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**Ecological studies on *Circulifer tenellus* (Hemiptera: Cicadellidae),
a vector of beet curly top virus**

By

HYOSEOK LEE
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Entomology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

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Abstract

Yield losses in crop production due to insect pests and plant diseases are a major threat to food security worldwide. Furthermore, some insect pests transmit plant pathogenic viruses, which are more difficult to control than other pests because of complex interactions among insect vectors, plant viruses, and host plants. Hemipterans (e.g., aphids, leafhoppers, mealybugs, planthoppers, psyllids, soft scales, and whiteflies) are the most common and efficient insect vectors because of their piercing-sucking mouthparts. Effective management of insect-vector plant viruses requires integration of diverse management practices and knowledge of their complex interactions. In this project, beet leafhopper, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae), and beet curly top virus (BCTV) were studied as a model insect vector-plant virus system. Beet leafhopper and BCTV have wide host ranges and cause yield losses in economically important crops such as sugar beet, tomato, pepper, melon, and common bean. For instance, BCTV caused ~\$100 million in losses to the California processing tomato industry in 2013. In California, beet leafhoppers overwinter in the foothills located on the western side of the Central Valley, and their progeny acquire BCTV from non-agricultural host plants and migrate to agricultural fields in spring. The Curly Top Virus Control Program run by the California Department of Food and Agriculture monitors populations using sweep net assessment and sprays malathion insecticide using airplanes to reduce beet leafhopper density in the foothills. Due to geographical scale, the program only covers small portions of the foothills and more efficient management strategies are needed to reduce BCTV incidence in crops.

This dissertation addresses questions to improve management of beet leafhopper in California through multiple approaches. Chapter 1 describes how BCTV affects beet leafhopper to enhance its propagation in tomato fields with barley or ribwort plantain as trap crops. BCTV

effects on host preference of beet leafhoppers were tested in dual- and no-choice experiments and simulation modeling predicted how they alter BCTV spread in tomato fields. Non-viruliferous beet leafhoppers preferred to probe on barley and ribwort plantain compared with tomato but viruliferous beet leafhoppers showed no probing preference. Simulation modeling revealed that this alteration may increase BCTV infection rate in tomato fields with trap crops. Chapter 2 describes oviposition of beet leafhopper on four common non-agricultural host plants [*Erodium cicutarium* (L.) L'Hér. (Geraniaceae), *Kochia scoparia* (L.) Schrader (Amaranthaceae), *Plantago ovata* Forsskál (Plantaginaceae), and *Salsola tragus* L. (Amaranthaceae)] at two temperatures (30 and 35 °C). In addition, oviposition models for each non-agricultural host plant were constructed and validated under fluctuating temperature conditions. *K. scoparia* was the most suitable host plant (highest number of eggs laid) followed by *P. ovata*, *E. cicutarium*, and *S. tragus*, and the optimal temperature for oviposition was 30.6 °C. Since beet leafhoppers in non-agricultural areas are the most important targets to prevent BCTV spread, this information is crucial for developing effective beet leafhopper management strategies. Chapter 3 describes beet leafhopper migration time and its association with BCTV outbreaks. Plant greenness effects on flight propensity of beet leafhopper were determined with two host plants, sugar beet and redstem filaree, under greenhouse conditions. In addition, spring migration of beet leafhoppers was monitored in the foothills for 2 years and vegetation greenness of study sites was calculated using satellite imagery. As plants in the foothills became dry in spring, beet leafhoppers started migrating to agricultural fields. Based on vegetation greenness, a spring migration model was developed to estimate beet leafhopper migration time. In addition, the spring migration model was implemented in a web-based system as a decision support tool for beet leafhopper management. Severe BCTV outbreaks were reported in the San Joaquin Valley and Sacramento

Valley in 2013 and 2021, respectively. In these years, early spring migration was estimated from the spring migration model, which supports the possibility of early spring migration as a key factor in causing BCTV outbreaks. The web-based mapping system not only aids in effective beet leafhopper management, but also provides valuable insight into BCTV epidemiology.

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Chapter 1: Insect vector manipulation by a plant virus and simulation modeling of its potential impact on crop infection

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Abstract

There is widespread evidence of plant viruses manipulating behavior of their insect vectors as a strategy to maximize infection of plants. Often, plant viruses and their insect vectors have multiple potential host plant species, and these may not overlap entirely. Moreover, insect vectors may not prefer plant species to which plant viruses are well-adapted. In such cases, can plant viruses manipulate their insect vectors to preferentially feed and oviposit on plant species, which are suitable for viral propagation but less suitable for themselves? To address this question, we conducted dual- and no-choice feeding studies (number and duration of probing events) and oviposition studies with non-viruliferous and viruliferous [carrying beet curly top virus (BCTV)] beet leafhoppers [*Circulifer tenellus* (Baker)] on three plant species: barley (*Hordeum vulgare* L.), ribwort plantain (*Plantago lanceolata* L.), and tomato (*Solanum lycopersicum* L.). Barley is not a host of BCTV, whereas ribwort plantain and tomato are susceptible to BCTV infection and develop a symptomless infection and severe curly top symptoms, respectively. Ribwort plantain plants can be used to maintain beet leafhopper colonies for multiple generations (suitable), whereas tomato plants cannot be used to maintain beet leafhopper colonies (unsuitable). Based on dual- and no-choice experiments, we demonstrated that BCTV appears to manipulate probing preference and behavior by beet leafhoppers, whereas there was no significant difference in oviposition preference. Simulation modeling predicted that BCTV infection rates would be higher in tomato fields with barley compared with ribwort plantain as a trap crop. Simulation model results supported the hypothesis that manipulation of probing preference and behavior may increase BCTV infection in tomato fields. Results presented were based on the BCTV-beet leafhopper pathosystem, but the approach taken (combination of experimental studies with complementary simulation modeling)

is widely applicable and relevant to other insect-vector plant pathogen systems involving multiple plant species.

Introduction

Most plant viruses require insect vectors (e.g., aphids, beetles, leafhoppers, thrips, and whiteflies) to be transmitted among plants¹. Efficiency and dynamics of plant virus acquisition and transmission by insect vectors are likely shaped by plant virus-insect vector coevolution^{2,3}. Insect vectors of plant viruses exhibit innate host preference to optimize fitness and that of their offspring^{4,5}, but such fitness optimization may not always align with host adaptation of the plant viruses they transmit^{6,7}. Consequently, insect vector manipulation should be viewed as a complex of conflicting selection pressures and optimizations between insect vectors and the plant viruses they transmit. Accordingly, plant viruses have evolved strategies to manipulate their insect vectors to prefer plant species suitable for virus propagation. Several important reviews have described these manipulations of both host plants and insect vectors by plant pathogenic microorganisms⁸⁻¹⁰. Throughout this article, “insect vector manipulation”, as opposed to “innate host plant preference”, refers to plant viruses manipulating feeding and oviposition of their insect vector in ways that are perceived to enhance fitness of a plant virus.

Manipulation of feeding behavior may be expressed in terms of altered probing preference when choices of plants are available to insect vectors. Additionally, under both choice- and no-choice conditions, duration of probing events may also be altered. Plant viruses may influence both numbers and duration of leaf probing events by piercing-sucking insect vectors¹¹. Plant viruses benefit from patterns of feeding behavior of insect vectors based on their modes of transmission⁹. Non- or semi-persistently transmitted plant viruses are transmitted

during brief probing events, whereas persistently transmitted plant viruses require longer-lasting feeding events¹². Thus, insect vector manipulation resulting in short and numerous probing events may optimize transmission of non- or semi-persistently transmitted plant viruses. In contrast, persistently transmitted plant viruses would have enhanced transmission by manipulating insect vectors to have longer feeding periods^{8,9}. Stafford, et al.¹³ showed that tomato spotted wilt virus (TSWV) manipulated probing behavior of its vector, Western flower thrips [*Frankliniella occidentalis* (Pergande)]. resulting in increased frequency of leaf probing events by males and enhanced transmission of the non-phloem-limited TSWV. Additionally, it has been demonstrated that non-viruliferous green peach aphids [*Myzus persicae* (Sulzer)] prefer host plants infected with potato leaf roll virus (PLRV), whereas viruliferous conspecifics prefer non-infected plants¹⁴. Thus, PLRV appears to be manipulating its insect vector to maximize transmission to non-infected plants.

