Development of an Individual-Based Simulation Model for the Spread of Citrus Greening Disease by the Vector Insect *Diaphorina citri*

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1. Introduction

To establish an Integrated Pest Management (IPM) system for crop pest insects and disease, risk assessment of relevant hindrances is necessary. Typically, the data for such risk assessment analysis is collected through a field survey or field experiments. However, the clarification of important factors often requires assessment of the interaction between such factors. Modelling provides a basis for the theoretical description of such interaction, based on field data, and for relevant simulations. We developed a simulation model which estimates the spread of citrus greening disease (Huanglongbing, HLB) by the vector insect *Diaphorina citri* Kuwayama. Then, we examined the parameters affecting the spread dynamics of the disease, using the model to inform risk assessment. We targeted the area of the Mekong Delta, Vietnam, one of the regions severely affected by the spread of HLB. At the beginning of this chapter, we describe the disease and the vector insect. Then we outline the developmental methodology for the HLB disease-spread estimation model. Finally, we provide suggestions aimed at preventing the spread of HLB, based on the disease-spread estimation results in several situations.

1.1 Citrus greening disease

Citrus greening disease is a limiting factor in citrus production worldwide (Bové, 2006). The symptoms of HLB are similar to those of nutritional stress (Halbert & Manjunath, 2004). A survey conducted over an eight-year period in Réunion Island, for example, indicated that 65% of the trees were badly damaged and rendered unproductive within seven years of planting (Aubert et al., 1996). In Thailand, citrus trees generally decline within 5-8 years after planting due to HLB (Roistacher, 1996). In his compilation of global infection statistics, Toorawa (1998) estimated that 50 million trees were infected in South and Southeast Asia, and 3 million were infected in Africa. In India and Saudi Arabia, there has been a marked decline in the citrus industry as a result of HLB.
The pathogens are phloem-inhabiting bacteria in the generous *Candidatus Liberibacter* (Halbert et al., 2004). Although these bacteria have hitherto not been sufficiently cultured for the application of Koch’s postulates, some experimental results have strongly suggested that they are the pathogen in HLB (Su et al., 1986; Buitendag & von Broembsen, 1993). There are two principal means of transmission for a healthy tree: graft transmission, whose frequency has been estimated in many previous studies (e.g., Lin & Lin, 1990; van Vuuren, 1993), but with widely varying values; and transmission through vector insects. In Asian countries, HLB is borne by the Asian citrus psyllid, *D. citri*.

Detection of the HLB pathogen has been achieved by several methods. DNA identification through the PCR method was used to detect the bacteria both in citrus plants and vector insects (Bové et al., 1993; Tian et al., 1996). Wang et al. (2006) conducted a study which developed a reproducible conventional PCR method with several primer sets, and two quantitative real-time PCR methods for detection and monitoring of the pathogen. The HLB pathogen also can be detected with an electron microscope, ELISA (Garnier & Bové, 1993).

**1.2 Life cycles of the vector insect and the disease**

The *D. citri* is a Hemipteran insect, measuring 3 to 4 mm in length, with piercing-sucking mouthparts that allow this pest to feed on the phloem of citrus spp. and other related rutaceous plants. The eggs of *D. citri* are laid on the new leaf growth of expanding terminals, in the folds of unfurled leaves, and behind developing leaf buds (Chavan & Summanwar, 1993). There are five nymphal instars (Aubert, 1987). Adults may live several months, and females may lay as many as 800 eggs in a lifetime, under artificial rearing conditions (Mead, 1977); however, the longevity and fecundity in actual field conditions are not well known. Temperature-dependent development of *D. citri* has been estimated (Liu & Tsai, 2000; Yang et al., 2006; Nakata, 2006), and the results reveal a relatively consistent trend, even though the host plants used in the experiments varied. In general, nymphs grew faster at higher temperatures, except for 32.5 °C. Nakata (2006) estimated the developmental zero and effective accumulative temperature of the egg as 13.7°C and 46.9 degree-days, respectively. The developmental zero and effective accumulative temperature of the nymphs were 11.6°C and 192.3 degree-days, respectively (Nakata, 2006).

