

 Open access • Journal Article • DOI:10.1093/JEE/TOY248

## Witch's Broom Disease of Lime (*Candidatus Phytoplasma aurantifolia*): Identifying High-Risk Areas by Climatic Mapping. — [Source link](#)

Philip Donkersley, Justine I. Blanford, Renan Batista Queiroz, Farley W. S. Silva ...+3 more authors

**Institutions:** Universidade Federal de Viçosa, Pennsylvania State University, Sultan Qaboos University

**Published on:** 14 Dec 2018 - Journal of Economic Entomology (Entomological Society of America)

**Topics:** Witch's broom

Related papers:

- [Transmission of 'Candidatus Phytoplasma aurantifolia' to Bakraee \(Citrus reticulata Hybrid\) by Feral Hishimonus phycitis Leafhoppers in Iran.](#)
- [Invasive mutualisms between a plant pathogen and insect vectors in the Middle East and Brazil.](#)
- [Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens.](#)
- [Witches' broom disease of Mexican lime trees: disaster to be addressed before it will be too late](#)
- [First report of a 'Candidatus Phytoplasma asteris' isolate associated with a witches' broom disease of alfalfa in Bolivia.](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/witch-s-broom-disease-of-lime-candidatus-phytoplasma-4da8niq3hj>

1 **Witch's Broom Disease of Lime (*Candidatus Phytoplasma***  
2 ***aurantifolia*): identifying high-risk areas by climatic**  
3 **mapping**

4 Philip Donkersley<sup>a\*</sup>, Justine M Blanford<sup>b</sup>, Renan Batista Queiroz<sup>ab</sup>, Farley W. S. Silva<sup>a</sup>,  
5 Claudine M. Carvalho<sup>c</sup>, Abdullah Mohammed Al-Sadi<sup>d</sup> & Simon L. Elliot<sup>a</sup>

6  
7 <sup>a</sup>Department of Entomology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

8 <sup>b</sup>Department of Geography, Penn State University, Pennsylvania, United States of America

9 <sup>c</sup>Department of Phytopathology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

10 <sup>d</sup>Department of Crop Sciences, College of Agricultural and Marine Sciences,

11 Sultan Qaboos University, PO Box 34, Al-Khod 123, Oman

12 \*Corresponding author: [donkersley@gmail.com](mailto:donkersley@gmail.com)

13

14

15 **Running title: Bioclimatic analysis of lime phytoplasma**

16 **Keywords:** bioclimatic modelling; witches' broom disease of lime; acid citrus lime; Middle  
17 East; Brazil.

18

19 **Abstract**

20 Biological invasions of vectorborne diseases can be devastating. Bioclimatic modelling  
21 provides an opportunity to assess and predict areas at risk from complex multi-trophic  
22 interactions of pathogens, highlighting areas in need of increased monitoring effort. Here, we  
23 model the distribution of an economically critical vectorborne plant pathogen '*Ca.*  
24 *Phytoplasma aurantifolia*', the etiological agent of Witches' Broom Disease of Lime (WBDL).

25 This disease is a significant limiting factor on acid lime production (*Citrus aurantifolia*) in the  
26 Middle East and threatens its production globally. We found that temperature, humidity and  
27 the vector populations significantly determine disease distribution. Following this, we used  
28 bioclimatic modelling to predict potential novel sites of infections. The model outputs  
29 identified potential novel sites of infection in the citrus producing regions of Brazil and China.  
30 We also used our model to explore sites in Oman where the pathogen may not be infectious,  
31 and suggest nurseries be established there. Recent major turbulence in the citrus agricultural  
32 economy has highlighted the importance of this work and the need for appropriate and targeted  
33 monitoring programs to safeguard lime production.

34

## 35 **Introduction**

36 Monitoring of animal and plant diseases vectored by insects is critical to their  
37 management. Insect vectors have a role in spreading pathogens amongst humans, animals and  
38 crop plants. Vectorborne pathogens create a worldwide strain on healthcare and food security.  
39 In the face of this challenge, bioclimatic niche models have become fundamental tools for  
40 exploring the potential spread of vectorborne diseases in medical, veterinary, or agricultural  
41 contexts (Kriticos et al. 2013).

42 Globalisation has increased the rate of spread of many pathogens (Perrings et al. 2005,  
43 Smith et al. 2007); yet our limited knowledge of the ranges of many arthropod-borne pathogens  
44 restricts our ability to respond to this growing issue. Climate has long been recognised as an  
45 important environmental determinant of the distribution of pests (Gregory et al. 2009), while  
46 niche models can be useful for projecting the distributions and relative abundances of a wide  
47 range of invasive insects, weeds and pathogens under both current and future climatic  
48 conditions (see (Hijmans et al. 2000, Yonow et al. 2004, Kriticos et al. 2005) for examples).

49           Phytoplasmas are plant pathogens that have been recognized in more than 700 host  
50 plant species (Lee et al. 1998, Berges et al. 2000; Bertaccini, 2007; Hogenhout et al., 2008).  
51 They are dependent on phloem-feeding insect vectors, such as the hemipteran leafhoppers,  
52 planthoppers and psyllids (Weintraub and Beanland 2006). Phytoplasmas demonstrate a  
53 variety of pathologies in their various host plants; a number of them represent major economic  
54 threats to agriculture globally (Berges et al. 2000). Here, we develop a bioclimatic model on  
55 the distribution and spread of an invasive vectorborne phytoplasma that has devastated lime  
56 agriculture in the Middle East and is a threat to Brazil (Ghosh et al. 2013).

57           The phytoplasma '*Candidatus* Phytoplasma aurantifolia' is the etiological agent of  
58 Witches' Broom Disease of Lime (WBDL). This disease has infected an estimated 98% of  
59 limes currently grown in Oman (Al-Yahyai et al. 2015). Lime production in the region  
60 principally employed acid lime (*Citrus aurantifolia* Swingle) and WBDL has spread across the  
61 Middle East and resulted in the destruction of more than 50% of the cultivated lime area and  
62 75% loss in production quantities (Al-Yahyai et al. 2015, FAO 2015). WBDL kills lime trees  
63 within 5 years of initial infection and has become a major limiting factor for lime production  
64 in the Middle East (Bove and Garnier 2000, Chung et al. 2009). The phytoplasma can be both  
65 insect-vectored and graft-vectored; insect vectors are known to include the planthopper  
66 *Hishmonus phycitis* and the psyllid *Diaphorina citri* (Salehi et al. 2007, Nascimento da Silva  
67 et al. 2015, Queiroz et al. 2016). Although these vectors are culpable in transmitting the  
68 phytoplasma between trees within a field, transmission through grafting of infected tissue is  
69 likely more important in moving the infections across international borders.

70           Recently an infection of acid lime by '*Ca. Phytoplasma aurantifolia*' was reported from  
71 São Paulo State, Brazil (Texeira et al. 2005, Silva et al. 2014). This infection was notably  
72 asymptomatic, indicating differences in host-pathogen interactions compared with the Middle  
73 East (Silva et al. 2014). Identification of phytoplasma-induced WBDL is primarily based on

74 symptoms (Ghosh et al. 1999), which is problematic for monitoring the spread of asymptomatic  
75 infections. Molecular tools have been developed for identification of WBDL from field  
76 samples (Ghosh et al. 2013, Al-Yahyai et al. 2015), but remain prohibitively expensive for  
77 widespread implementation by growers. Considerable research effort and resources have been  
78 devoted to development of on-the-spot diagnostics in plant pathology, and have shown success  
79 in control and monitoring the spread of some plant diseases (e.g. *Potato Virus Y*), but do not  
80 exist for phytoplasmas yet (De Boer and López 2011). *In-situ* kits exist for testing  
81 phytoplasmas using immunofluorescence, but have not been adopted for widespread use thus  
82 far (Rad et al. 2012, Kashyap et al. 2016). Novel sites of infection, expense of monitoring and  
83 damage the pathogen has already caused highlights the importance of using bioclimatic models  
84 for identifying potential areas at risk by this phytoplasma.

