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

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Abstract

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

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

Introduction

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

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

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

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

Recently an infection of acid lime by '*Ca*. Phytoplasma aurantifolia' was reported from São Paulo State, Brazil (Texeira et al. 2005, Silva et al. 2014). This infection was notably asymptomatic, indicating differences in host-pathogen interactions compared with the Middle 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 40ºN to 40ºS (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

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

Materials and Methods

Factors determining distribution of phytoplasma in Oman

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

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

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

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

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

147 refered 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^{-2} (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) and Surface Temperature [Day] (http://neo.sci.gsfc.nasa.gov/
167 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.

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

Results

(1) Temperature and humidity determines distribution of 'Ca. *Phytoplasma aurantifolia' in Oman*

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

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

The greatest probability of detection occurred at higher humidity (>40%) and at temperatures between 10ºC and 25ºC. A significant temporal effect on infection likelihood was 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 ± 1.634, t = 2.006, P = 0.045; Lon: -2.793 ±
201 0.658, t = -4.247, P < 0.001), but not temporally (Date: 0.001 ± 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 ± 0.327, t = -2.523, P = 0.012; Lon: -1.819
204 ± 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 ± 0.0132, t = -8.860, P < 0.001; Date²: 0.003 ± 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 $$P(WBDL) = -5.458 + 0.179(AVD) + 0.246(T) - 0.008(T^2) + 0.024(Hphycitis) + 0.004(Dcitri)$$

216 [Equation
217 1]

218 *P(WBDL)* = infection likelihood

219 *AVD* = atmospheric water density (gm^{-3})

220 *T* = mean diurnal temperature (°C)

Hphycitis = population density of *Hishimonus phycitis* (individuals per km^2)

Dcitri = population density of *Diaphorina citri* (individuals per km^2)

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

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

Discussion

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

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

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

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

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

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

Australia presents another interesting potential story regarding the distribution of '*Ca.* 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* (trifoliate 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 ($200km^{-2}$). 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 unifected 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).

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

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

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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 *H. phycitis**	Total count *D. citri**
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

(a)	Coefficient (logit)	SD	z	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
Temperature²	-0.008	0.001	-5.285	<0.001
H. phycitis	0.024	0.010	2.401	0.016
D. citri	0.004	0.001	2.379	0.017
Date	-0.072	0.012	-5.813	<0.001

588

(b)	Coefficient (logit)	SD	z	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
Temperature²	-0.009	0.002	-5.922	<0.001
H. phycitis	0.030	0.010	3.010	0.002
D. citri	0.004	0.001	2.468	0.013
Date	-0.074	0.011	-6.720	<0.001

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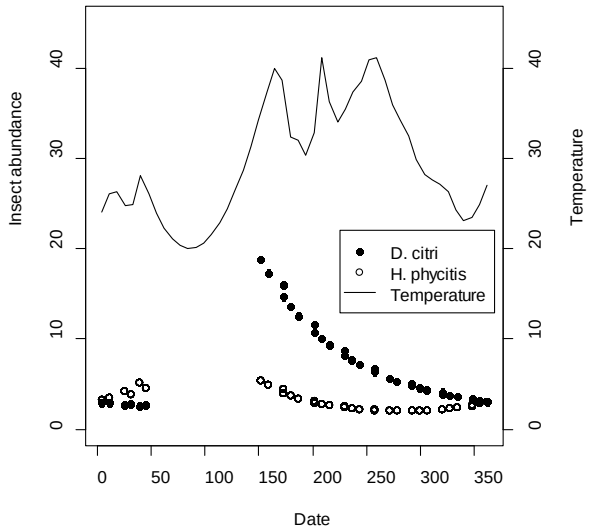

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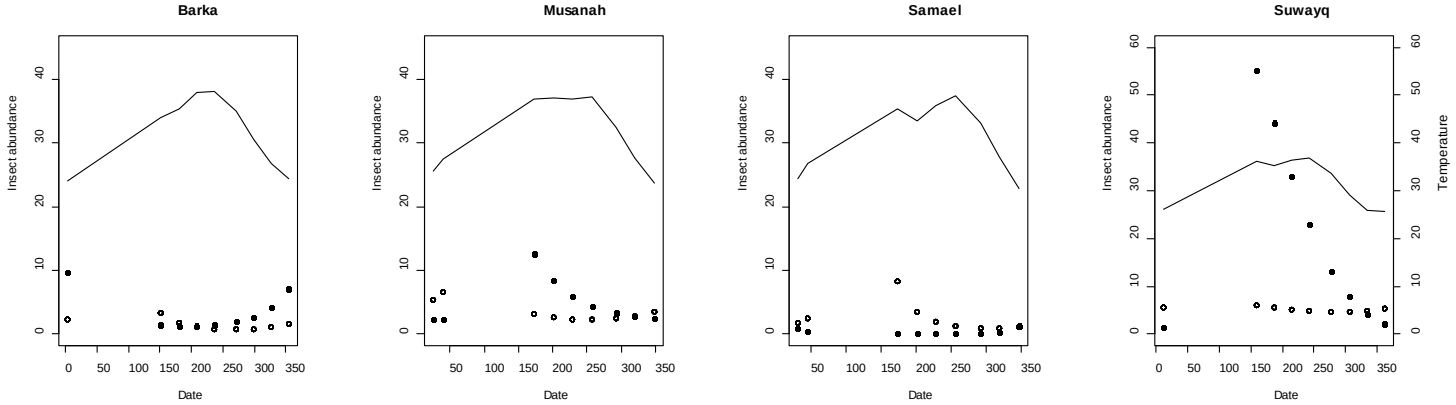