Regarding manipulation of oviposition, it has been demonstrated that *Bemisia tabaci* (Gennadius) Mediterranean (MED) vectoring tomato yellow leaf curl virus (TYLCV) laid more eggs and gained more weight than conspecifics feeding on non-infected plants¹⁵. In addition, Chen, et al.¹⁶ found that MED preferentially settled and oviposited on TYLCV-infected plants than on non-infected plants. From an evolutionary standpoint, TYCLV benefits from manipulation of both settling and oviposition preferences, because TYLCV can also be transmitted transovarially by MED¹⁷. In short, a growing body of knowledge about plant virus manipulation of both plants and insect vectors provides critical insights into complex species interactions and behavioral patterns.

Once insect vector manipulation has been identified and characterized, simulation models can be developed to predict broader epidemiological implications and ultimately how such insect

vector manipulation may affect crop production. A few but important studies have described use of simulation models to quantify possible effects of insect vector manipulation. For instance, Ogada, et al.¹⁸ predicted that insect vector manipulation of Western flower thrips increased TSWV transmission rate up to 33%. In addition, simulation model can be used to characterize effects of insect vector manipulation under various environmental conditions¹⁹. For example, simulation model may be used to examine effects of insect vector manipulation on infection of crop plants in trap cropping systems²⁰. Here, trap cropping refers to managed cropping systems, in which a given plant species is used as a “decoy” (trap crop) to attract insect pests, so that a main crop experiences reduced insect pest infestation. In some cases, trap crops can be the same as the main crop but be more attractive due to different planting date or altered management (i.e. different fertilization/irrigation regimes). A trap crop may also be a different variety or plant species with higher relative attractiveness than the main crop. In all trap cropping systems, a common factor is that insect pests are “offered choices” of feeding and oviposition plants at a landscape level. Thus, performance of a given trap cropping approach to insect pest management depends on an expected host preference of target insect pests, but insect vector manipulation may alter such innate host preference by insect-vector-borne pathogens.

In the present study, a phloem-limited plant virus [beet curly top virus (BCTV)] and its insect vector, the beet leafhopper [*Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae)], were used as a model pathosystem to study insect vector manipulation. The beet leafhopper is the only known vector of BCTV in North America, and BCTV is transmitted in a persistent circulative manner. BCTV is the type species of the genus *Curtovirus*, family *Geminiviridae*. The beet leafhopper and BCTV have wide host ranges^{21,22}, and BCTV causes yield losses in economically important crops, including tomato, sugar beet, pepper, spinach, and common bean^{23,24}. The study

objectives were: 1) to experimentally determine if beet leafhoppers carrying BCTV (viruliferous) show manipulated probing/oviposition preference or probing behavior on three plant species: barley (*Hordeum vulgare* L.), ribwort plantain (*Plantago lanceolata* L.), and tomato (*Solanum lycopersicum* L.), as compared with non-viruliferous beet leafhoppers. Barley is not a host of BCTV, whereas ribwort plantain and tomato are susceptible to BCTV infection and develop a symptomless infection and severe curly top symptoms, respectively. Ribwort plantain plants can be used to maintain beet leafhopper colonies for multiple generations (suitable), whereas tomato plants cannot be used to maintain beet leafhopper colonies (unsuitable)²⁵ (Fig. S1); and 2) utilize a simulation model to assess potential effects of insect vector manipulation on BCTV spread in tomato fields with various percentages of barley or ribwort plantain as trap crop. We predicted insect vector manipulation effects by incorporating probing preference and behavior of viruliferous beet leafhoppers and those of non-viruliferous conspecifics as manipulated and innate preference and behavior.

Most studies of insect vector manipulation focus on a single plant species, although plant communities in natural and agricultural environments possess varying degrees of diversity. Thus, under real-world conditions, insect vectors face choices regarding feeding and oviposition plants. Furthermore, most plant viruses and their insect vectors have wide host ranges. We therefore investigated the role of insect vector manipulation in the BCTV etiology in tomato, a plant that is not a preferred plant species of the beet leafhopper but is highly suitable for BCTV.

Materials and methods

Insects and plants

Non-viruliferous and viruliferous beet leafhoppers were originally obtained from colonies maintained in the laboratory of R. L. Gilbertson in the Department of Plant Pathology at the University of California, Davis. The colonies were maintained in BugDorm mesh cages (61 cm × 61 cm × 61 cm, Megaview Science, Taichung, Taiwan) with non-infected sugar beet (*Beta vulgaris* L. cultivar Saccharifera) or sugar beet infected with an isolate of the BCTV severe-type strain in separate greenhouses (25 ± 5 °C and 80 ± 10% relative humidity). BCTV-infection of sugar beet plants and beet leafhoppers was confirmed with a well-established PCR-based method²⁶.

Barley (cultivar Champion), ribwort plantain, and tomato (cultivar Apt 410) were grown in four-inch plastic pots with a mixture of 1:1:1:1 ratio of pumice: sand: sphagnum peat moss: redwood sawdust in a greenhouse at 25 ± 5 °C and 80 ± 10% relative humidity. All plants were watered daily and fertilized with 0.5% soluble N-P-K fertilizer (6:1:4) in 200 ml of water. One- to two-month-old plants were used for all experiments. Barley and ribwort plantain were selected among trap crop candidates based on probing preference of non-viruliferous beet leafhoppers (Fig. S2). All protocols using insects and plants in this study complied with relevant institutional, national, and international guidelines and legislations.

Dual-choice experiments

Using dual-choice experiments (i.e., tomato vs. ribwort plantain and tomato vs. barley), we investigated probing and oviposition preference of non-viruliferous and viruliferous beet leafhoppers. Dual-choice experiments were conducted in clear plastic tubes (28 cm × 6 cm; L × D), in which single leaves/leaflets were inserted in either end, and beet leafhopper individuals

were released in the middle (Fig. 1a). Each leaf/leaflet was placed into a vial (8 cm x 2.5 cm; L x D) filled with water and held in place with cotton. For each dual-choice bioassay, three pairs (six individuals) of newly emerged (<72 hours) non-viruliferous or viruliferous adult beet leafhoppers were starved for four hours at 25 °C and then released into plastic tubes and kept in a controlled environment chamber (25 ± 0.5°C and 50 ± 5% relative humidity). Similar to previously published studies^{27,28}, probing events were counted based on McBryde staining²⁹. Moreover, after 24 hours of exposure to feeding beet leafhoppers, leaves/leaflets were collected and stained with McBryde's solution³⁰, which is 0.2% (wt/vol) acid fuchsin in a mixture of 95% ethanol and glacial acetic acid (1:1 vol/vol), for 24 hours. Leaves/leaflets were then transferred to a clearing solution of distilled water, 99% glycerol, and 95% lactic acid (1:1:1 vol/vol/vol) at 95 °C for four hours. Stained probing events and eggs were counted under a binocular stereomicroscope (Olympus SZ51; Olympus, Tokyo, Japan).

No-choice experiments

Total number of probing events and mean duration of each probing event by non-viruliferous and viruliferous beet leafhoppers were recorded on each plant species. Single non-viruliferous and viruliferous beet leafhoppers were starved for four hours at 25 °C and afterwards transferred to a glass cage (3 cm × 2 cm × 0.3 cm) attached to abaxial sides of leaf/leaflet from each plant species (Fig. 1b). Probing behavior was video recorded for 30 minutes with a Canon EOS 70D fitted with a Canon MP-E 65mm macro lens (Canon, Huntington, NY, USA) (<https://youtu.be/HLdBd4grQ34>). The confined width of the cage was allowed for focusing of the

camera on the mouth part of beet leafhopper. Duration of probing events was determined as the time period in which stylets were inserted into leaves/leaflets¹¹.

Statistical analyses

All statistical analyses were performed with R software (version 3.6.1)³¹. Arcsine-transformed percentages of probing events and eggs laid on each plant species were compared to determine preference of viruliferous and non-viruliferous beet leafhoppers. Insect vector manipulation was determined by using two-way analysis of variance (ANOVA) followed by Tukey's HSD test. Significant differences were determined at the 0.05 level. Normality of data was checked with Shapiro-Wilk normality test.

Regarding data from video recordings of probing behavior, total numbers of probing events were square root transformed for statistical analysis. Normality of mean duration of probing events and transformed numbers of probing events on different plant species were examined based on Shapiro-Wilk normality test. Statistical comparison of average probing behavior by viruliferous and non-viruliferous beet leafhoppers among all plant species was based on two-way ANOVA followed by Tukey's HSD test.

