}_j$: Mean clustering index value over the gap units; $P\overline{v}_j$: P value of $\overline{v}_j$

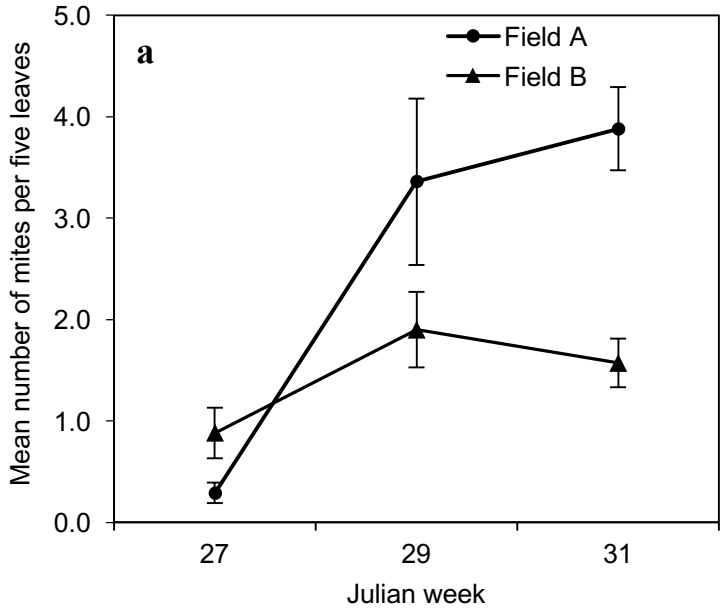
$\overline{v}_i$: Mean clustering index value over the patch units; $P\overline{v}_i$: P value of $\overline{v}_i$

Table 3. SADIE spatial association parameters for *T. urticae* population between two consecutive samplings in peppermint in northern California

Field	Sampling	Index of association (*X*)	*P*-value
Field A	JW 27 *vs.* JW 29	-0.185	0.414
	JW 29 *vs.* JW 31	-0.025	0.542
Field B	JW 27 *vs.* JW 29	0.3448	0.032
	JW 29 *vs.* JW 31	0.006	0.404
Field C	JW 26 *vs.* JW 27	0.931	<0.001**
	JW 27 *vs.* JW 28	0.935	<0.001**
	JW 28 *vs.* JW 29	0.862	<0.001**
	JW 29 *vs.* JW 30	0.574	<0.001**
	JW 30 *vs.* JW 31	0.650	<0.001**
Field D	JW 27 *vs.* JW 28	0.421	0.006*
	JW 28 *vs.* JW 29	0.336	0.009*
	JW 29 *vs.* JW 30	0.330	0.013*
	JW 30 *vs.* JW 31	0.037	0.332

* Significant at $P < 0.025$, ** significant at $P < 0.001$

Fig. 1. Mean (±SE) number of a) motile *T. urticae*, and b) eggs of *T. urticae*, in peppermint in Tulelake, CA, 2014



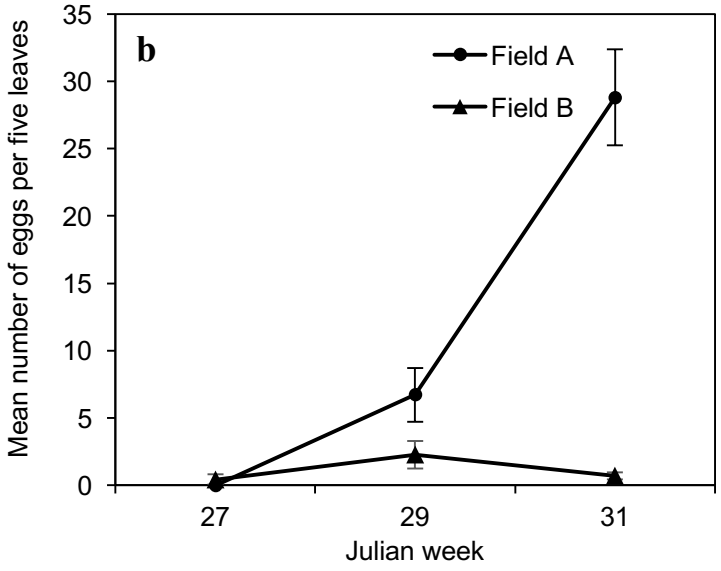


Fig. 2. Mean (±SE) number of motile *T. urticae*, and b) eggs of *T. urticae*, in peppermint in McArthur, CA, 2014