85         The centre of origin for citrus and *D. citri* is in South-east Asia, New Caledonia and  
86 Australia (Swingle 1967) and lime is generally cultivated within the tropical, subtropical and  
87 temperate regions from 40°N to 40°S (Samson 1986, Mukhopadhyay 2004). Despite this, our  
88 records indicate that '*Ca. Phytoplasma aurantifolia*' has almost exclusively been detected in  
89 Oman (Bove 1986), the United Arab Emirates and Iran (Mardi et al. 2011), while related strains  
90 of the pathogen in the Nagpur region of India in 1999 (Ghosh et al. 1999). Considering the  
91 centres of origin and current cultivated distribution of lime, it becomes evident that the  
92 phytoplasma may be present far beyond its current detected range. The Middle East, India,  
93 Pakistan, Brazil, Argentina and Mexico grow lime as a key part of their agricultural economy  
94 (Liu et al. 2012, Al-Yahyai et al. 2015). The infection has been detected recently in Brazil  
95 (Silva et al. 2014), which highlights concerns that this pathogen may have already spread  
96 beyond its current known range.

97         In this study, we have gathered data on the distribution of phytoplasma-infected and  
98 uninfected lime trees in orchards in Oman as well as environmental variables to better

99 understand how these may influence its distributions. We used our findings to develop a model  
100 that could then be used to predict the likely distribution of the phytoplasma based on  
101 environmental data and explore areas of risk in different parts of the world. A Geographic  
102 Information System (GIS) was used to map and explore, globally, areas at risk.

103

## 104 **Materials and Methods**

### 105 *Factors determining distribution of phytoplasma in Oman*

106 The distribution of ‘*Ca. Phytoplasma aurantifolia*’ was surveyed across 12 lime  
107 orchards in Oman (Burka, Musanah, Samael, Suwayq). Infection incidence (proportion of  
108 trees), vector abundance (counts of individuals) and environmental data were collected weekly  
109 from June 2013 to March 2014, with each orchard sampled every four weeks. Because of the  
110 cryptic nature of some phytoplasma infections, plant material from lime trees was tested by  
111 nested PCR. Leaf tissue was macerated in liquid nitrogen using a pestle and mortar, 0.1 g of  
112 leaf tissue was used for total DNA extraction using the NucleoSpin Plant II Kit (Macherey-  
113 Nagel, Düren, Germany) according to the manufacturer’s specifications. Nested PCR reactions  
114 used primer sets P1/P7 (Deng and Hiruki 1991) and R16F2n/R16R2 (Gundersen and Lee 1996)  
115 and followed reactions detailed in Silva *et al.* (2015).

116 The population densities of hemipteran phytoplasma vectors were surveyed  
117 simultaneously with pathogen sampling. Sticky yellow traps (24x12 cm) were also deployed  
118 to record population fluctuations of the leafhopper *Hishmonus phycitis* and the psyllid  
119 *Diaphorina citri*, the main vectors of phytoplasma in this system (Queiroz et al. 2016). 15-20  
120 traps were used per farm (relative to the number of lime trees), distributed in a grid 10m from  
121 one another. The sum of vector catches relative to the total area covered by these traps within  
122 each farm were calculated to produce a population density (km<sup>-2</sup>) for each vector.

123 Environmental data were measured during each discrete sampling occasion (i.e. when  
124 insect traps and leaf samples were taken) using an Omega Wireless Temperature/Humidity  
125 Data Logger (OM-EL-WIFI-TH; Omega Engineering Inc., Connecticut, USA). Mean diurnal  
126 (over 24 hours on each sampling occasion) temperature and humidity were recorded.  
127 Supplementary data for wind speed, direction and air pressure were retrieved from a weather  
128 station at Sultan Qaabos Unviersity in Oman for June 2013 to March 2014. The station was  
129 located at a minimum of 21.03 km and maximum of 111.13 km from the farms.

130 To compliment local weather station data, we obtained remote sensed environmental  
131 data from the National Oceanic and Atmospheric Administration (NOAA) National Centre for  
132 Environmental Information (NCEI; <https://www.ncdc.noaa.gov/cdo-web/>) weather station  
133 network (NOAA 2015). These were monthly mean diurnal temperature (°C) and atmospheric  
134 water density ( $\text{gm}^{-3}$ ) between June 2013 to March 2014 (NOAA 2015). In order to maintain  
135 comparison with field collected meteorological data, we had to covert the atmospheric water  
136 density data to humidity. We compared the field measured temperatures with each of the  
137 stations' temperature and humidity data to assess differences/errors between data sets  
138 (averaged over each month for comparability; see supplementary materials).

139 We analysed climatic correlates of disease and insect distributions using the statistical  
140 software R (release 3.1.1) (R Core Team, 2013). Phytoplasma infection presence/absence  
141 frequencies were analysed using a generalised linear model, assuming a binomial error and  
142 a logit-link function to estimate response curves with environmental variables, vector  
143 populations and Julian day as a temporal variable. We compared models produced using field  
144 logged environmental data with models using NCEI data. We then calculated the root-mean-  
145 square error (RMSE) between the two outputs. The model using NCEI data was used so that it  
146 could be applied globally. For the remainder of this study, this generalised linear model will be

147 referred to as 'the bioclimatic model'. The bioclimatic model will provide a value for infection  
148 probability for a farm with known climate and vector population density values.

149

#### 150 *Development of the global bioclimatic model*

151 Potential phytoplasma risk at a global level was examined by integrating the bioclimatic  
152 model developed from field collected data with satellite climatic data (temperature and  
153 humidity) in a Geographic Information System (GIS). As there is no accurate account of the  
154 global distribution of the insect vectors, we produced maps with universal vector densities that  
155 ranged from 0 to 200 km<sup>2</sup> (according to variance in population densities detected from  
156 preliminary surveys in Oman). We compared model predictions of pathogen incidence for the  
157 original sampling sites with the field collected data of these sites to determine the accuracy  
158 using an RMSE. Global mapped outputs were used to explore the potential risk in key lime  
159 growing regions of the world.

160 To produce global models, meteorological (temperature and humidity) data were  
161 obtained from the NASA Earth Observations (NEO) global satellite imagery database  
162 (<http://neo.sci.gsfc.nasa.gov/>). As with previous studies in bioclimatic modelling, we used data  
163 downloaded as floating point GeoTIFF files at a resolution of 0.1 degrees in 8-day cycles from  
164 02-June-2013 to 30-March-2014 (Peng et al. 2014, Noi et al. 2016). Data sets used were the  
165 atmospheric Water Vapour ([http://neo.sci.gsfc.nasa.gov/view.php?datasetId  
166 =MYDAL2\\_E\\_SKY\\_W](http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MYDAL2_E_SKY_W)) and Surface Temperature [Day] ([http://neo.sci.gsfc.nasa.gov/  
167 view.php?datasetId=MOD11C1\\_M\\_LSTDA](http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MOD11C1_M_LSTDA)).

168 Weekly pathogen monitoring, vector population estimates and environmental data from  
169 field sampling were matched with the resolution of bioclimatic modelling. The bioclimatic  
170 model was run for each 8-day data cycle. All of these 45 pathogen risk maps were deposited  
171 as a data archive in the supplementary materials.



172 Pathogen-likelihood incidences were calculated in ArcGIS 10.0 (ESRI, Redlands, CA,  
173 USA). Environmental data from NEO databases were input into the bioclimatic model. Since  
174 the model produced from field data uses the “logit-link” function, the raster files generated  
175 from this calculation were then back-transformed using the inverse-logit function to scale the  
176 probability of infection between 0 and 1. Model accuracy was compared against the predictions  
177 using the data collected at the field site in Oman. An RMSE was used to determine the  
178 goodness-of-fit of the model.

179

## 180 **Results**

181 *(1) Temperature and humidity determines distribution of ‘Ca. Phytoplasma aurantifolia’ in*  
182 *Oman*

183 The presence of ‘*Ca. Phytoplasma aurantifolia*’ was confirmed in 10 of the 12 lime  
184 orchards in Oman; the phytoplasma was not be detected on a farm in the Barka and one in the  
185 Suwayq regions (Table 1, Figure 1). None of the farms that were uninfected developed  
186 infections during the survey period, nor did the infected farms cease to be infected (i.e. infection  
187 status was consistent throughout the survey period).

188 Modelling the distribution of these infections against field surveyed environmental data  
189 demonstrated a significant nonlinear effect of temperature and positive effect of humidity  
190 (Table 2a) on likelihood of infection. We found the same effects when modelling with NCEI  
191 satellite data and a low RMSE value of 0.329 was calculated between these two models,  
192 demonstrating low variation and therefore indicating that both are valid (Table 2b, Figure S1).