Fourth and fifth instar nymphs and adults of *D. citri* can acquire the pathogen, and emerged adults that have fed on infected plants as nymphs can transmit the pathogen to healthy plants (Capoor et al., 1974; Xu et al., 1988). Once the bacterium is acquired, the psyllid will retain and transmit the bacterium throughout its life. Multiplication of the pathogen in individual *D. citri* was investigated by Inoue et al. (2009). The efficiency of transmission to healthy plants by the virulent adult *D. citri* was estimated in several studies. The virulence of vectors grown on infected trees, and the transmission rate of virulent vectors to healthy host trees, were different in each report. Inoue et al. (2009) estimated a successful transmission of 67% (for test plants) by inoculative adult *D. citri* that were given acquisition feeding in the nymphal developmental period, suggesting that the pathogenic bacteria was present in the salivary glands of these psyllids. In another study, the transmission rate of virulent *D. citri* to healthy plants was estimated 1% (Huang et al., 1984). In our previous study, the virulence of vectors grown on infected trees, and the transmission rate of virulent vectors to healthy host trees, were almost 90% and almost 25%, respectively (Ohto & Kobori, unpublished data).
2. The model

Until quite recently, almost all models of pathogens and hosts were developed within the framework of mean-field models, which assumed that the respective individuals were uniformly distributed and that the respective interactions occurred uniformly. In recent years, however, the increased calculation speed of computers has enabled the development of an Individual-Based Model (IBM), wherein each individual, of the pathogen and the vector, behave individually, acting according to a predefined set of rules. The model is able to examine disease-spread dynamics in a simulation field, by calculating the cumulative results of the individual behaviors. Because it can treat the vector and host individually, the IBM offers a new and powerful tool in the study of insect borne plant disease. We therefore employed this technique to develop an HLB disease-spread model based on the D. citri vector, with reference to the pine wilt disease-spread model developed by Takasu (2009). The C language used in writing the source code for the model was based on the C language technology of simulated individuals, published electronically by Takasu (2008).

2.1 Framework of the model and estimation of the parameters

In this simulation, we targeted the region of the Mekong Delta, Vietnam, in order to establish the basic parameters. Some parameters were provided by previous reports and some by our own observations in the target area. The model is summarized in Fig. 1.

![Diagram of disease spread](image)

From Kobori et al. (2010).

Fig. 1. (a) Change in tree status over time. (b) Summary of citrus greening disease-spread simulation by our model.
We assumed that the citrus trees were arranged in a two-dimensional regular lattice, with the position of a given tree as '(x, y)', where x and y are integers, and \(-L \leq x \leq L\) and \(-M \leq y \leq M\) determines the spatial dimensions of the lattice. The distance between trees was assumed to be 2.5 m, in accordance with the common planting regime in the region. We assumed a discrete time progression defined by one month (t).

Tree status was defined as one of the following: (1) healthy period (H), when the tree is healthy and has the potential to be infected with HLB by the feeding of virulent D. citri; (2) latent period (LP), when the tree has been infected with HLB by D. citri but still does not have infective ability; (3) infectious period (IP), when the D. citri growing on the tree have the potential to transmit HLB with a probability of \(V\); and (4) dead period (D), when the tree is dead or there is no tree at the given point in the lattice. The value \(V\) was estimated at 0.9 from our aforementioned previous study (Ohto & Kobori, unpublished data). Transition through these tree statuses is irreversible: \(H \rightarrow LP \rightarrow IP \rightarrow D\). There have been few previous reports that estimated the latent and infectious periods of HLB. The growth stage, cultivar and environmental differences of the host plant may affect these, but we do not know the details. Hence, in this model, we estimated the default values, based on our own field observation and a survey questionnaire conducted in Vietnam, as the following: the LP tree essentially changes status to IP in 3t, and the IP to D in 45t (Fig. 1 (a)).