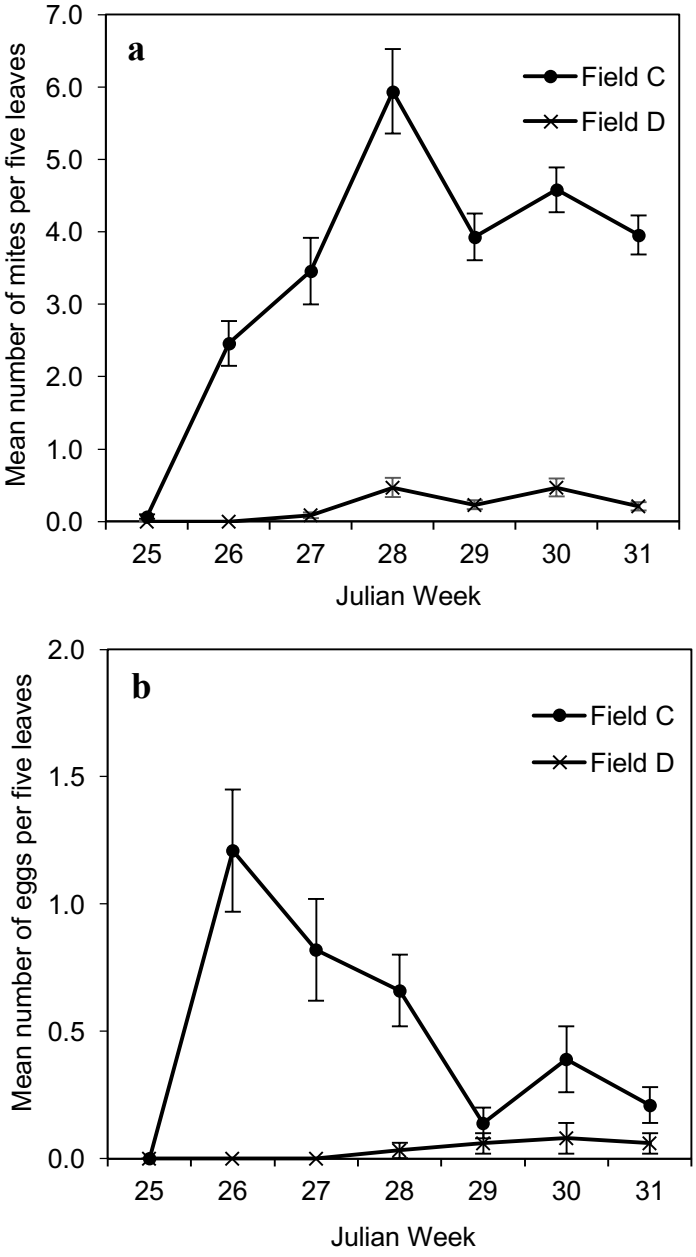


Fig. 3. Estimated distribution map (developed by using clustering indices) of spider mite infestation across different sampling dates from peppermint fields in Tulelake, California. The dark colored area represents aggregation with high infestation "patch, and the light colored area represents aggregation with low infestation "gap".