193 The greatest probability of detection occurred at higher humidity (>40%) and at  
194 temperatures between 10°C and 25°C. A significant temporal effect on infection likelihood was  
195 found (Table 2), likely reflecting the well documented variation in vector abundances. We also

196 found a significant positive correlation between both *D. citri* and *H. phycitis* abundances and  
197 phytoplasma infection likelihood (Table 2a).

198 The abundances of insect vectors also demonstrated significant spatiotemporal  
199 variation across the farms. *Diaphorina citri* counts varied geographically, with higher  
200 abundances in the northwestern farms (Lat:  $3.277 \pm 1.634$ ,  $t = 2.006$ ,  $P = 0.045$ ; Lon:  $-2.793 \pm$   
201  $0.658$ ,  $t = -4.247$ ,  $P < 0.001$ ), but not temporally (Date:  $0.001 \pm 0.001$ ,  $t = 1.383$ ,  $P = 0.167$ ).  
202 *Hishimonus phycitis* counts also varied geographically, showing significantly higher  
203 abundances in the southwestern farms (Lat:  $-0.826 \pm 0.327$ ,  $t = -2.523$ ,  $P = 0.012$ ; Lon:  $-1.819$   
204  $\pm 0.224$ ,  $t = -8.131$ ,  $P < 0.001$ ). Significant non-linear temporal variation in vector abundance  
205 was also found (Figure 1), with lowest abundances occurring in November 2013 and highest  
206 in March 2014 (Date:  $-0.117 \pm 0.0132$ ,  $t = -8.860$ ,  $P < 0.001$ ; Date<sup>2</sup>:  $0.003 \pm 0.001$ ,  $t = 8.731$ ,  
207  $P < 0.001$ ).

208 From the environmental coefficients shown to affect WBDL in Oman (Table 2), we  
209 developed a probabilistic model to predict the likelihood of infection. (Equation 1); since  
210 outputs were in logit units, these were back-transformed using the inverse-logit function. We  
211 assessed model fit by comparing our field-level phytoplasma infection data with the model  
212 outputs derived from NOAA meteorological station data from Oman (Figure 2) and determined  
213 an RMSE value of 0.584.

214

$$215 \quad P(WBDL) = -5.458 + 0.179(AVD) + 0.246(T) - 0.008(T^2) + 0.024(Hphycitis) + 0.004(Dcitri)$$

216 [Equation

217 1]

218  $P(WBDL)$  = infection likelihood

219  $AVD$  = atmospheric water density ( $\text{gm}^{-3}$ )

220  $T$  = mean diurnal temperature ( $^{\circ}\text{C}$ )

221 *Hphycitis* = population density of *Hishimonus phycitis* (individuals per km<sup>2</sup>)

222 *Dcitri* = population density of *Diaphorina citri* (individuals per km<sup>2</sup>)

223

224 (2)Modelling the distribution of 'Ca. *Phytoplasma aurantifolia*' infection

225 We expanded this model outside Oman to examine areas suitable for 'Ca. *Phytoplasma*  
226 *aurantifolia*'. We used temperature and humidity data from NASA Earth Observations (NEO)  
227 to examine regions outside of the current known distribution of the pathogen (the Middle East  
228 and Brazil) that may be susceptible. The insect vectors of this pathogen are most active during  
229 March-April in the Middle East (Pande 1971, Shabani et al. 2013) and June-July in Brazil  
230 (Yamamoto et al. 2001), hence the primary outputs presented here (Figure 3) are mean  
231 infection likelihoods across this period. These models were then reproduced under the low (10  
232 km<sup>2</sup>) and high (200km<sup>2</sup>) hypothetical vector densities (Figure 3a-b). Frontier zones (i.e. where  
233 vector abundance rather than climatic conditions determine infection likelihood) may be a key  
234 factor in monitoring the spread of the pathogen. We calculated the difference between low  
235 vector density (Figure 3a) and high vector density (Figure 3b), which indicate where insect  
236 abundance is the key driving factor in disease spread (Figure 3c).

237

## 238 **Discussion**

239 In this study we assessed climatic variables that determine the infection-likelihood by  
240 a phytoplasma pathogen of lime. The key findings are that infections are more likely to occur  
241 in environments with humidity above 40%, but prevalence is lower at temperatures around 15-  
242 25°C. Infection probabilities increase in the presence of either insect vectors (*D. citri* and *H.*  
243 *phycitis*). Hypothetical bioclimatic models indicate areas in Brazil and Oman that are most at  
244 threat from the phytoplasma and areas in China and Central America that may be susceptible  
245 to future infection spread.

246           Before drawing any conclusions from this work however, it is crucial to be aware of  
247 the resolution and limitations of spatial models based on the output from global climate models.  
248 Our models indicate climatic potential for transmission and infection of lime trees by ‘*Ca.*  
249 *Phytoplasma aurantifolia*’. Bioclimatic models do not generate predictions, but rather suggest  
250 a trajectory of change under current conditions. Due to non-uniform errors, there are many  
251 sources of uncertainty in predicting biological responses to global climatic variables (Martens  
252 et al. 1999, Thomas et al. 2004) and thus there are caveats to be considered when interpreting  
253 the results presented here. First, there is limited comprehensive information about real-world  
254 distributions of agricultural land that is capable of growing lime; citrus fructiculture requires  
255 level land with sufficient drainage, (Monter and Aguilera 2011, Evans et al. 2014), but the  
256 variability in environmental tolerances of each of the citrus rootstocks makes the crop adaptable  
257 to any land that can be sufficiently modified (Castle 2010, Evans et al. 2014, Snoussi-Trifa et  
258 al. 2015). Next, we used climate data interpolated from satellite readings to give values over  
259 areas of 11 km x 11 km (0.1 latitude–longitude grid cells). There can be a great deal of local  
260 variation in temperature, rainfall, land use and tree planting density within this spatial  
261 resolution; bioclimatic models of malaria distributions have drawn significant criticism over  
262 inferences made over finer spatial scales (Hay et al. 2002, Patz et al. 2002). Third, at finer  
263 grains of spatial resolution, other factors can dominate; where climate is suitable, local  
264 transmission may be determined principally by social, demographic, economic and ecological  
265 factors (Frison and Taher 1991, Thomas et al. 2004, Huber et al. 2012). Finally, a lack of  
266 current comprehensively surveying of WBDL beyond the Middle East limits our ability to  
267 ground-truth Brazilian and global distribution predictions from the model.

268           Here we have developed a model to examine areas suitable for ‘*Ca.* *Phytoplasma*  
269 *aurantifolia*’; from this, we constructed maps based on the significant bioclimatic variables  
270 using global satellite data. The resulting bioclimatic model indicated areas within Oman and

271 Brazil, where the pathogen is currently detected, that are at a greater risk of infection by ‘*Ca.*  
272 *Phytoplasma aurantifolia*’ (Figure 3). Within Oman, the geographic models indicate that the  
273 coastal areas in the North (Figure 2) are the most likely to be infected by ‘*Ca. Phytoplasma*  
274 *aurantifolia*’, whereas it is much more unlikely in the cooler inland and upland areas to the  
275 west. In Brazil, infection probabilities were highest in the south and southeast, in the Rio  
276 Grande do Sul and São Paulo states (Figure 2), where ‘*Ca. Phytoplasma aurantifolia*’ has  
277 already been detected (Teixeira et al. 2005), and also in Minas Gerais and Rio de Janeiro states  
278 in the southeast.

279         Despite these limitations to the model, our results are supported by the limited real  
280 world data on geographical distributions of WBDL. Specifically, previous studies in Oman  
281 have demonstrated an increased level of detection in trees located in the north of Oman, with  
282 highest in the Ibri, Suwaiq and Mahadha regions (Al-Sadi et al. 2012). Furthermore, although  
283 limited data on its distribution in Brazil is available, the most comprehensively studied case is  
284 in São Paulo state (Silva et al. 2014), which corresponds to hotspots in both local and global  
285 models of WBDL bioclimatic models (Teixeira et al. 2005, Teixeira et al. 2008).