The vector insects are assumed to move around according to a given dispersal kernel. We discuss the determination of this kernel in Section 2.2. If the insect is virulent, it transmits HLB with a probability of \(Tr\) to tree \(H\). In this report, we set \(Tr\) at 0.3, in accordance with our aforementioned previous study (Ohto & Kobori, unpublished data). A vector insect individual produces an average of \(Rp\) next-generation individuals according to Gaussian distribution. In this report we assumed an \(Rp\)-value of 10, in accordance with our life-table study in Japan (Kobori, un-published data) and similar observations in Vietnam. We also established a threshold number, of 100 individuals of the next generation on a given tree, from our observations in Vietnam.

To exclude edge and corner effects associated with a rectangular two-dimensional space, the model space was assumed to be a torus. In addition, we distributed enough D trees around the simulation to prevent adults from arriving at the edge.

2.2 Determination of the dispersal kernel

To determine the dispersal kernel of the D. citri, we carried out choice and no-choice tests in a glass house, and artificial release experiments in the experimental field in Japan.

In the glass house, we estimated factors affecting the dispersal activity of D. citri, first by means of a no-choice test. Movement from a ‘growth’ tree (that is, one on which the D. citri has grown from egg to adult) to another tree was observed, in time, for each situation (Kobori et al., 2011a). We examined high – low insect density situations, and new-bud – no-new-bud situations, for the growth tree. The dispersal movement of the adult D. citri was greater for trees with new buds than for trees without new buds (Fig. 2). The effects of D. citri density were not detected in this experiment. Additionally, we investigated host-plant selection by D. citri through choice tests (Fig. 3). Choice testing between trees with and without buds revealed that released adults preferred trees with buds during the testing period. This preference disappeared when both trees were treated with a sticky spray to
prevent adult movement between trees. These results suggest that adult *D. citri* do not select trees with new buds from a distance but recognize them by means of random movement between trees.

Through artificial release experiments, we estimated mean dispersal distance, frequency of movement and dispersal direction (Kobori et al., 2011b). We artificially released, in separate experiments, 10,000 and 1,000 adult *D. citri*, marked in pink, at the center of the experimental field, in which 33 X 33 potted host plants were set out in a grid at 2.5 m intervals (for the marking method, see Nakata, 2008).

In the 10,000-adult experiment, the mean dispersal distance from the release point was 5-6 m, and in the 1,000-adult experiment it was 6-12 m. In each case, the proportion of *D. citri* found on different days over the experimental periods declined with increasing distance from the release point, and did not change substantially over time (Fig. 4). These results suggested that once the adult *D. citri* arrived on the host plants they hardly moved again. Moreover, the center of distribution would be expected to move with the wind direction, if each *D. citri* continued its random movement, because the same wind direction was observed throughout most of each experimental period; however, the center of distribution in fact stayed close to the release point throughout the experimental periods (Fig. 5). Individuals that moved more than 7.5 m from the release point were found on the lee side. These results suggested that once *D. citri*, here carried by the wind, had arrived on the host plants, they too hardly moved again.
Tree with buds: 8 to 10 buds present on the tree; tree without buds: no buds on the tree; other: individuals found on the side wall of the glasshouse or missing inside the glasshouse. From Kobori et al. (2011a).

Fig. 3. Results of a choice test with adult *D. citri* (mean ± S.E.).

In light of the above results, we determined the dispersal kernel. We did not include the effects of vector insect density in the frequency of movement in the kernel. On the other hand, it is highly possible that the existence of new buds affects movement behavior. However, in the Mekong Delta, Vietnam, our targeted area, there are new buds on the host trees almost the entire year. Thus, we did not include bud effects in our model. The direction of movement was isotropic, and the distance of a given movement, *r*, follows a probability distribution *w*(*r*). For the dispersal kernel, we approximated mobility by means of the formula:

\[
w(r) = \gamma e^{-\gamma r}
\]  

(1)
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$\gamma$ was estimated at 0.8 from previous experiments (Fig. 4). As a result of a given movement, the insect lands on the nearest-neighbour host tree. If the insect lands on a *D* tree, the individual moves again without feeding or reproducing, in accordance with the kernel.