Simulation of BCTV spread in tomato fields

Although beet leafhoppers can transmit BCTV to tomato plants, few eggs are laid on tomato plants²⁵, and newly hatched nymphs don't carry BCTV. Therefore, we only considered probing preference and duration of probing of beet leafhoppers in model simulations. We examined two

tomato field scenarios with either barley or ribwort plantain as a trap crop. In both scenarios, total number of plants was held constant at 100, but percentage of trap crop plants represented percentages ranging from 0% (i.e., tomato only) to 90% (only 10% tomato plants). We assumed settlement of viruliferous beet leafhoppers to be driven by two independent variables: 1) probing preference for plant species and 2) percentages of available plant species¹⁹ such that:

$$\alpha_{y,s} = \frac{\gamma_s P_{y,s} D_s}{\sum_{s=1}^S \sum_{z=1}^Z \gamma_s D_s P_{z,s}} \quad (1)$$

where $\alpha_{y,s}$ denotes the proportion of viruliferous beet leafhoppers that probe on plant species, s (barley [b], ribwort plantain [p], and tomato [t]) of infection status y (non-infected [n] or infected [i]). γ_s denotes probing preference of viruliferous beet leafhoppers for plant species s . $P_{y,s}$ denotes total number of plant species, s , of infection status, y , and $s = 1, \dots, S$ indexes all plant species, while $z = 1, \dots, Z$ indexes all plant infection statuses (infected or non-infected). D_s denotes probing duration (day^{-1}) on plant species, s . Incorporating probing preference and probing behavior yields¹⁹:

$$\frac{dP_{n,s}}{dt} = -\beta \alpha_{n,s} V \quad (\text{for non-infected plant } s) \quad (2)$$

$$\frac{dP_{i,s}}{dt} = \beta \alpha_{n,s} V \quad (\text{for infected plant } s) \quad (3)$$

β denotes transmission rate coefficient from a viruliferous beet leafhopper to a non-infected plant and determined as $\beta = 0.38$. β value was identified as the value Stafford, et al.³² measured inoculation success rate of one individual beet leafhopper. $\alpha_{n,s}$ denotes proportion of viruliferous beet leafhoppers that probe on non-infected plant species, s . V denotes number of viruliferous beet leafhoppers.

Each simulation began with non-infected plants and 10 viruliferous beet leafhoppers. We quantified BCTV spread in tomato fields based on assumption of innate probing preference and behavior or presence of insect vector manipulation. Innate probing preference and behavior equals probing preference: $\gamma_{i,p} = 0.63$, $\gamma_{i,t} = 0.37$; $\gamma_{i,b} = 0.6$, $\gamma_{i,t} = 0.4$ and probing behavior: $D_p = 0.0122$, $D_t = 0.0120$, $D_b = 0.0109$. Insect vector manipulation equals probing preference: $\gamma_{m,p} = 0.53$, $\gamma_{m,t} = 0.47$; $\gamma_{m,b} = 0.47$, $\gamma_{m,t} = 0.53$ and probing behavior: $D_p = 0.0139$, $D_t = 0.0150$, $D_b = 0.0055$. Percentages of BCTV-infected tomato plants and time to 20% infection were calculated in the scenarios. All simulations were performed in R.

Results

Probing and oviposition preference

In dual-choice experiments, percentage of leaf probing events on each plant species was used as an indicator of probing preference by non-viruliferous and viruliferous beet leafhoppers (Fig. 2). Actual numbers of probing events and eggs laid are presented in Table S1. A significant difference in probing preference between non-viruliferous and viruliferous beet leafhoppers ($F = 8.22$, $df = 1,96$, $P = 0.005$) was observed in the ribwort plantain vs. tomato combination. Thus, a post hoc Tukey's HSD test revealed that non-viruliferous beet leafhoppers probed ribwort plantain significantly more than tomato, whereas viruliferous beet leafhoppers showed no significance (non-viruliferous: $P < 0.001$; viruliferous: $P = 0.776$). There was also a significant difference in probing preference between non-viruliferous and viruliferous beet leafhoppers in the barley vs. tomato combination ($F = 23.98$, $df = 1,96$, $P < 0.001$). Here, non-viruliferous beet leafhoppers probed barley significantly more than tomato (Tukey's HSD; $P < 0.001$), whereas

viruliferous beet leafhoppers showed no preference (Tukey's HSD; $P = 0.298$). Therefore, viruliferous beet leafhoppers were less selective in their host plant choice (i.e., reduced preference) compared to non-viruliferous conspecifics.

Oviposition preference of non-viruliferous and viruliferous beet leafhoppers was determined by comparing percentages of eggs laid on choices between two plant species (Fig. 3). There was no significant difference in oviposition preference between non-viruliferous and viruliferous beet leafhoppers in neither the ribwort plantain vs. tomato combination ($F = 2.76$, $df = 1,84$, $P = 0.1$) nor barley vs. tomato ($F = 3.858$, $df = 1,60$, $P = 0.054$). A post hoc Tukey's HSD test revealed that both viruliferous and non-viruliferous beet leafhoppers preferred ribwort plantain over tomato for oviposition (both viruliferous and non-viruliferous: $P < 0.001$). In contrast, tomato was preferred over barley by viruliferous and non-viruliferous beet leafhoppers in the tomato vs. barley combination (Tukey's HSD; $P < 0.001$). Thus, viruliferous and non-viruliferous beet leafhoppers showed the same oviposition preference in the two combinations of plant species.

Probing behavior on each plant species

Viruliferous beet leafhoppers showed a significant difference in total numbers of probing events on barley compared with ribwort plantain and tomato ($F = 7.436$, $df = 2, 79$, $P = 0.001$) (Fig. 4a), whereas this was not seen for non-viruliferous beet leafhoppers. A post hoc Tukey's HSD test revealed that viruliferous leafhoppers probed barley 1.9 more times than non-viruliferous conspecifics ($P < 0.001$). Moreover, only viruliferous beet leafhoppers showed a significant reduced mean duration of probing event on barley compared with ribwort plantain and tomato (F

= 5.122, $df = 2, 79$, $P = 0.008$) (Fig. 4b). The probing duration of viruliferous beet leafhoppers on barley was 0.5 times shorter than that of non-viruliferous conspecifics (Tukey's HSD; $P = 0.007$). The difference was not observed for ribwort plantain or tomato.

Simulation of BCTV spread in tomato fields

Based on simulation modeling of BCTV spread in scenarios with different percentages of trap crops, we observed that assumption of insect vector manipulation increased the risk of tomato plant infection in terms of the percentage of infected tomato plants and time needed to reach 20% infection (Fig. 5). Viruliferous beet leafhoppers with manipulated probing preference resulted in higher percentages of infected tomato plants than conspecifics with innate probing preference in the tomato fields with barley or ribwort plantain as a trap crop (Fig. 5a). In addition, shorter probing times on barley were associated with increased BCTV infection in tomato as compared to tomato with ribwort plantain trap crop. Insect vector manipulation was also predicted to accelerate the spread of BCTV, with the effect decreasing as the ratio of tomato plants to trap crops increased (Fig. 5b). We observed a linear relationship between trap crop percentage and BCTV spread, in which it would take 2-8 days for 20% of tomato plants to be infected depending on trap crop species. In tomato fields with barley trap crops, the time to reach 20% infection was greater than in simulations with ribwort plantain as a trap crop.

Discussion

In this study, we demonstrated that BCTV appears to manipulate probing preference and behavior by beet leafhoppers, whereas there was no significant difference in oviposition

preference. BCTV is only transmitted during probing but not transovarially transmitted from infected female beet leafhoppers to their progeny²⁶. Therefore, BCTV would have less evolutionary benefit from manipulating oviposition preference versus probing preference of beet leafhoppers. Indeed, we observed that non-viruliferous beet leafhoppers preferred to probe on barley and ribwort plantain compared with tomato, whereas viruliferous beet leafhoppers showed no preference. In addition, mean probing duration was shorter for viruliferous beet leafhoppers on barley compared with non-viruliferous conspecifics. This alteration of probing preference and behavior in viruliferous beet leafhoppers may result in enhanced BCTV transmission in tomato fields, and that was examined based on a simulation model of tomato fields under different trap crop scenarios. Simulation modeling based on limited parameters predicted that BCTV infection rates would be higher in tomato fields with barley compared with ribwort plantain as a trap crop. Simulation results supported the hypothesis that manipulation of probing preference and behavior could increase BCTV infection in tomato fields. Finally, these results suggest that beet leafhopper manipulation by BCTV following virus acquisition under natural conditions (i.e., high plant diversity) may accelerate the spread of BCTV in tomato fields. BCTV outbreaks in tomato are associated with high densities of viruliferous beet leafhoppers^{33,34}. Therefore, viruliferous beet leafhopper populations, which have acquired BCTV from symptomless weeds outside tomato fields, would already have altered probing preference and behavior, further contributing to BCTV outbreaks in tomato crops.