286         We also predict WBDL spreading to China, which has struggled with managing  
287 Huanglongbing, and the likelihood of a coinfection of the two pathogens hints at a possible  
288 repeat of invasion, spread and infection of lime orchards that happened in Brazil (Teixeira et  
289 al. 2008, Silva et al. 2014, Queiroz et al. 2016). Furthermore, the current lack of testing,  
290 monitoring or phytosanitary programs for WBDL in China, combined with the absence of any  
291 confirmation of presence, despite the presence of its hosts, vectors and sufficient climatic  
292 conditions, is deeply concerning and highlights the need to begin testing, and the importance  
293 of the bioclimatic mapping presented here.

294         Australia presents another interesting potential story regarding the distribution of ‘*Ca.*  
295 *Phytoplasma aurantifolia*’. As the centre of origin for *D. citri*, a key vector of this pathogen

296 (Swingle 1967) and showing high infection likelihood (Figure 3), it seems that this area may  
297 also come under threat in the future. Although ‘*Ca. Phytoplasma aurantifolia*’ is capable of  
298 infecting other citrus species, including *Citrus trifoliata* (trifoliolate orange) and *C. hystrix* (kaffir  
299 lime) (Donkersley et al. 2018); other lime species, including Tahiti limes (*C. latifolia*) and  
300 sweet lime (*C. limetta*), may not be susceptible to the pathogen (Chung et al. 2006). Alternative  
301 cultivars such as these may have an important role in establishing disease free areas for citrus  
302 industries destroyed by WBDL. Although it is not a major producer of lime in the global  
303 market, a rarely cultivated species of lime, the finger lime (*Citrus australasica* F.Muell.) is  
304 native to Australia (Mabberley 2004). Future studies could usefully examine the potential for  
305 interactions between the phytoplasma and this lime species that shares a centre of origin with  
306 one of its primary vectors.

307         Both *H. phycitis* and *D. citri* are known vectors of WBDL in *C. aurantiifolia* (Salehi et  
308 al. 2007, Queiroz et al. 2016). These two are considered the most serious pests of citrus when  
309 in the presence of transmittable pathogens (Grafton-Cardwell et al. 2013, Queiroz et al. 2016,  
310 Donkersley et al. 2018); although if no pathogens are present, they are usually minor pests  
311 (Halbert and Manjunath 2004). Based on the bioclimatic model, we have found areas that  
312 become highly susceptible in the presence of a high density of these vectors (200km<sup>-2</sup>). In  
313 particular, frontiers were found in South Brazil, Argentina, West China and Europe (Figure  
314 3c). The global distribution of these vectors raises further concerns over the potential spread of  
315 the phytoplasma (Chung et al. 2009). Given the prohibitive cost of molecular methods for  
316 identification of WBDL from field samples (Ghosh et al. 2013, Al-Yahyai et al. 2015), these  
317 remain prohibitively expensive for widespread implementation and are unlikely in the near  
318 future. Evidently therefore, a more effective way to protect these areas from phytoplasma  
319 spread would require a vector monitoring and control program (Perring et al. 1999). Here, we  
320 deployed between 15 and 20 yellow-sticky traps in an approximately 10x10m grid within each

321 farm; from these we calculate that the number of insects that need to be captured using this  
322 protocol in order to reach a 50% infection likelihood were 307 and 24 *D. citri* and *H. phycitis*  
323 respectively. Further research could usefully examine this relationship and develop a protocol  
324 for monitoring these vectors in these frontier infection zones.

325 Results of our bioclimatic modelling in Brazil identifies key areas of lime production  
326 that have a high phytoplasma infection-likelihood, such as São Paulo, Santa Catarina and Rio  
327 Grande do Sul states , in addition to Uruguay (Figure 2). Recently an asymptomatic infection  
328 of lime by '*Ca. Phytoplasma aurantifolia*' was reported from São Paulo State, Brazil (Silva et  
329 al. 2014). Furthermore, in countries with asymptomatic infections, monitoring systems are  
330 reliant on molecular probes, which are expensive and difficult to implement over a broad spatial  
331 scale (Taheri et al. 2011, Rad et al. 2012). By identifying key areas that may be more  
332 susceptible to the spread of a pathogen, the results of this study may reduce the costs of a  
333 monitoring program for '*Ca. Phytoplasma aurantifolia*'. An economically sustainable  
334 monitoring program for this pathogen may also avoid a situation similar to the previous failed  
335 management of the pathogen '*Ca. Liberibacter americanus*', the etiological agent of  
336 Huanglongbing of citrus (Belasque Jr et al. 2010).

337 Re-establishing citrus production in the Middle East is dependent on production of  
338 disease-free stocks, which is in turn dependent on identifying regions where new infections of  
339 '*Ca. Phytoplasma aurantifolia*' are less likely to arise. Acid lime production in most regions of  
340 Oman is not currently viable due to WBDL, and there are considerable cultural motives to  
341 prefer acid lime over other limes (Donkersley et al. 2018). Therefore, focusing on the regions  
342 identified by climatic mapping here (Figure 2), where infection rates are predicted to be lower  
343 will likely be an important first step in future efforts to re-establish WBDL-free lime production  
344 in the Middle East. This will, however, naturally require significant investment in empirical  
345 data from these locations to confirm our predicted probability of novel phytoplasma infections.

346 Previous research has also suggested that WBDL transmission potential varies seasonally, and  
347 is significantly lower in the Omani winter (Queiroz et al. 2016). Therefore, in addition to  
348 limited production of disease-free germplasm stocks to the cooler areas in the Middle East,  
349 movement of plant material and establishing new growing sites could be restricted to the winter  
350 season, to further limit the probably of spreading the pathogen. Naturally, recommendations of  
351 this nature require more detailed analyses and communication with growers and other  
352 stakeholders in the region.

353 We have also reviewed citrus pest and pathogen distributions and found these countries  
354 to be global hotspots of biological and abiotic threats to lime production (Donkersley et al.  
355 2018). Global climate modelling indicates that major lime producing nations (China, Mexico  
356 and Argentina) show suitable climatic conditions for infection by Witches Broom Disease of  
357 Lime (Figure 2), yet the etiological agent has yet to be detected in these countries (EPPO 2006).  
358 Another key citrus-producing region, Australia, also has a suitable climate for WBDL (Figure  
359 2) and is the centre of origin of one of the major vectors of this pathogen (*D. citri*).

360 The expanding range of WBDL is therefore of real concern, even where it may initially  
361 appear not to be a great problem. This expansion is especially troubling, given that population  
362 growth rates of the vector *D. citri* on phytoplasma-infected acid lime are double those on  
363 uninfected trees, both in Oman where the trees were suffering from WBDL and in Brazil where  
364 infections are entirely asymptomatic (Queiroz et al. 2016). This is important not just for the  
365 spread of the phytoplasma but also other pathogens that may be vectored by *D. citri* (e.g.  
366 Huanglongbing).

367 Over the last 30 years, global lime production has been damaged by severe weather  
368 events, invasive insect pests and novel insect-pathogen interactions (Crane et al. 1993, Grafton-  
369 Cardwell et al. 2013). The damage has been so severe as to render lime production in Florida,  
370 once one of the biggest producers in the world, no longer financially viable (Evans et al. 2014).



371 These crises have opened the possibility for new producers to invest in lime production;  
372 currently the majority of the gap in the market left by Florida has been filled by Mexico (Spreen  
373 2000).

374 Although our conclusions are based on a limited set of preliminary data, the problem  
375 this pathogen presents is so serious and the system so intractable that growers and managers  
376 cannot await validation. Considering acid lime is a perennial plant and the potential gravity of  
377 the disease, it stands to reason that Integrated Disease Management must be developed. The  
378 tools we developed here may become a part of that. With new major producers of lime  
379 emerging, the potential for invasive disease vectors and accompanying pathogens highlights  
380 the importance of appropriate and targeted monitoring programs to safeguard food security.

381

## 382 **Acknowledgements**

383 The authors would like to thank Vale S.A. for financial support. We also thank Sultan Qaabos  
384 University for facilitating the research. SLE is in receipt of a productivity grant from Conselho  
385 Nacional de Pesquisa (CNPq), Brazil.