These values were calculated in a one-dimensional cross-section from south to north in the field. Density of adult insects = (number of individuals / total number of the individuals in field) × 100. ▲: 3 days, ■: 7 days, ×: 14 days, ●: (a) 20 days, (b) 21 days after release. From Kobori et al. (2011b).

Fig. 4. The density of *D. citri* (*D*) found at different time periods, plotted against distance from the release point; (a) 10,000 adults released, (b) 1,000 adults released.

When the individual lands on an *H*, *LP* or *IP* tree, it does not move again. In the process of moving, individuals have a certain probability ($D_m$) of dying. After reproduction, individuals have a certain probability ($D_l$) of dying; individuals remaining alive (probability
of 1-Dl) are integrated into the next generation. There is insufficient previous research to provide estimates for these parameters, and we could not estimate their value by certain observation or experiment. Thus, we roughly estimated these parameters at $Dm$: 0.7 and $Dl$: 0.9 by speculative inference from life-table analysis and our own limited observation in this report (e.g. Mead, 1977; Tsai et al., 2002).

Empty and solid circles indicate 10,000 adults and 1,000 adults released, respectively. The two arrows (solid: 10,000 adults; dashed: 1,000 adults) indicate the composite wind vectors over the experimental periods, as calculated from the daily dominant wind directions. The average velocities were 2.42 m/s in the case of 10,000 adults released, and 3.98 m/s in the case of 1,000 adults released. From Kobori et al. (2011b).

Fig. 5. Center of distribution, over the experimental period, of insects released at the zero coordinate.

3. HLB disease-spread simulation using the model

3.1 Spread of HLB in a field under default conditions

We simulated HLB spread under default conditions. Field size was set at $L, M = 100$. Healthy trees ($H$) were distributed at the center of the field ($L, M = 20$). The remaining trees were $D$. Then, 100 virulent vector insects were distributed at the center of the $H$ field. We set the model calculation period for 84 months.
Within $12t$, more than 10 newly infected trees had appeared in the given field (Fig. 6 (a)). The spread speed increased with time (Fig. 6 (b)). By the end of the calculation period, more than half of the $H$ trees had changed status to $LP$, $IP$ or $D$.

Fig. 6. (a) Snapshots of tree-status distribution for $t=12, 24, 36, 48, 56, 68, 72, 84$. (b) Number of trees newly infected in the field over time.

Blue dot indicates Latent Period ($LP$); red: Infectious Period ($IP$), black: Dead ($D$). Healthy ($H$) trees are not shown. Solid line: number of trees newly infected; dotted line: number of initial $H$ trees in the simulated field (1,681 trees).
3.2 Effectiveness of delaying the latent period (LP), and of removing infectious (IP) trees, in suppressing the spread rate of HLB in an orchard

We estimated the effectiveness of delaying the latent period (LP) and removing infectious (IP) trees in suppressing the spread rate of HLB in an orchard, compared with the default situation. The field was defined according to the default conditions above. Then, 100 virulent vector insects were distributed at the center of the H field. We set the model calculation period for 84 months.

First, we changed the number of time steps required to transition from LP to IP from the default (3t) to 6, 12 and 24t, respectively, in order to estimate the effects of delaying the latent period. The model estimation suggested that the spread rate of HLB in the orchard slowed as the time from LP to IP increased (Fig. 7). The theory that mean generation time has more affect on the intrinsic rate of natural increase than the reproductive rate, would account for this result. Our estimation suggested that cultivars in which the transition from LP to IP is delayed might show resistance to HLB and thus suppress the rate of disease spread.