Phytophagous insects commonly use chemical cues to locate and accept host plants³⁵, which suggests that viruliferous status of insect vectors may affect how olfactory and/or gustatory cues are processed³⁶. Processing of volatile plant cues by insects is mediated by soluble binding proteins, found in olfactory and gustatory organs³⁷. Chemosensory proteins (CSPs) and

odorant-binding proteins (OBPs) were identified as the major soluble proteins found in sensillar lymph of insects³⁸. Those proteins are conserved across insect species, suggesting they may play an important role in host selection by insects. Indeed, it has been shown that these proteins affect host preference of some insects, including *Drosophila sechellia*³⁹, *Adelphocoris fasciaticollis*⁴⁰, and *Nilaparvata lugens*⁴¹. Thus, plant viruses may alter the expression of CSPs and OBPs, and consequently the host preference. Hu, et al. ⁴² reported that viruliferous *Sogatella furcifera* (carrying southern rice black-streaked dwarf virus) exhibited decreased expression levels of OBPs, which altered host preference of the viruliferous *S. furcifera*. Hence, future research comparing the expression levels of CSPs and OBPs between viruliferous and nonviruliferous beet leafhoppers may reveal molecular mechanisms of probing preference manipulation.

Viruliferous beet leafhoppers showed an increase in the number of probing events, but a decrease in the mean duration of each probing event only on barley, which is not a host for BCTV. This suggests the possibility of plant species-specific manipulation of probing behavior of viruliferous beet leafhoppers. Broadly, insects tend to shorten probing duration on less suitable plant species, which implies that mean duration of probing events can be used as an indicator of feeding host suitability^{43,44}. Stafford and Walker ⁴⁵ characterized the feeding behavior of beet leafhoppers using an electrical penetration graph (EPG). They classified feeding into three phases: pathway phase, non-phloem ingestion phase, and phloem phase, and measured mean duration of each phase. In addition, time from onset of probing to phloem phase was measured for critical stylet penetration behavior associated with inoculation of BCTV³². Mean duration of probing events of the viruliferous beet leafhoppers on barley was similar to the median time to phloem salivation, as determined by Stafford, et al. ³². Thus, we suspect that the viruliferous beet leafhoppers rejected barley at the phloem phase, and that the chemical

composition of the phloem sap may be involved in the BCTV-induced host rejection process. In future work, it would be informative to confirm in which feeding phase beet leafhoppers reject plants by using EPG.

Most insect vector manipulation studies with plant viruses include only a single host plant species with different infection status⁴⁶. However, more studies are needed that incorporate multiple plant species because insect vector manipulation could be species-specific and most plant viruses have variable host ranges⁴⁷. Shoemaker, et al.¹⁹ found a decrease in overall plant pathogen spread through multi-host plant species due to insect vector manipulation. However, when only infection of the main crop (i.e., tomato) was considered, our simulation modeling showed an increase in the spread of BCTV. In addition, the rate of BCTV spread was affected depending on trap crop species. Therefore, multiple plant systems provide an important opportunity to better understand insect vector manipulation effects on the spread of plant viruses in natural and agricultural environments.

In summary, we experimentally examined insect vector manipulation and predicted its impact in tomato fields using simulation modeling. Although three plant species were used in this preliminary study, future work with additional plant species will be necessary to address the wide host ranges of beet leafhopper and BCTV^{21,22} and their influence on infection of tomato crops. Results presented in the current study were based on the BCTV-beet leafhopper pathosystem, and this study combining experimental studies with complementary simulation modeling can contribute to more thorough understanding of the dynamics driving not only beet leafhopper transmission of BCTV, by providing information on specific key factors contributing to virus epidemiology. Further, such a system can also be relevant to other insect-vectored plant pathogen systems involving multiple plant species.

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Data availability

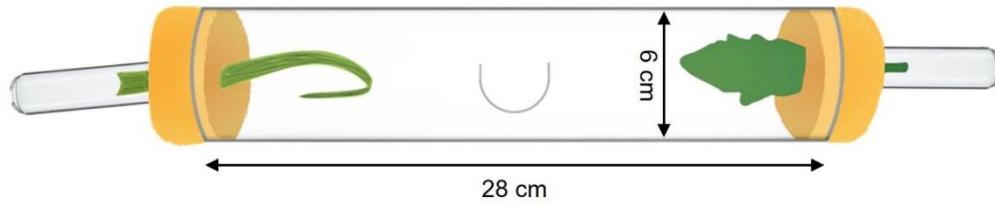
All data relevant to the study are included in the article or uploaded as supplementary information. Model code is available on GitHub

(https://github.com/hyoseoklee23/Vector_manipulation).

Figure legends

Figure 1. Diagrammatic illustrations of (a) feeding and oviposition preference experiments and (b) feeding behavior.

(a)



(b)

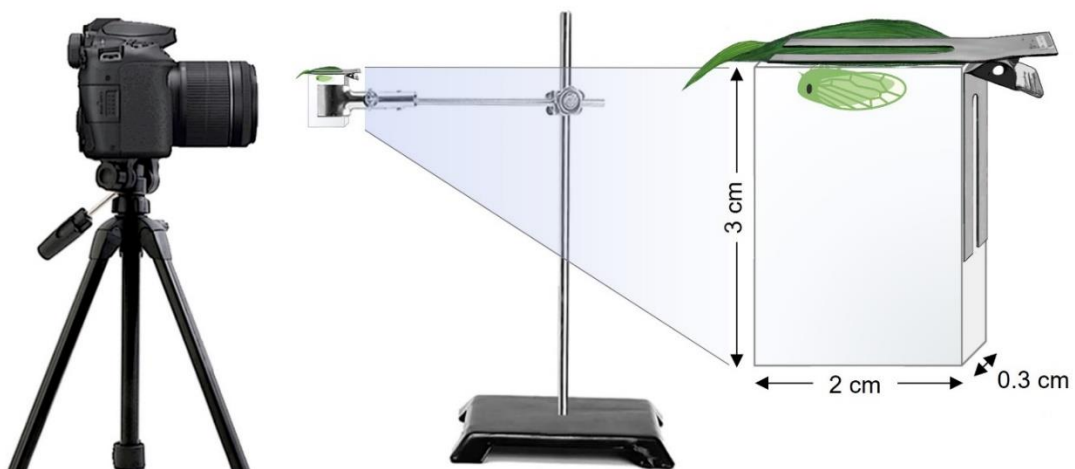


Figure 2. Probing events by non-viruliferous and viruliferous beet leafhoppers to leaves/leaflets in the dual-choice arena. Data are presented as mean percentages \pm SE. Asterisks indicate significant differences ($*p < 0.05$). NS: No significant difference ($p > 0.05$).

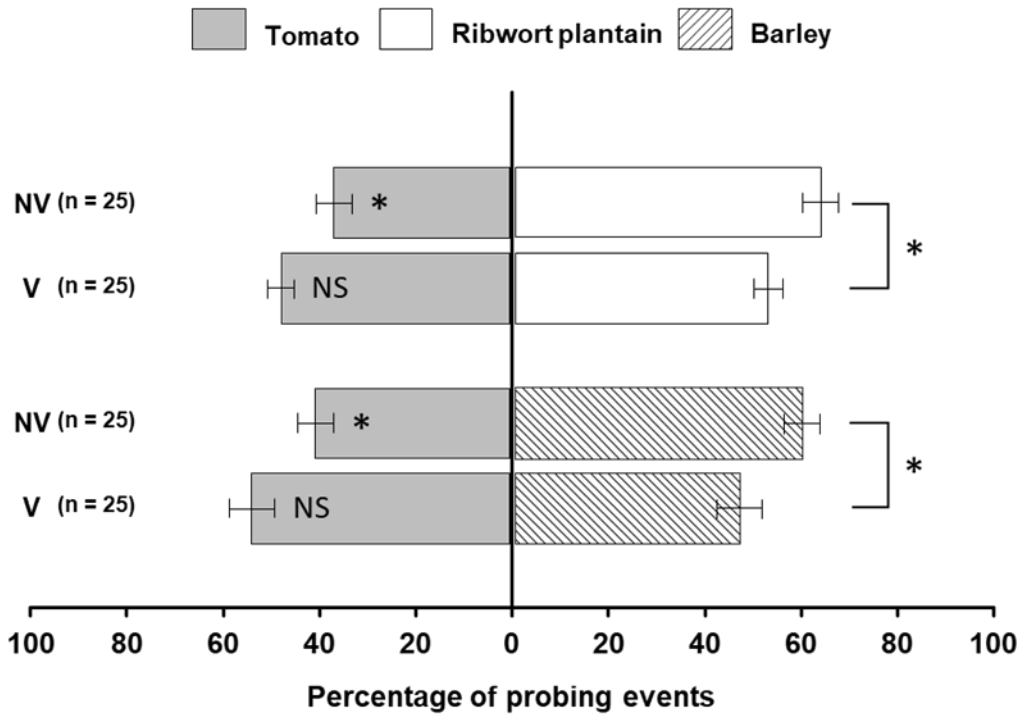


Figure 3. Oviposition by non-viruliferous and viruliferous beet leafhoppers on leaves/leaflets in the dual-choice experiments. Data are presented as mean percentages \pm SE. Asterisks indicate significant differences (***) ($p < 0.001$). NS: No significant difference ($p > 0.05$).