386

## 387 **References**

- 388 Al-Sadi, A., H. Al-Moqbali, R. Al-Yahyai, and F. Al-Said. 2012. AFLP data suggest a  
389 potential role for the low genetic diversity of acid lime (*Citrus aurantifolia* Swingle)  
390 in Oman in the outbreak of witches' broom disease of lime. *Euphytica* **188**:285-297.
- 391 Al-Yahyai, R. A., A. M. Al-Sadi, F. A. Al-Said, Z. H. Al-Kalbani, C. M. Carvalho, S. L.  
392 Elliot, and A. Bertaccini. 2015. Development and morphological changes in leaves  
393 and branches of acid lime (*Citrus aurantifolia*) affected by witches' broom.  
394 *Phytopathologia Mediterranea* **54**:133-139.
- 395 Bassanezi, R. B., L. H. Montesino, and E. S. Stuchi. 2009. Effects of Huanglongbing on fruit  
396 quality of sweet orange cultivars in Brazil. *European Journal of Plant Pathology*  
397 **125**:565-572.
- 398 Belasque Jr, J., R. Bassanezi, P. Yamamoto, A. Ayres, A. Tachibana, A. Violante, A. Tank Jr,  
399 F. Di Giorgi, F. Tersi, and G. Menezes. 2010. Lessons from Huanglongbing  
400 management in São Paulo state, Brazil. *Journal of Plant Pathology*:285-302.
- 401 Bertaccini A. (2007) Phytoplasmas: diversity, taxonomy, and epidemiology. *Frontiers in*  
402 *Bioscience* **12**:673-689.

- 403 Berges, R., M. Rott, and E. Seemüller. 2000. Range of phytoplasma concentrations in various  
404 plant hosts as determined by competitive polymerase chain reaction. *Phytopathology*  
405 **90**:1145-1152.
- 406 Bove, J. 1986. Outbreaks and new records. Oman. Witches' broom disease of lime. *FAO*  
407 *Plant Protection Bulletin* **34**:217-218.
- 408 Bové, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of  
409 citrus. *Journal of Plant Pathology*:7-37.
- 410 Bove, J. M., and M. Garnier. 2000. Witches' broom disease of lime. *Arab Journal of Plant*  
411 *Protection* **18**:148-152.
- 412 Castle, W. S. 2010. A career perspective on citrus rootstocks, their development, and  
413 commercialization. *Hortscience* **45**:11-15.
- 414 Chung, K.-R., I. Khan, and R. Brlansky. 2009. Citrus diseases exotic to Florida: Witches'  
415 Broom Disease of Lime (WBDL). Fact Sheet pp228 Institute of Food and  
416 Agricultural Sciences, University of Florida.
- 417 Chung, K., I. Khan, and R. Brlansky. 2006. Citrus diseases exotic to Florida: Witches' Broom  
418 Disease of Lime (WBDL). Florida: IFAS Extension.
- 419 Crane, J. H., R. J. Campbell, and C. F. Balerdi. 1993. Effect of hurricane Andrew on tropical  
420 fruit trees. pp 139-142 *in* Proceedings of Florida State Horticultural Society. Florida  
421 State Horticultural Society.
- 422 da Graça, J. V. 2004. Etiology, history and world situation of citrus Huanglongbing *in*  
423 *Diseases of Fruits and Vegetables: Diagnosis and Management, Vol 1*.
- 424 De Boer, S. H., and M. M. López. 2011. New grower-friendly methods for plant pathogen  
425 monitoring. *Annual Review of Phytopathology* **50**:197-218.
- 426 Deng, S., and C. Hiruki. 1991. Amplification of 16S rRNA genes from culturable and  
427 nonculturable mollicutes. *Journal of Microbiological Methods* **14**:53-61.
- 428 Donkersley, P., F. Silva, I. Al-Mahmmoli, C. Carvalho, and S. Elliot. (2018) Biological,  
429 environmental and socioeconomic threats to lime production. *Journal of Plant*  
430 *Diseases and Protection*, pp1-18. <https://doi.org/10.1007/s41348-018-0160-x>
- 431 EPPO. 2006. *Candidatus* Phytoplasma aurantifolia. *EPPO Bulletin* **36**:117-119.
- 432 EPPO. 2015. PQR - EPPO Plant Quarantine Data Retrieval system.  
433 <http://www.eppo.int/DATABASES/pqr/pqr.htm>.
- 434 Evans, E. A., F. H. Ballen, and J. H. Crane. 2014. Economic potential of producing Tahiti  
435 limes in Southern Florida in the presence of citrus canker and citrus greening.  
436 *HortTechnology* **24**:99-106.
- 437 FAO. 2015. FAOSTAT: Food and Agriculture Organization of the United Nations Statistics  
438 Division. <http://faostat3.fao.org>.
- 439 Frison, E., and M. Taher. 1991. FAO/IBPGR technical guidelines for the safe movement of  
440 citrus germplasm. Bioversity International.
- 441 Ghosh, D., S. Bhose, R. Manimekalai, and S. Gowda. 2013. Molecular detection of  
442 *Candidatus* Phytoplasma spp. causing witches' broom disease of acid lime (*Citrus*  
443 *aurantifolia*) in India. *Journal of Plant Biochemistry and Biotechnology* **22**:343-347.
- 444 Ghosh, D., A. Das, S. Singh, S. Singh, and Y. Ahlawat. 1999. Occurrence of Witches'-  
445 Broom, a new phytoplasma disease of acid lime (*Citrus aurantifolia*) in India. *Plant*  
446 *Disease* **83**:302-302.
- 447 Gottwald, T. R., J. V. da Graça, and R. B. Bassanezi. 2007. Citrus Huanglongbing: the  
448 pathogen and its impact. *Plant Health Progress* **6**.
- 449 Grafton-Cardwell, E. E., L. L. Stelinski, and P. A. Stansly. 2013. Biology and management of  
450 Asian Citrus Psyllid, vector of the Huanglongbing pathogens. *Annual Review of*  
451 *Entomology* **58**:413-432.

- 452 Gregory, P. J., S. N. Johnson, A. C. Newton, and J. S. Ingram. 2009. Integrating pests and  
453 pathogens into the climate change/food security debate. *Journal of Experimental*  
454 *Botany* **60**:2827-2838.
- 455 Gundersen, D., and I.-M. Lee. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR  
456 assays using two universal primer pairs. *Phytopathologia Mediterranea*: **35**:144-151.
- 457 Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae)  
458 and greening disease of citrus: a literature review and assessment of risk in Florida.  
459 *Florida Entomologist* **87**:330-353.
- 460 Hay, S. I., J. Cox, D. J. Rogers, S. E. Randolph, D. I. Stern, G. D. Shanks, M. F. Myers, and  
461 R. W. Snow. 2002. Climate change and the resurgence of malaria in the East African  
462 highlands. *Nature* **415**:905-909.
- 463 Hijmans, R., G. Forbes, and T. Walker. 2000. Estimating the global severity of potato late  
464 blight with GIS-linked disease forecast models. *Plant Pathology* **49**:697-705.
- 465 Hogenhout S.A., Oshima K., Ammar, E.D., Kakizawa, S., Kingdom, H.N., Namba S. (2008)  
466 Phytoplasmas: bacteria that manipulate plants and insects. *Molecular Plant Pathology*  
467 **9**:403-423.
- 468 Huber, D., V. Römheld, and M. Weinmann. 2012. Chapter 10 - Relationship between  
469 Nutrition, Plant Diseases and Pests. Pages 283-298 *in* P. Marschner, editor.  
470 Marschner's Mineral Nutrition of Higher Plants (Third Edition). Academic Press, San  
471 Diego.
- 472 Kashyap, P. L., S. Kumar, and A. K. Srivastava. 2016. Nanodiagnostics for plant pathogens.  
473 *Environmental Chemistry Letters*:1-7.
- 474 Kriticos, D. J., A. Leriche, D. J. Palmer, D. C. Cook, E. G. Brockerhoff, A. E. A. Stephens,  
475 and M. S. Watt. 2013. Linking climate suitability, spread rates and host-impact when  
476 estimating the potential costs of invasive pests. *PLoS One* **8**:e54861.
- 477 Kriticos, D. J., T. Yonow, and R. E. McFadyen. 2005. The potential distribution of  
478 *Chromolaena odorata* (Siam weed) in relation to climate. *Weed Research* **45**:246-  
479 254.
- 480 Lee, I.-M., D. E. Gundersen-Rindal, and A. Bertaccini. 1998. Phytoplasma: ecology and  
481 genomic diversity. *Phytopathology* **88**:1359-1366.
- 482 Liu, Y., E. Heying, and S. A. Tanumihardjo. 2012. History, global distribution, and  
483 nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and*  
484 *Food Safety* **11**:530-545.
- 485 Mabberley, D. 2004. Citrus (Rutaceae): a review of recent advances in etymology,  
486 systematics and medical applications. *Blumea-Biodiversity, Evolution and*  
487 *Biogeography of Plants* **49**:481-498.
- 488 Mardi, M., S. Khayam Nekouei, L. K. Farsad, F. Ehya, M. Shabani, M. Shafiee, M.  
489 Tabatabaei, M. R. Safarnejad, G. Salehi Jouzani, and G. Hosseini Salekdeh. 2011.  
490 Witches' broom disease of Mexican lime trees: disaster to be addressed before it will  
491 be too late. Pages S205-S206 *in* *Bulletin of Insectology*. Department of  
492 Agroenvironmental Sciences and Technologies.
- 493 Martens, P., R. Kovats, S. Nijhof, P. De Vries, M. Livermore, D. Bradley, J. Cox, and A.  
494 McMichael. 1999. Climate change and future populations at risk of malaria. *Global*  
495 *Environmental Change* **9**:S89-S107.
- 496 Monter, A. V., and A. M. Aguilera. 2011. Advances in Fruiticulture. *Revista Brasileira De*  
497 *Fruticultura* **33**:179-186.
- 498 Mukhopadhyay, S. 2004. Diseases of Citrus and their management. Pages 90-196 *Citrus:*  
499 *production, postharvest, disease and pest management*. Science Publishers, Enfield  
500 (NH), USA.