Second, we estimated the effects of removing infectious (IP) trees. We assumed that the IP trees were removed at 24, 12 and 6t, respectively, after their status had changed from LP (default: trees were removed at 45t, when their status changed to D), and we compared the spread rates of HLB. Under the no-removal scenario (default), the number of newly infected
trees was more than 1,000 (Fig. 8). When we removed the $IP$ trees $24t$ after their status change, the number of newly infected trees was almost same as under the default condition. However, when we removed the $IP$ trees $9t$ after their status change, the number of newly infected trees decreased relative to the default condition. Moreover, when we removed the $IP$ trees $6t$ after their status change, the number of newly infected trees barely increased. We may thus predict that the removal of infectious trees will be efficacious in preventing the spread of HLB in a field.

![Graph showing the number of newly infected trees over time](image)

**Fig. 8. Effects of infectious tree ($IP$) removal in the field.**

3.3 Attempts to simulate more realistic scenarios

The vector insect population follows seasonal trends (e.g., Nakahira et al., 2011). Thus, we established high/low reproductive periods and conducted the simulation. The number of newly infected trees increased with time without immigration of virulent vector insects. The model forecast that more than 80% of trees were infected with HLB by $84t$. Hence, we suggest that the use of disease-free seedlings offers a fundamental technique for preventing HLB spread in an orchard. Additionally, we estimated the suppression of the HLB spread rate through systemic pesticide treatment in a newly planted orchard. Several reports have indicated that systemic pesticide treatment causes high mortality rates in $D. citri$ (e.g., Ichinose et al., 2010). We assumed that pesticide applied in the new orchard would be effective for two years. Hence, we assumed that, until $24t$ after planting, 100% of the insects...
that had migrated from an infected orchard into the newly planted orchard would die after feeding (if an individual was virulent it might transmit HLB, but would not reproduce on the tree). After this period, individuals migrating from the infected orchard would reproduce in the newly planted orchard. The number of newly infected trees was lower in the plantings treated with systemic pesticide than in those with the negative controls. We concluded that treatment with systemic pesticide was effective in suppressing the spread rate of HLB in a newly planted orchard, and the effect was definitive when the virulent _D. citri_ migrated from a nearby orchard (Fig. 9). Part of this study have been reported in Kobori et al. (2011c).

![Graph](image)

Dashed line, negative control (did not treat systemic pesticide); soiled line, treated with systemic pesticide (effective for 24t). Number of initial _H_ trees in the simulated field was 256. No. of newly infected trees = LP + IP + D (excluding initial _D_ trees). Modified from Kobori et al. (2011).

Fig. 9. Effects of systemic pesticide to suppress HLB in the field.

Although these results generated valuable suggestions, there was a problem in our simulated conditions. In a number of replications, extinction of the vector insect occurred. In actual observation, even insects not found in a given season could be found in later observations. One cause of this may be the closed nature of our simulation system. In reality, vector insects could immigrate into the field. Currently, we are expanding the model to incorporate this possibility. In addition, the dispersal kernel was developed for calculations of single-field behavior. We must revise the kernel to simulate larger scenarios, such as those involving multiple, separated fields.
4. Conclusion

This chapter described the development of an individual-based modelling technique to simulate disease-spread dynamics. Our model was able to provide parameters for each individual citrus tree and the respective vector insect, and thereby to examine disease-spread dynamics in the simulation field by calculating the cumulative results of the individual behaviors. This model can be applied to many diseases vectored by insects, although we developed the model for HLB.

Our simulated results suggested that both delaying the transition from latent period (LP) to infectious period (IP), and removing infectious (IP) trees, suppressed the spread rate of HLB in an orchard. Additionally, the results of our a preliminary trial suggested that cooperative control of D. citri on the part of orchard owners in a given area, to reduce the number of virulent individuals, may be effective in suppressing the spread rate of HLB.

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6. References

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The present book is not a classical manual on Zoology and the reader should not expect to find the usual treatment of animal groups. As a consequence, some people may feel disappointed when consulting the index, mainly if searching for something that is considered standard. But the reader, if interested in Zoology, should not be disappointed when trying to find novelties on different topics that will help to improve the knowledge on animals. This book is a compendium of contributions to some of the many different topics related to the knowledge of animals. Individual chapters represent recent contributions to Zoology illustrating the diversity of research conducted in this discipline and providing new data to be considered in future overall publications.

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