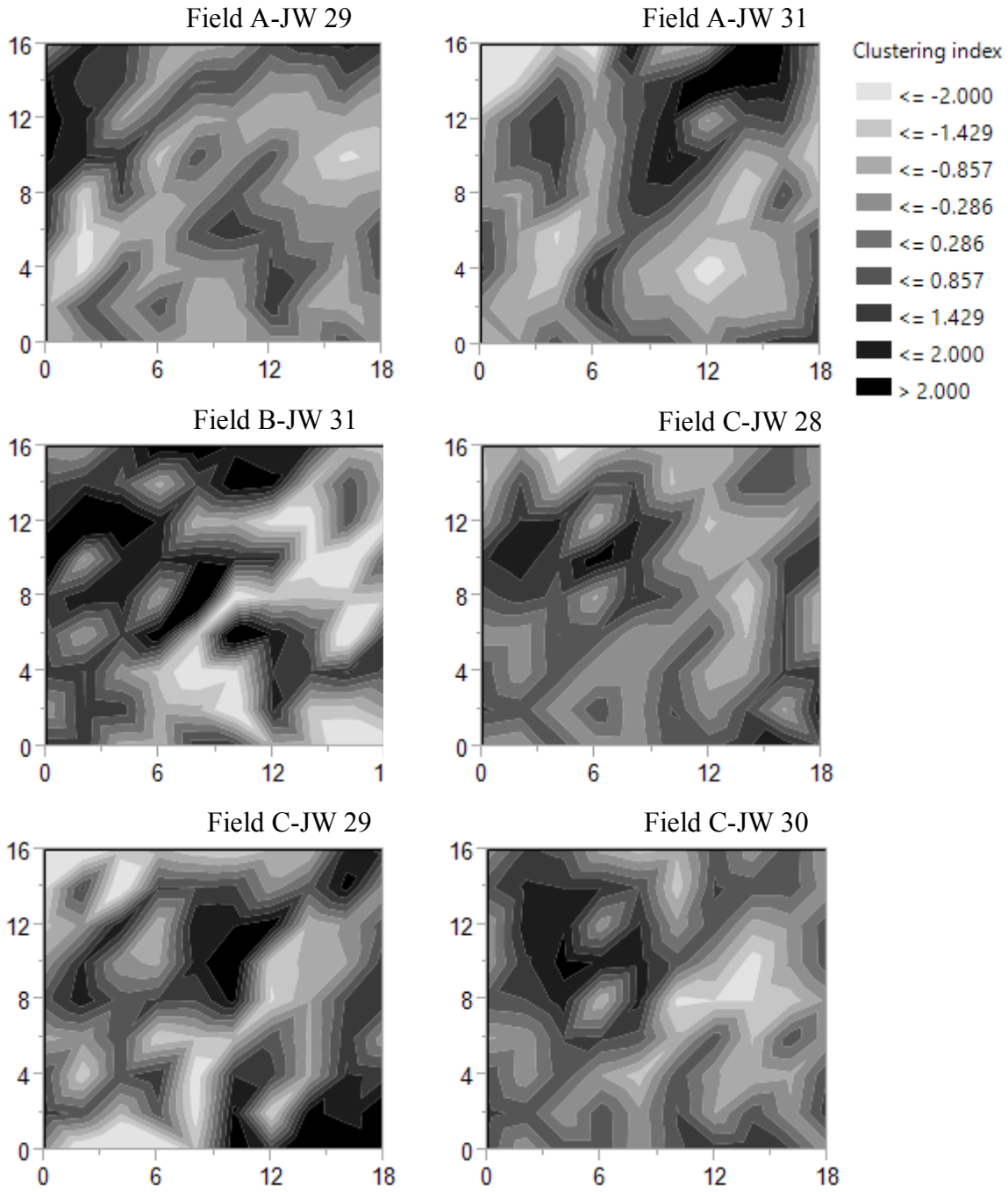


Fig. 4. Estimated distribution map (developed by using clustering indices) of spider mite infestation across different sampling dates from peppermint fields in McArthur, California. The dark colored area represents aggregation with high infestation "patch, and the light colored area represents aggregation with low infestation "gap".