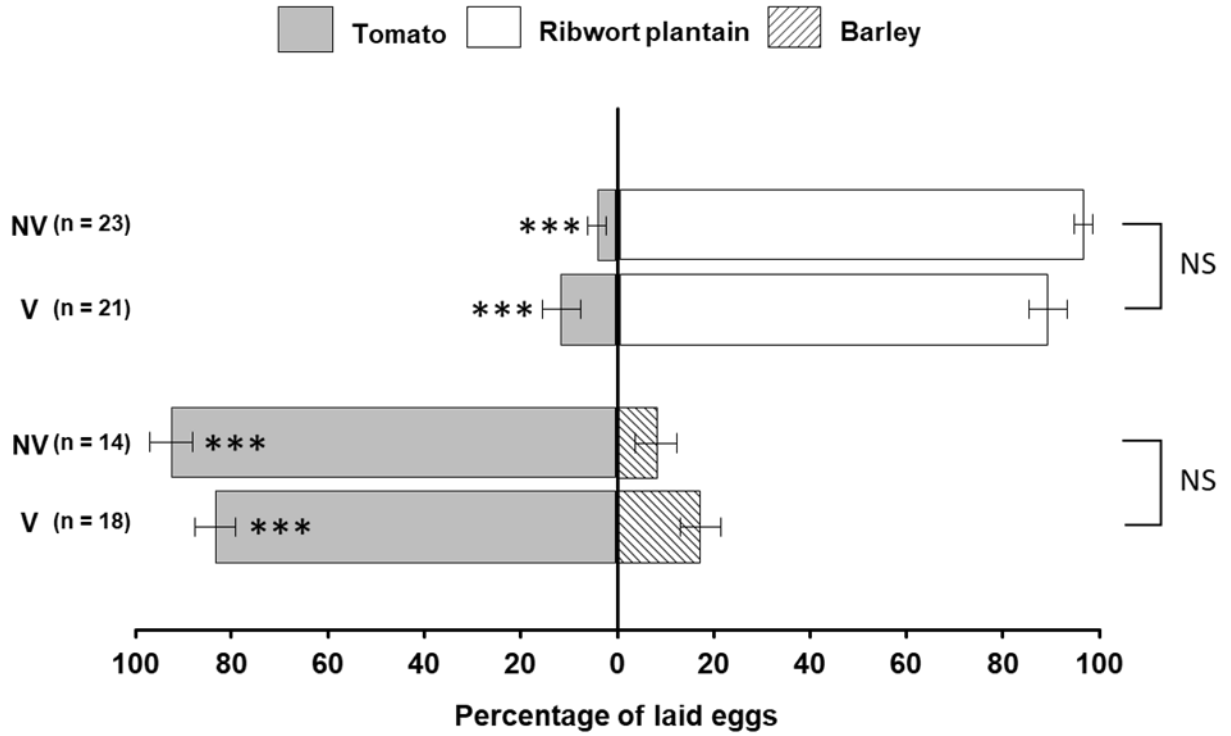


Figure 4. Probing events by non-viruliferous and viruliferous beet leafhoppers on barley, ribwort plantain, and tomato. (a) Total number of probing events; (b) mean duration per probing event. Letters indicate significant among plant species (Tukey test, $p < 0.05$). Asterisks indicate statistically significant differences between non-viruliferous and viruliferous beet leafhoppers (** $p < 0.01$, *** $p < 0.001$). NS: No significant difference ($p > 0.05$).

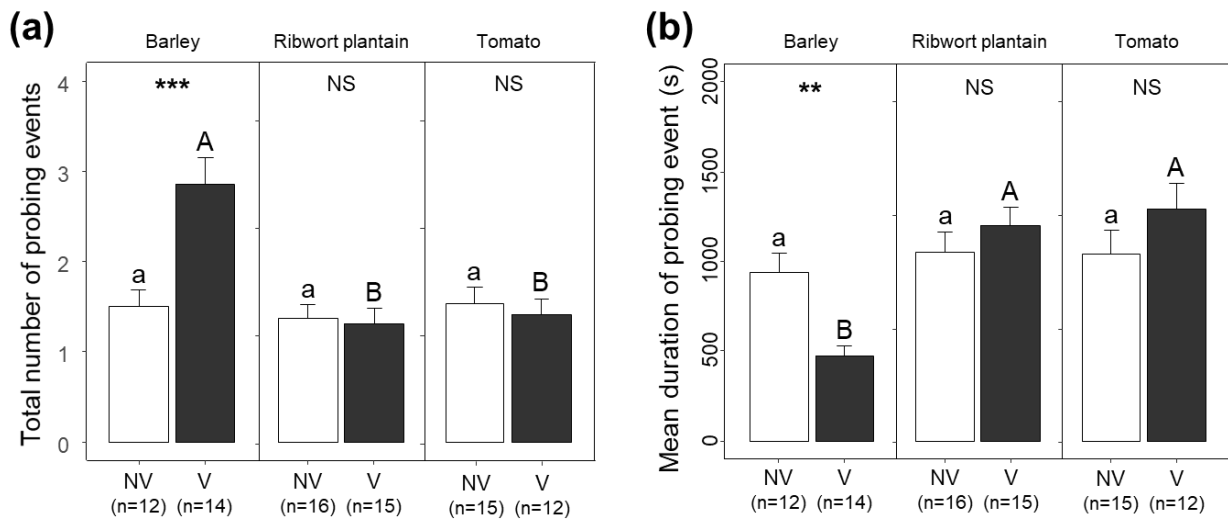
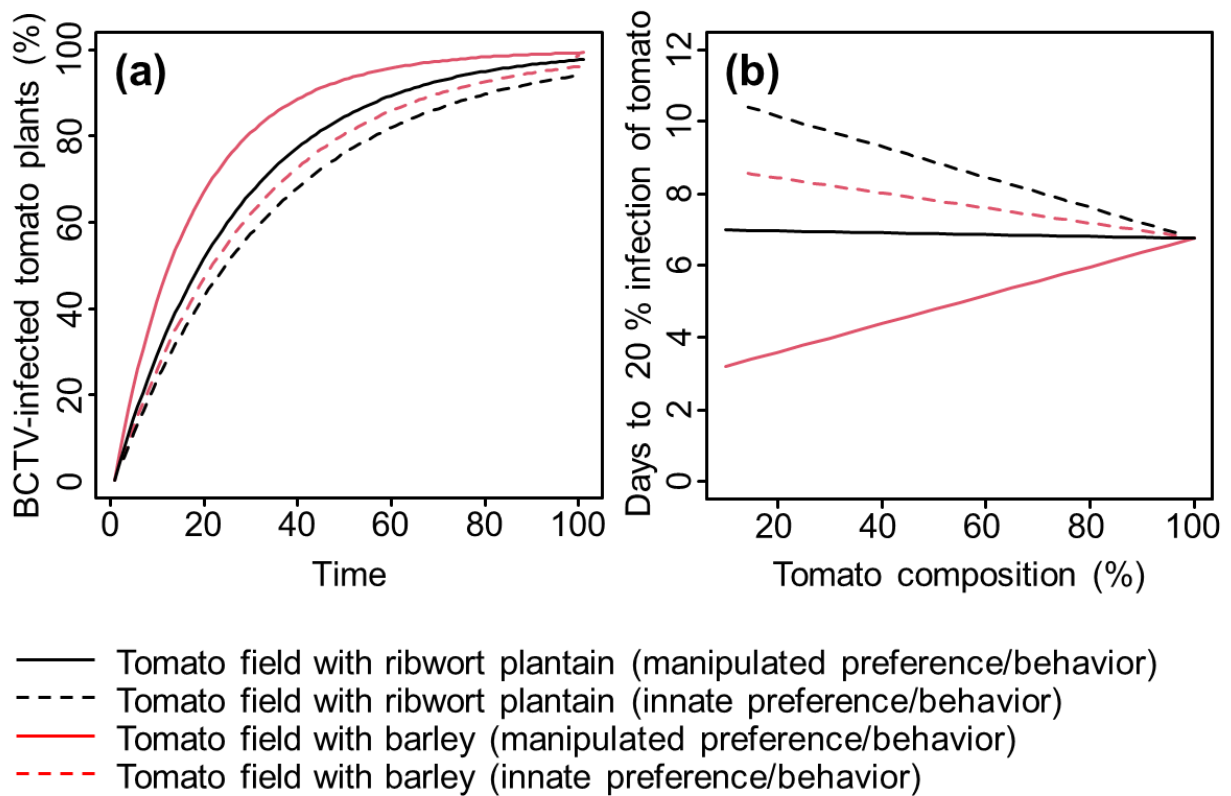


Figure 5. BCTV manipulation effects on (a) the percentage of BCTV-infected tomato plants and (b) the time to 20% infection of tomato plants across various tomato composition were examined in tomato fields with ribwort plantain or barley as a trap crop. The cases with manipulated preference and innate (without manipulated) preference are shown in solid and dashed lines, respectively. Black lines represent tomato fields with ribwort plantain as a trap crop and red lines represent tomato fields with barley as a trap crop.