501 Nascimento da Silva, F., A. Neves de Souza, I. Al-Mahmooli, A. M. Al-Sa'di, and C. M.  
502 Carvalho. 2015. A new disease in *Citrus aurantifolia* in Oman, "sudden decline", is  
503 associated with a pathogen complex including a 16SrII group phytoplasma.  
504 *Phytopathogenic Mollicutes* **5**:S101-S102.

505 NOAA. 2015. NOAA National Centers for Environmental Information: Custom Monthly  
506 Summaries. National Oceanic and Atmospheric Administration (NOAA).

507 Noi, P., M. Kappas, and J. Degener. 2016. Estimating daily maximum and minimum land air  
508 surface temperature using MODIS land surface temperature data and ground truth  
509 data in Northern Vietnam. *Remote Sensing* **8**:1002.

510 Pande, Y. 1971. Biology of citrus psylla, *Diaphorina citri* Kuw. (Hemiptera: Psyllidae). *Israel*  
511 *Journal of Entomology* **6**:307-311.

512 Patz, J. A., M. Hulme, C. Rosenzweig, T. D. Mitchell, R. A. Goldberg, A. K. Githeko, S.  
513 Lele, A. J. McMichael, and D. Le Sueur. 2002. Climate change (Communication  
514 arising): Regional warming and malaria resurgence. *Nature* **420**:627-628.

515 Peng, S.-S., S. Piao, Z. Zeng, P. Ciais, L. Zhou, L. Z. X. Li, R. B. Myneni, Y. Yin, and H.  
516 Zeng. 2014. Afforestation in China cools local land surface temperature. *Proceedings*  
517 *of the National Academy of Sciences* **111**:2915-2919.

518 Perring, T. M., N. M. Gruenhagen, and C. A. Farrar. 1999. Management of plant viral  
519 diseases through chemical control of insect vectors. *Annual Review of Entomology*  
520 **44**:457-481.

521 Perrings, C., K. Dehnen-Schmutz, J. Touza, and M. Williamson. 2005. How to manage  
522 biological invasions under globalization. *Trends in Ecology & Evolution* **20**:212-215.

523 Queiroz, R. B., P. Donkersley, F. N. Silva, I. H. Al-Mahmmoli, A. M. Al-Sadi, C. M.  
524 Carvalho, and S. L. Elliot. 2016. Invasive mutualisms between a plant pathogen and  
525 insect vectors in the Middle East and Brazil. *Open Science* **3**:160557.

526 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation  
527 for Statistical Computing, Vienna, Austria.

528 Rad, F., A. Mohsenifar, M. Tabatabaei, M. Safarnejad, F. Shahryari, H. Safarpour, A.  
529 Foroutan, M. Mardi, D. Davoudi, and M. Fotokian. 2012. Detection of '*Candidatus*  
530 *phytoplasma aurantifolia*' with a quantum dots FRET-based biosensor. *Journal of*  
531 *Plant Pathology* **94**:525-534.

532 Salehi, M., K. Izadpanah, M. Siampour, A. Bagheri, and S. Faghihi. 2007. Transmission of  
533 '*Candidatus* *Phytoplasma aurantifolia*' to Bakraee (*Citrus reticulata* hybrid) by feral  
534 *Hishimonus phycitis* leafhoppers in Iran. *Plant Disease* **91**:466-466.

535 Samson, J. 1986. *Citrus*. Longman Scientific and Technical, Essex, New York.

536 Shabani, M., C. Bertheau, M. Zeinalabedini, A. Sarafrazi, M. Mardi, S. M. Naraghi, H.  
537 Rahimian, and M. Shojaee. 2013. Population genetic structure and ecological niche  
538 modelling of the leafhopper *Hishimonus phycitis*. *Journal of Pest Science* **86**:173-183.

539 Silva, F., R. Queiroz, A. Souza, A. Al-Sadi, D. Siqueira, S. Elliot, and C. Carvalho. 2014.  
540 First report of a 16SrII-C *Phytoplasma* associated with asymptomatic acid lime  
541 (*Citrus aurantifolia*) in Brazil. *Plant Disease* **98**:1577-1577.

542 Smith, K. F., D. F. Sax, S. D. Gaines, V. Guernier, and J.-F. Guégan. 2007. Globalization of  
543 human infectious disease. *Ecology* **88**:1903-1910.

544 Snoussi-Trifa, H., M. F. Duval, A. Garcia-Lor, X. Perrier, J. P. Jacquemoud-Collet, L.  
545 Navarro, and P. Ollitrault. 2015. Analysis of genetic diversity in Tunisian citrus  
546 rootstocks. Pages 147-154 in B. SabaterMunoz, P. Moreno, L. Pena, and L. Navarro,  
547 editors. Xii International Citrus Congress - International Society of Citriculture. Int  
548 Soc Horticultural Science, Leuven 1.

549 Spreen, T. H. 2000. The citrus industries of the United States and Mexico after NAFTA,  
550 *Revista Chapingo Serie Horticultura* **VI**:145-152.

- 551 Swingle, W. T. 1967. The botany of Citrus and its wild relatives. The citrus industry:190-430.
- 552 Taheri, F., G. Nematzadeh, M. G. Zamharir, M. K. Nekouei, M. Naghavi, M. Mardi, and G.
- 553 H. Salekdeh. 2011. Proteomic analysis of the Mexican lime tree response to
- 554 '*Candidatus* Phytoplasma aurantifolia' infection. *Molecular BioSystems* **7**:3028-3035.
- 555 Teixeira, D., N. Wulff, E. Martins, E. Kitajima, R. Bassanezi, A. Ayres, S. Eveillard, C.
- 556 Saillard, and J. Bové. 2008. A phytoplasma closely related to the Pigeon Pea
- 557 Witches'-Broom Phytoplasma (16Sr IX) is associated with citrus huanglongbing
- 558 symptoms in the state of São Paulo, Brazil. *Phytopathology* **98**:977-984.
- 559 Texeira, D. C., J. Ayres, E. W. Kitajima, L. Danet, S. Jagoueix-Eveillard, C. Saillard, and J.
- 560 M. Bové. 2005. First report of a Huanglongbing-like disease of citrus in Sao Paulo
- 561 state, Brazil and association of a new liberibacter species, "*Candidatus* Liberibacter
- 562 americanus", with the disease. *Plant Disease* **89**:107-107.
- 563 Thomas, C. J., G. Davies, and C. E. Dunn. 2004. Mixed picture for changes in stable malaria
- 564 distribution with future climate in Africa. *Trends in Parasitology* **20**:216-220.
- 565 Weintraub, P. G., and L. Beanland. 2006. Insect vectors of phytoplasmas. *Annual Review of*
- 566 *Entomology* **51**:91-111.
- 567 Yamamoto, P. T., P. E. Paiva, and S. Gravena. 2001. Population dynamics of *Diaphorina*
- 568 *citri* Kuwayama (Hemiptera: psyllidae) in citrus orchards in the North of Sao Paulo
- 569 State, Brazil. *Neotropical Entomology* **30**:165-170.
- 570 Yonow, T., D. J. Kriticos, and R. W. Medd. 2004. The potential geographic range of
- 571 *Pyrenophora semeniperda*. *Phytopathology* **94**:805-812.
- 572
- 573