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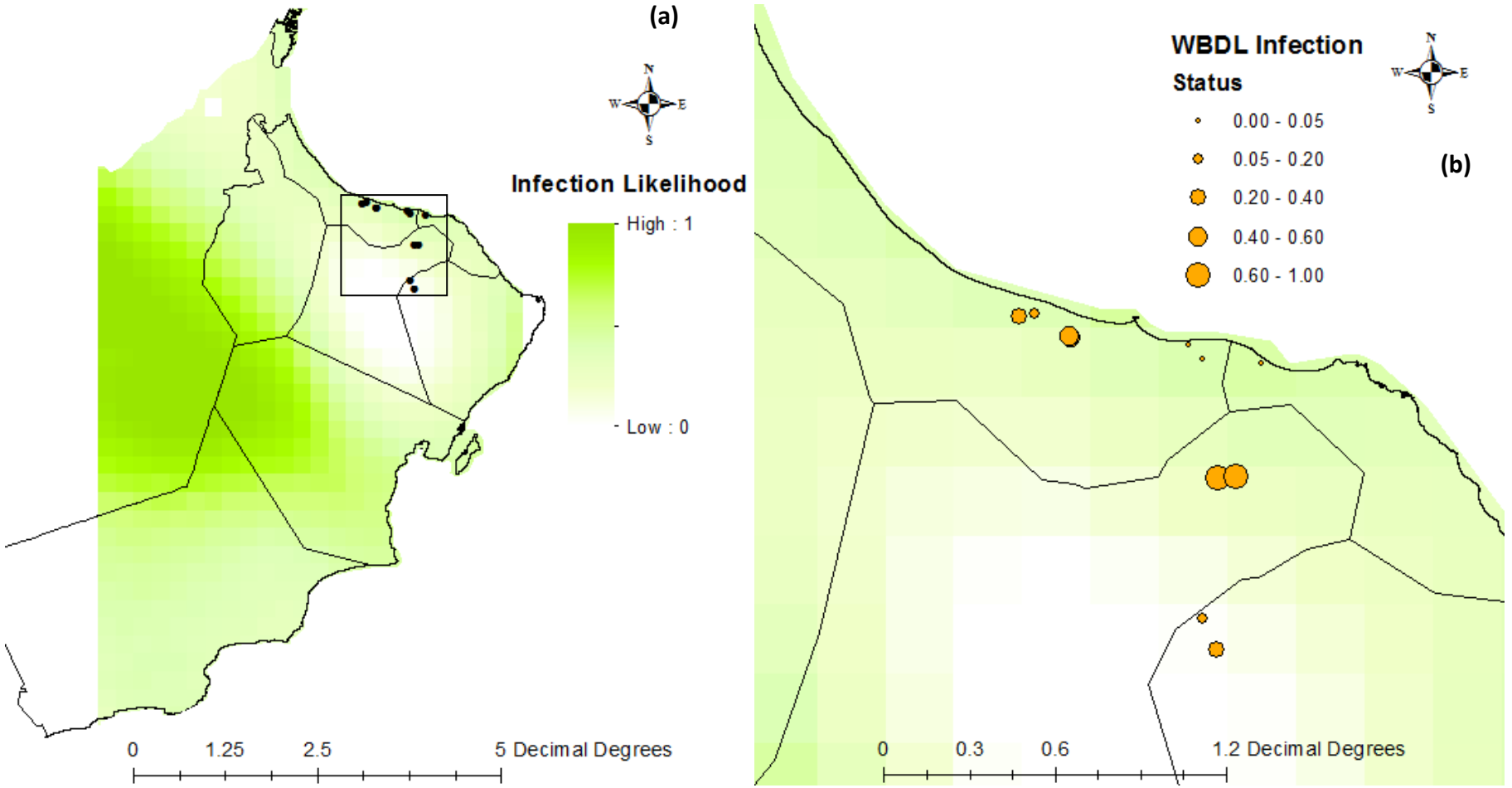
596 Figure 1. Population density variation of the Hemipteran vectors *Diaphorina citri* (black) and *Hishimonus phycitis* (hollow) across Oman and
597 within each state. Mean temperature measured at each sampling occasion is also shown (line).