Supplementary materials

Figure S1. Adult survivorship of non-viruliferous beet leafhoppers confined on barley, ribwort plantain, and tomato plants for 40 days. Newly emerged adults were reared at 27 °C, 50% relative humidity, and a photoperiod of 16:8 (L:D) h. The experiment was replicated 10 times for each plant species and 15 beet leafhoppers were used for each replication. The median longevity on barley, ribwort plantain, and tomato plants were 8.2, 42, and 2.2 days, respectively. The data for sugar beet was acquired from Munyaneza and Upton ¹

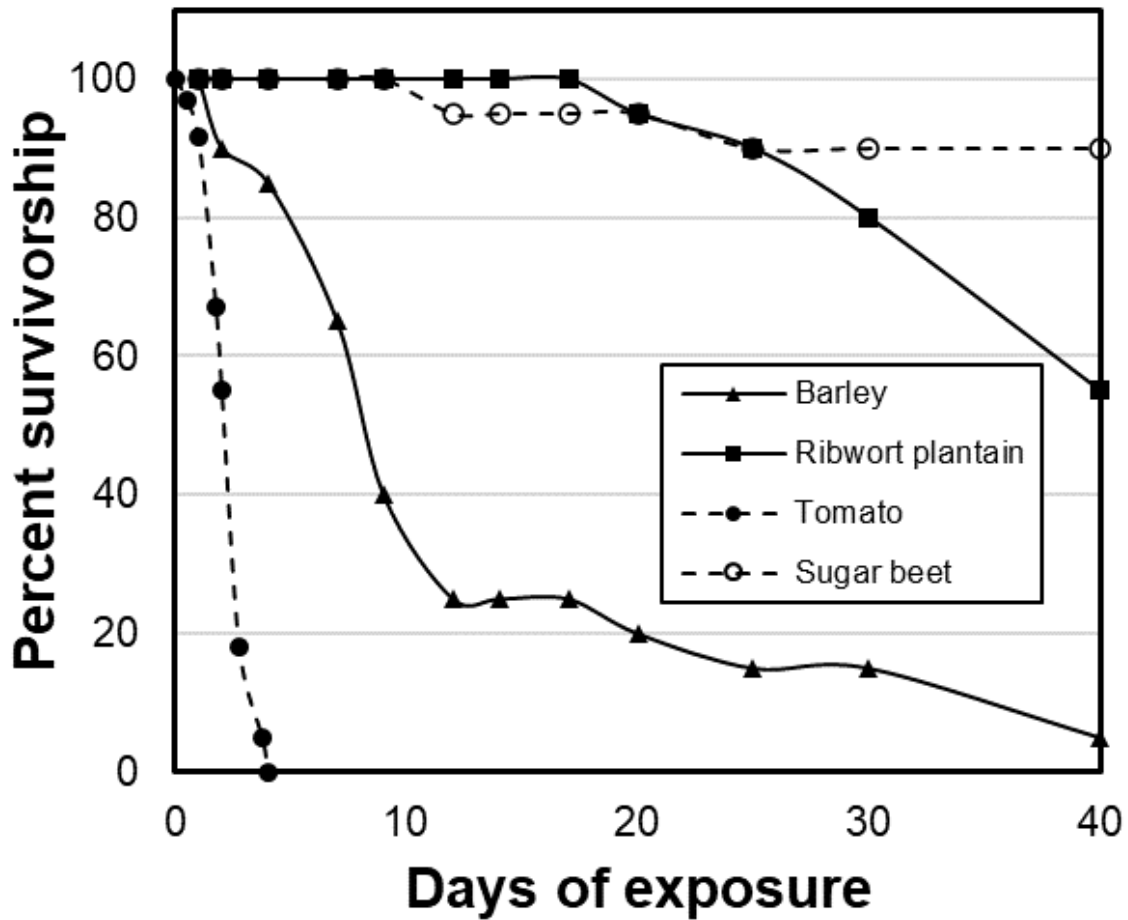


Figure S2. Probing preference of non-viruliferous beet leafhoppers between tomato and trap crop candidates in dual-choice experiments. Data are presented as mean \pm SE. Asterisks indicate significant differences (* p <0.05, ** p <0.01, *** p <0.001, NS not significant).

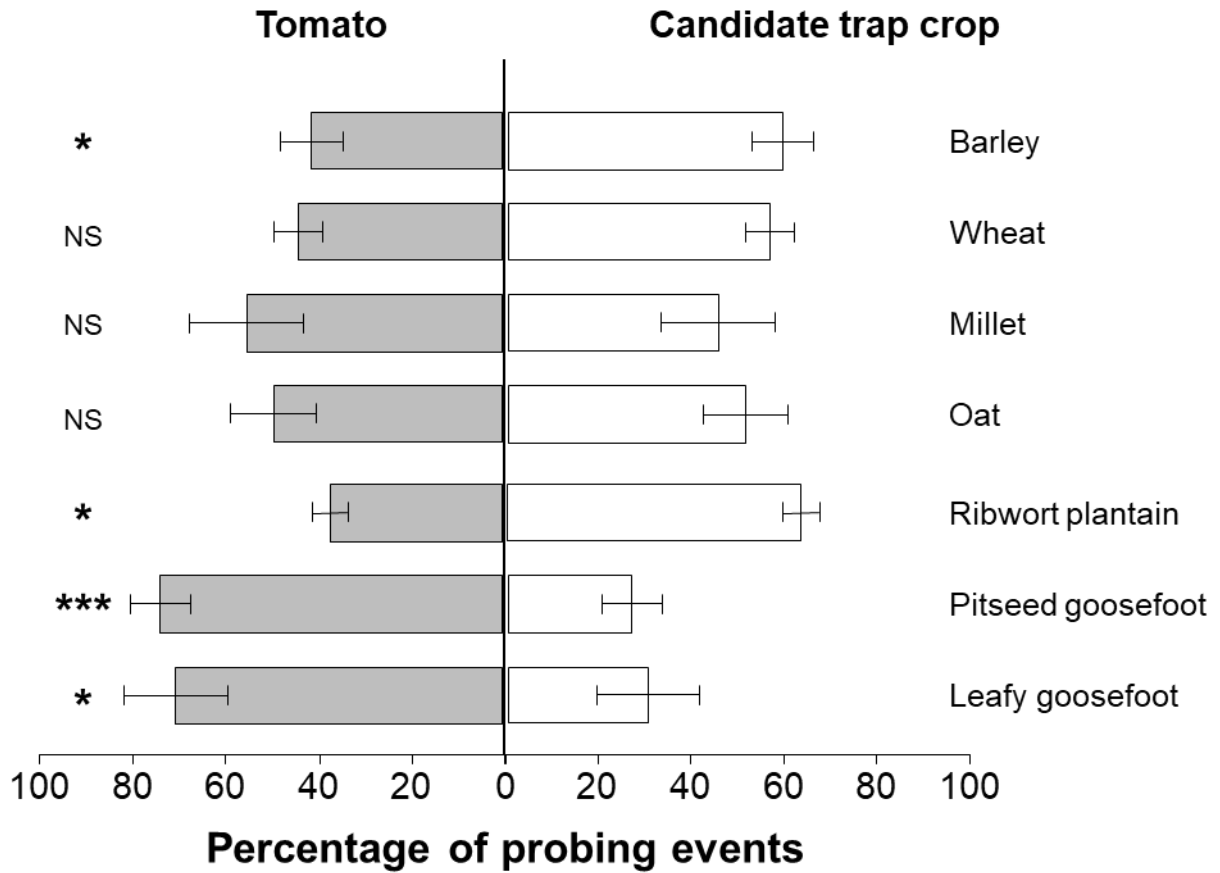


Table S1. Number of probing events and eggs (mean \pm SE) on each plant species in the dual-choice experiments.