574 **Table 1.** Infection occurrence of witches' broom disease of lime (*Ca. Phytoplasma*  
575 *aurantifolia*) in Omani farms. Infection data demonstrate the proportion of trees sampled that  
576 were confirmed infected in June 2013-March 2014.  
577

Region	Farm	Proportion infected†	No. trees sampled	Total count <i>H. phycitis</i> *	Total count <i>D. citri</i> *
Barka	1	0.00	180	111	418
Barka	2	0.20	180	432	382
Barka	3	0.05	180	254	1037
Musanah	4	0.53	135	247	1378
Musanah	5	0.33	135	271	176
Musanah	6	0.60	135	872	429
Samael	7	1.00	135	399	8
Samael	8	0.40	135	378	112
Samael	9	1.00	135	206	4
Suwayq	10	0.20	180	1854	354
Suwayq	11	0.00	135	157	47
Suwayq	12	0.33	108	165	8249

578 † Proportion infected is the relative to the total number of *C. aurantifolia* trees tested

579 \* Vector densities (*Diaphorina citri* and *Hishimonus phycitis*) are total counts for each farm  
580 in the study

581

582

583 Table 2. Coefficients of phytoplasma infection detection in Omani lime orchards. Logistic  
 584 regression produces coefficients in logits. Coefficients derived from (a) field station data and  
 585 (b) NCEI satellite data. The variance between these two models provides an RMSE value of  
 586 0.329.  
 587

<b>(a)</b>	Coefficient (logit)	SD	<i>z</i>	P
Intercept	-5.458	0.961	-5.683	<0.001
Water density	0.179	0.066	9.915	<0.001
Temperature	0.246	0.071	3.447	<0.001
Temperature <sup>2</sup>	-0.008	0.001	-5.285	<0.001
<i>H. phycitis</i>	0.024	0.010	2.401	0.016
<i>D. citri</i>	0.004	0.001	2.379	0.017
Date	-0.072	0.012	-5.813	<0.001

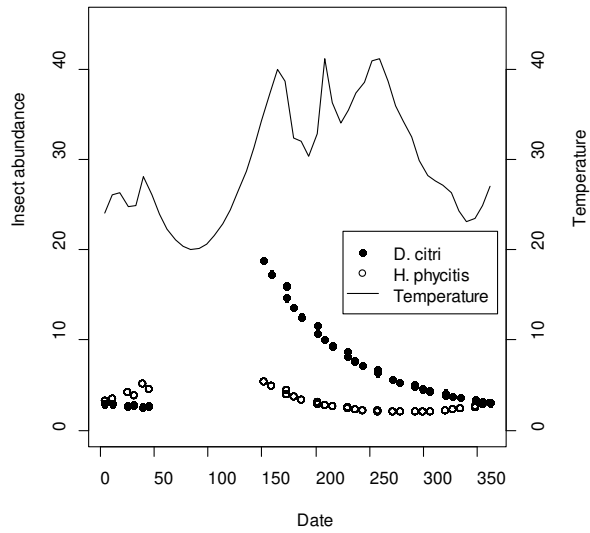
588

<b>(b)</b>	Coefficient (logit)	SD	<i>z</i>	P
Intercept	-0.661	1.072	-0.617	0.537
Water density	0.888	0.170	5.226	<0.001
Temperature	0.218	0.071	3.080	0.002
Temperature <sup>2</sup>	-0.009	0.002	-5.922	<0.001
<i>H. phycitis</i>	0.030	0.010	3.010	0.002
<i>D. citri</i>	0.004	0.001	2.468	0.013
Date	-0.074	0.011	-6.720	<0.001