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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.

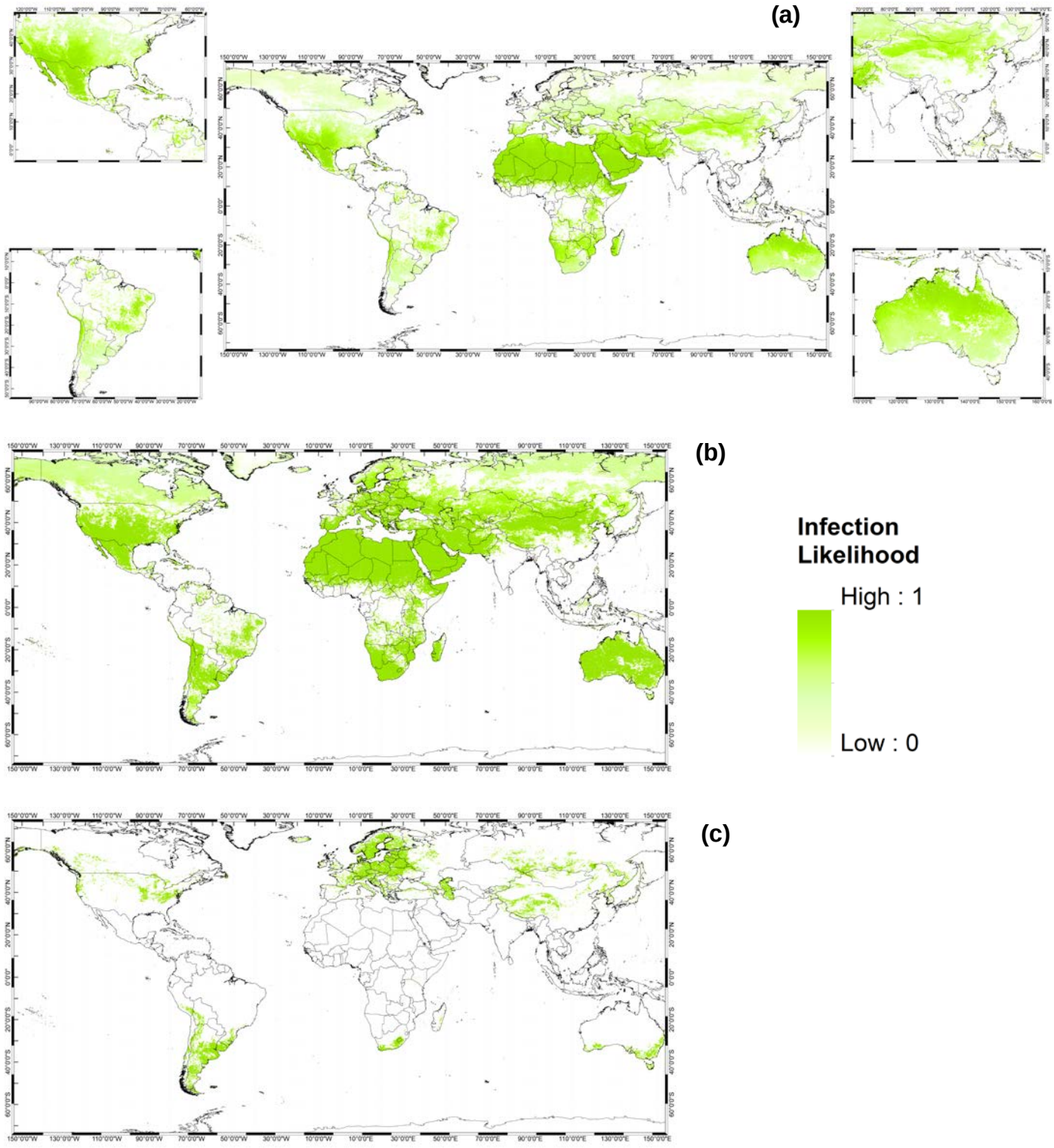

Figure 3. Global bioclimatic models for distributions of Witches Broom Disease of Lime (*Ca.* Phytoplasma aurantifolia), averaged from outputs between March-July, when the insect vectors (*Diaphorina citri* and *Hishimonus phycitis*) of this pathogen are most active. Variable vector abundances are presented: (a) 10 and (b) 200 *H. phycitis* and *D, citri* per km² and (c) key frontier zones for where the infection spread is predicted to be due to insect population density (i.e. not due to climate).