Plant combination	Beet leafhopper	Plant	Number of probing events ¹	Number of eggs ²
Tomato	Viruliferous	Tomato	103.1 \pm 15.68ab	1.2 \pm 0.40ab
vs.		Ribwort plantain	99.3 \pm 9.64	10.72 \pm 1.38
Ribwort plantain	Nonviruliferous	Tomato	74.9 \pm 10.85a	0.76 \pm 0.32a
		Ribwort plantain	117.4 \pm 10.96	11.76 \pm 2.03
Tomato	Viruliferous	Tomato	144.4 \pm 13.23b	2.52 \pm 0.55b
vs.		Barley	123.3 \pm 12.73	0.48 \pm 0.13
Barley	Nonviruliferous	Tomato	107.5 \pm 7.52ab	1.52 \pm 0.44ab
		Barley	165.9 \pm 12.17	0.2 \pm 0.10

^{1,2}Within a column means for tomato with the same letter indicates no significant difference in ANOVA, Tukey test ($P < 0.05$). There was no significant difference in the number of probing events and eggs for other plants ($P > 0.05$).

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This chapter is formatted for submission to *PLoS ONE*

Chapter 2: Modeling and validation of oviposition by a polyphagous insect pest as a function of temperature and host plant species

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Keywords: Beet leafhopper; Fecundity; Normalized age; Survival; Longevity

Abstract

The number of eggs laid by female insects on a plant within a given time period is largely a function of non-linear relationships among insect age, temperature, and host plant suitability. Modeling oviposition as a function of these variables may provide valuable insight into insect population growth of polyphagous insect pests at a landscape level. Based on modeling, we quantified oviposition of beet leafhoppers, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae), on four common non-agricultural host plant species [*Erodium cicutarium* (L.) L'Hér. (Geraniaceae), *Kochia scoparia* (L.) Schrader (Amaranthaceae), *Plantago ovata* Forsskál (Plantaginaceae), and *Salsola tragus* L. (Amaranthaceae)] at two constant temperature conditions. Additionally, temperature-based oviposition models for each plant species were validated, as host plants with beet leaf hoppers were maintained in meshed cages under semi-field and greenhouse conditions. We found that *K. scoparia* was the most attractive host plant for oviposition, and the optimal temperature for oviposition was estimated to be 30.6 °C. Accordingly, beet leafhoppers appear to be well-adapted to high-temperature conditions, so increasing temperature due to climate change may favor population growth in non-agricultural areas. Maximum total fecundity (R_m) is an indicator of the relative attractiveness of host plants. *S. tragus* has been considered an important non-agricultural host plant; however, we found that *S. tragus* and *E. cicutarium* have a lower R_m compared to *K. scoparia* and *P. ovata*. The combination of detailed oviposition bioassays, modeling, and model validation should be widely relevant and applicable to host plant assessments of other polyphagous insect pests.

Introduction

Polyphagy is a significant challenge regarding development of effective management strategies for economically important insect pests of agricultural crops [1], as it may require coordination of management efforts across multiple crops and possibly in both agricultural and non-agricultural habitats. For these insect pests, it is of paramount importance to characterize and ideally quantify relative importance and potential of different host plant species [2]. Stated boldly, if an insect pest is known to be able to successfully complete its life cycle on multiple plant species, are these plant species contributing equally to the overall insect pest population growth? This is one of the basic questions addressed in this study, and a reasonable initial approach is to compare oviposition by individual female insects on multiple host plant species. Knowledge of how suitable host plants are for oviposition is of great importance in designing vegetation management strategies to control polyphagous insect pests [3]. Saeed et al. (4) tested oviposition of diamond-back moth, *Plutella xylostella* (Lepidoptera: Plutellidae), on six different crops including cabbage (*Brassica oleracea capitata*), cauliflower (*Brassica oleracea botrytis*), radish (*Raphanus sativus*), turnip (*Brassica rapa*), mustard (*Brassica campestris*), and canola (*Brassica napus* var. *canola*). The authors concluded that canola was the most suitable host plant for population growth of diamond-back moth due to higher oviposition. In addition, life-table data for tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), were collected on multiple green manure cover crops including sesbania (*Sesbania roxburghii*), sunn hemp (*Crotalaria juncea*), and rapeseed (*Brassicae campestris*) [5]. Tobacco cutworms laid about 1.5 times more eggs on sesbania than other green manure cover crops. The authors emphasized importance of sesbania fields as major sources of tobacco cutworm and need of an area-wide pest management program based on host suitability. Additionally, assessments of relative

suitability of host plant species can be used to characterize and model insect pest population dynamics as a function of vegetation composition.

In addition to suitability of host plant species, oviposition by insects is influenced by age/development and temperature, and the relationship of these variables with oviposition is typically asymmetric and unimodal [6, 7]. Taylor (8) measured insect age by using the physiological time concept, accumulation of temperature-dependent aging (development) rate at each instant in time. Accordingly, insect oviposition under fluctuating temperature conditions has been described by three components: temperature-dependent total fecundity, age-specific oviposition rate, and age-specific survival of female adults [8-10]. Age-specific oviposition rate and survival have not been investigated in many insects, but such data are needed to describe insect oviposition under fluctuating temperature conditions. For describing the relationship between temperature and total fecundity, non-linear functions such as Lactin [11], extreme value [10, 12], and Gaussian [13] functions have been used. Age-specific oviposition rate and survival have been modeled using Gompertz [14], sigmoid [13], or Weibull [15] functions.

The model insect for this study is beet leafhopper, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae). Beet leafhoppers have a wide host range, including a number of crops, and non-agricultural trees, shrubs, and annual plant species [16, 17]. Beet leafhoppers are economically important insect pests as they vector beet curly top virus (BCTV), which can cause significant yield losses in economically important crops including tomato (*Solanum lycopersicum* L.), sugar beet (*Beta vulgaris* L.), pepper (*Capsicum annuum* L.), spinach (*Spinacia oleracea* L.), and common bean (*Phaseolus vulgaris* L.) [18]. In North America, beet leafhoppers are the only known vectors of BCTV [19], and they transmit BCTV to crops from non-agricultural host plants in spring, after migrating from non-agricultural habitats into agricultural landscapes [20].

Thus, evaluation of suitability of non-agricultural host plants for oviposition of beet leafhopper is needed to effectively characterize and model beet leafhopper population growth and ultimately, to develop sustainable management strategies for this important insect pest. In the current study, we quantified oviposition of beet leafhoppers on four non-agricultural host plant species, *Erodium cicutarium* (L.) L'Hér. (Geraniaceae), *Kochia scoparia* (L.) Schrader (Amaranthaceae), *Plantago ovata* Forsskál (Plantaginaceae), and *Salsola tragus* L. (Amaranthaceae). These host plants were selected because beet leafhoppers commonly use *E. cicutarium*, *P. ovata*, and *S. tragus* as winter and spring non-agricultural host plants and *K. scoparia* as a summer non-agricultural host plant [16]. Oviposition models (i.e., temperature-dependent total fecundity, age-specific oviposition rate, and age-specific survival) were constructed for each of non-agricultural host plant species. In addition, these models were validated under fluctuating temperature conditions (semi-field and greenhouse conditions), and we discussed relative importance of the non-agricultural host plant species in terms of beet leafhopper management.

Materials and methods

Oviposition and longevity experiments

Beet leafhopper colonies were reared on *B. vulgaris*. Four non-agricultural host plants, *E. cicutarium*, *K. scoparia*, *P. ovata*, and *S. tragus*, were grown in pots (d = 11 cm, h = 9.5 cm) under greenhouse conditions (25 ± 5 °C, 50 ± 10 RH). Individual pairs of newly emerged beet leafhoppers (< 24 h-old) were transferred into mesh cages (d = 6 cm, h = 10 cm) containing a leaves of each non-agricultural host plant. In total, 110 mesh cages were maintained at two constant temperatures (30 or 35 °C) in growth chambers. Males that died during early stages of the experiment (< 1 week) were replaced with new males to ensure mating. Leaves were

collected weekly, and mesh cages were transferred to new leaves until females died. Counting of eggs laid was facilitated by staining of leaves using a modified McBryde's solution [21], consisting of 0.2% (wt/vol) acid fuchsin in a mixture of 95% ethanol and glacial acetic acid (1:1 vol/vol) for 24 hours [22]. Leaves were cleared in a clearing solution consisting of distilled water, 99% glycerol, and 95% lactic acid (1:1:1 vol/vol/vol) at 95 °C for four hours. Stained eggs were counted under a binocular stereomicroscope (Olympus SZ51; Olympus, Tokyo, Japan). Oviposition rate and female survival were recorded every week for each non-agricultural host plant at each temperature condition (n = 11 to 15 cages/temperature/plant). Data were analyzed by analysis of variance using R software [23] to determine differences in total fecundity and longevity among the two different temperature conditions and four non-agricultural host plant species. Means were separated using a Tukey studentized range test at $\alpha = 0.05$.