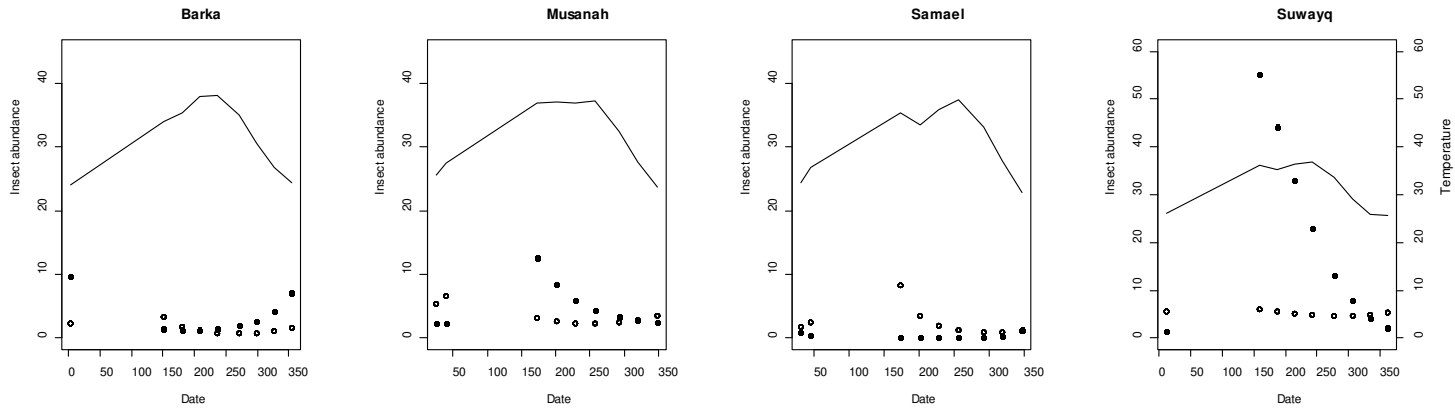
589

590

591



592



593

594

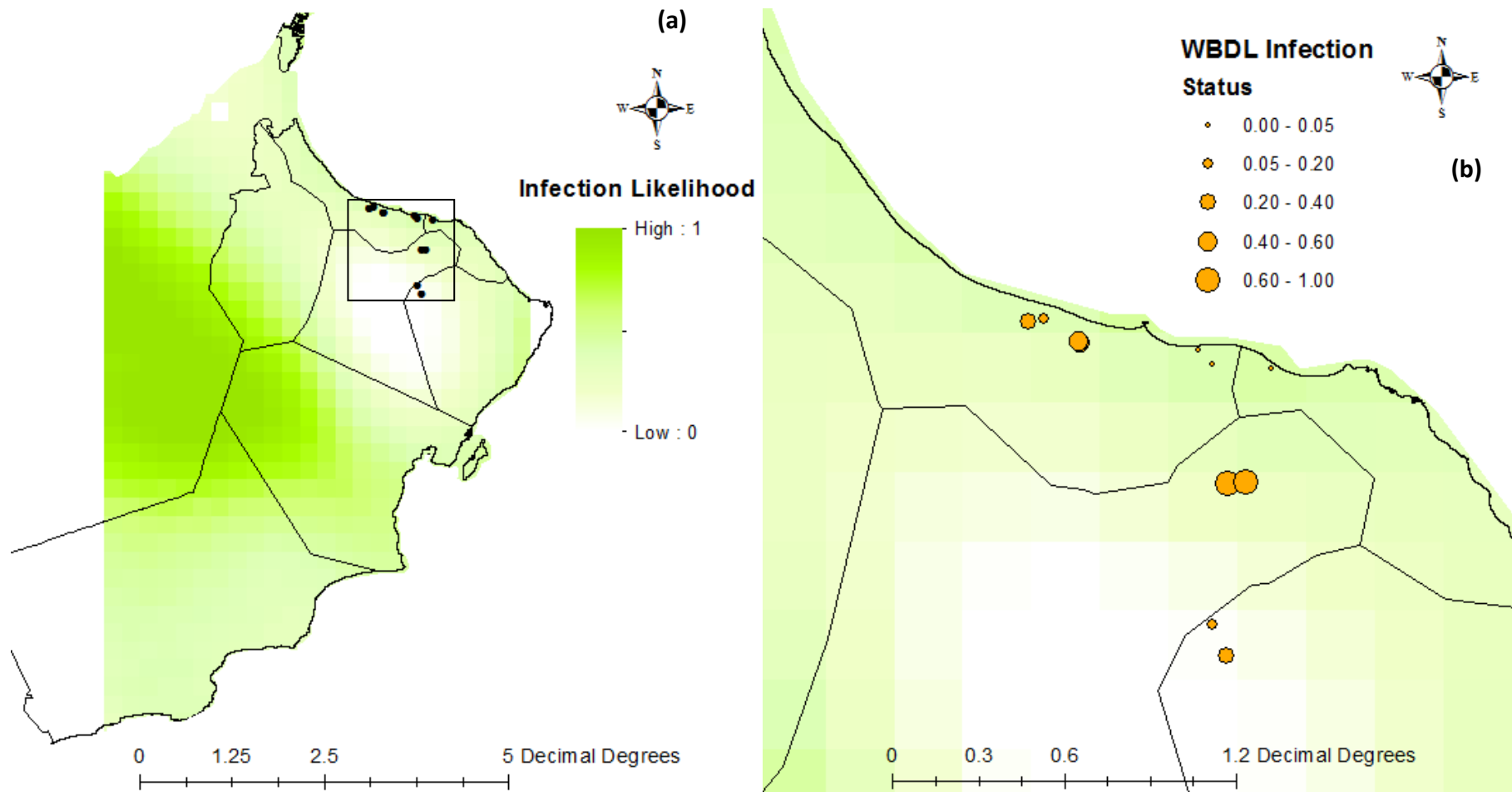
595

596

597

Figure 1. Population density variation of the Hemipteran vectors *Diaphorina citri* (black) and *Hishimonus phycitis* (hollow) across Oman and within each state. Mean temperature measured at each sampling occasion is also shown (line).



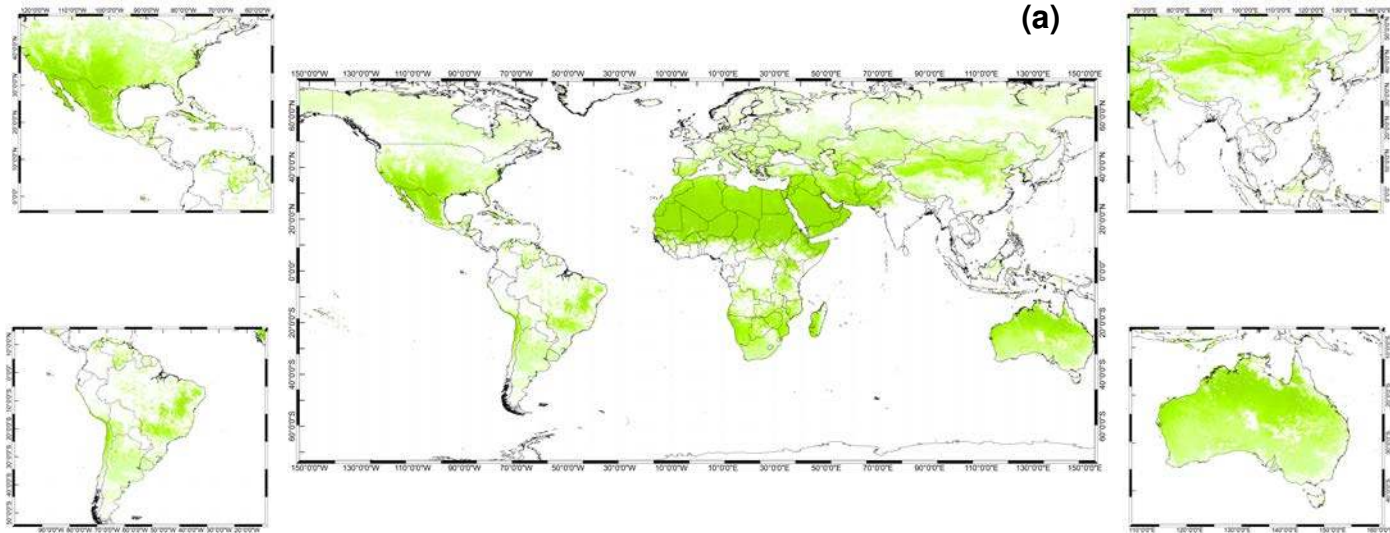


598

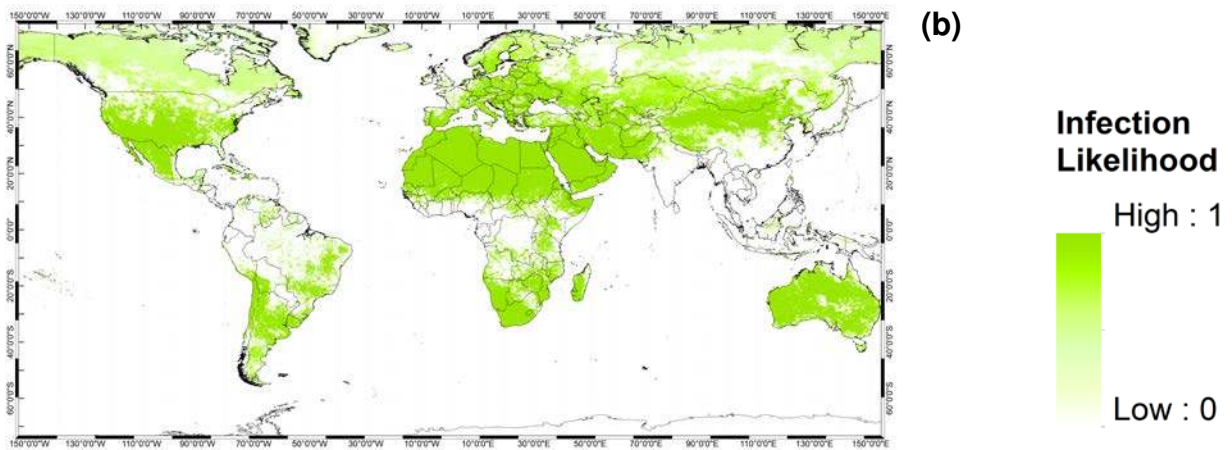
599

600 Figure 2. Bioclimatic model output from Oman, using NOAA meteorological station data. (a) Farm locations and (b) proportion of *Citrus*  
 601 *aurantifolia* trees infected with “*Ca. Phytoplasma aurantifolia*” from data from field in June 2013 - March 2014 within each are also displayed to  
 602 compare with the modelled results. Polygons within Oman are the regional governances.

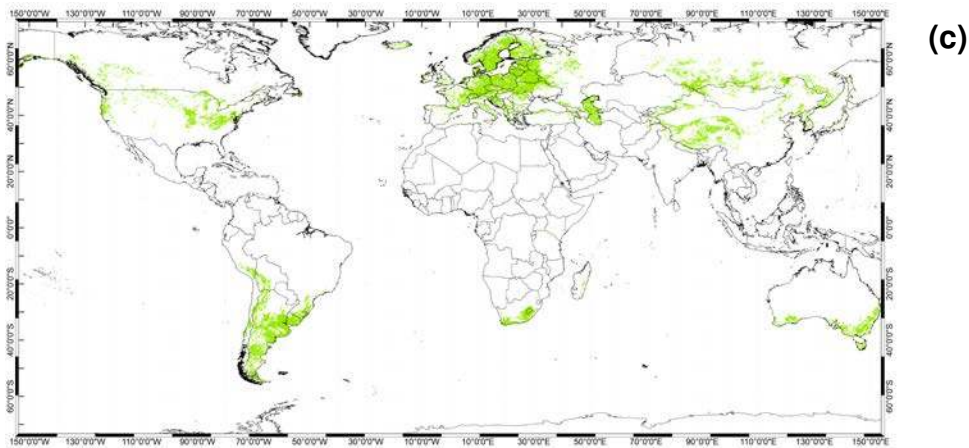
603



604



605



606

607 Figure 3. Global bioclimatic models for distributions of Witches Broom Disease of Lime (*Ca.*  
608 *Phytoplasma aurantifolia*), averaged from outputs between March-July, when the insect vectors  
609 (*Diaphorina citri* and *Hishimonus phycitis*) of this pathogen are most active. Variable vector  
610 abundances are presented: (a) 10 and (b) 200 *H. phycitis* and *D. citri* per km<sup>2</sup> and (c) key frontier  
611 zones for where the infection spread is predicted to be due to insect population density (i.e. not  
612 due to climate).

613