Oviposition model components

Temperature-dependent total fecundity

Total fecundity is here defined as total number of eggs laid per female during her entire adult female lifespan, and its relationship with temperature was described by a Gaussian function [8]:

$$f(T) = R_m e^{[-\frac{1}{2}((T-T_{max})/k)^2]} \quad (1)$$

where $f(T)$ is the total number of eggs laid per female at temperature T (°C), R_m is the maximum total fecundity, T_{max} is the temperature (°C) at which the maximum fecundity occurs, and k is an estimated parameter defining curve steepness. Total fecundity of beet leafhoppers reared on *B. vulgaris* was obtained from Harries and Douglass (1948) [24] and fitted to Equation (1) to

estimate parameters. Regarding non-agricultural host plant species, only R_m was re-estimated with the same T_{max} and k parameters estimated as for *B. vulgaris*.

Normalized age

Temperature-dependent adult aging rate was modeled based on reciprocals of beet leafhopper longevity reared on *B. vulgaris* and non-agricultural host plant species. The following equation was used to describe adult aging rate as a function of temperature [25]:

$$r(T) = \sigma * e^{(a+bT^{2.5}+cT^3)} \quad (2)$$

where $r(T)$ is the adult aging rate (1/days) at temperature T (°C), σ is a host-specific parameter indicating a relative influence of non-agricultural host plant species compared to *B. vulgaris* (i.e., 1 for *B. vulgaris*), and a , b , and c are estimated parameters. The data for modeling adult aging rate of beet leafhoppers on *B. vulgaris* was obtained from Harries and Douglass (1948). Temperature-dependent adult aging rate on *B. vulgaris* was fitted to estimate a , b , and c parameters. Subsequently, σ was estimated for each non-agricultural host plant species with the same a , b , and c parameters estimated as for *B. vulgaris*:

Adult age (P_x) was normalized by accumulating adult aging rate as the following equation [9].

$$P_x = \sum_{i=0}^n r(T_i) \quad (3)$$

where n is days from emergence, $r(T_i)$ is the adult aging rate at temperature T (°C) of i th day after emergence.

Age-specific oviposition rate

Age-specific cumulative oviposition rate of beet leafhopper was modeled using the Weibull function [13]:

$$p(P_x) = 1 - e^{-(P_x/\alpha)^\beta} \quad (4)$$

where $p(P_x)$ is the cumulative rate of eggs laid at normalized age P_x , and α and β are estimated parameters. As adult age was normalized, oviposition data obtained under all temperature and non-agricultural host plant conditions were combined and modeled together.

Age-specific survival

Age-specific survival of adult beet leafhopper was modeled using a sigmoid function [13]:

$$s(P_x) = \frac{1}{1 + e^{(\gamma - P_x)/\delta}} \quad (5)$$

where $s(P_x)$ is the proportion of live females at normalized age P_x , γ is the normalized age at 50% survival, and δ is an estimated parameter. Survival data under all temperature and non-agricultural host plant conditions were combined and modeled together.

Model validation

Semi-field and greenhouse data collection

We measured oviposition of beet leafhoppers reared on non-agricultural host plant species under fluctuating temperature conditions. Individual pairs of newly emerged beet leafhoppers (< 24 h-old) were released into mesh cages (d = 6 cm, h = 10 cm) containing leaves of each non-agricultural host plant (two replicates for each host plant species under different

temperature conditions). For *E. cicutarium*, *P. ovata*, and *S. tragus*, mesh cages were maintained in an experimental field setting at the University of California, Davis during winter months. Mesh cages for *K. scoparia* were maintained in a greenhouse to mimic summer temperature conditions. Leaves were collected weekly for a total of 10 to 15 weeks, and eggs were stained and counted as described above. Daily ambient temperatures were recorded using Hobo loggers (Onset Computer, Co., Bourne, MA, USA) and used to predict beet leafhopper oviposition.

Simulation and validation

Daily oviposition at i th day on each non-agricultural host plant was the product of temperature-dependent total fecundity [$f(T_i)$], change in age-specific cumulative oviposition [$p(P_{x_{i+1}}) - p(P_{x_i})$], and survival $s(P_x)$. Weekly oviposition at n th week [$F(n)$] was simulated by adding up daily oviposition according to the following equation:

$$F(n) = \sum_{i=n}^{n+6} f(T_i)[p(P_{x_{i+1}}) - p(P_{x_i})] \quad (6)$$

Oviposition model outputs were compared with the semi-field and greenhouse observation data.

Results

Total fecundity and longevity on non-agricultural host plants

The total fecundity and longevity of beet leafhoppers reared on the four non-agricultural host plants under two temperature conditions are shown in Table 1. Both host plant species and temperature significantly affected the total fecundity (host plant species: $F[3,102] = 185.9$, $p < 0.001$; temperature: $F[1,102] = 32.8$, $p < 0.001$). Beet leafhoppers laid most eggs when reared on *K. scoparia* at both temperatures. Longevity was also significantly influenced by both host plant

species and temperature (host plant species: $F[3,102] = 10.7, p < 0.001$; temperature: $F[1,102] = 55.9, p < 0.001$). Beet leafhoppers reared on *K. scoparia* had the greatest longevity under both temperature conditions.

Table 1. Total fecundity (mean \pm SD) and longevity (mean \pm SD) of beet leafhoppers on four common non-agricultural host plants at constant temperatures.

Plant species	Total fecundity (eggs/female)		Longevity (weeks)	
	30°C	35°C	30°C	35°C
<i>Erodium cicutarium</i>	27.2 \pm 10.38cA (13)	17.3 \pm 4.92bB (15)	5.3 \pm 1.38bA (13)	3.2 \pm 0.77cB (15)
<i>Kochia scoparia</i>	137.6 \pm 20.80aA (14)	102.2 \pm 39.49aB (12)	6.4 \pm 1.09aA (14)	5.0 \pm 1.60aB (12)
<i>Plantago ovata</i>	106.6 \pm 13.61bA (15)	79.27 \pm 23.86aB (15)	5.5 \pm 0.92abA (15)	4.3 \pm 0.80abB (15)
<i>Salsola tragus</i>	30.1 \pm 5.56cA (15)	20.6 \pm 7.83bB (11)	5.1 \pm 0.92bA (15)	3.8 \pm 0.98bcB (11)

Different lowercase letters (a-c) indicate significant differences in total fecundity or longevity of beet leafhoppers on different plant species under the same temperature. Different capital letters (A-B) indicate significant differences in total fecundity or longevity of beet leafhoppers at different temperatures within the same plant species. Values in parentheses are sample sizes.

Oviposition model components

Total fecundity and aging rate models

Temperature-dependent total fecundity of beet leafhoppers reared on *B. vulgaris* was well described by the Gaussian function ($F[2,8] = 116.02$, $p < 0.001$, $adj-r^2 = 0.966$) (Fig 1A, Table 2). Temperature (T_{max}) with the maximum total fecundity was estimated to be 30.6 °C. Total fecundity on the non-agricultural host plant species was also well-described by the Gaussian function (1) with the same T_{max} and various k values (Table 2). *K. scoparia* showed the highest R_m value among the non-agricultural host plants ($F[4,7] = 608.96$, $p < 0.001$).

Table 2. Estimated parameters of the total fecundity models for beet leafhoppers on five host plant species.

Host plant	Parameter	Estimate (SE)	Adjusted r^2
<i>Beta vulgaris</i>	R_m	216.98 (9.876)	0.966
	T_{max}	30.6 (0.397)	
	K	7.18 (0.362)	
<i>Erodium cicutarium</i>	R_m	24.87 (2.871)	0.717
<i>Kochia scoparia</i>	R_m	133.05 (5.895)	0.908
<i>Plantago ovata</i>	R_m	103.15 (4.507)	0.910
<i>Salsola tragus</i>	R_m	28.61 (2.603)	0.777

The adult aging rate model effectively described aging rates (1/longevity) of beet leafhoppers reared on *B. vulgaris* ($F[2,7] = 1010.4$, $p < 0.001$, $adj-r^2 = 0.997$) (Fig 1B, Table 3). Regarding aging rate models for beet leafhoppers reared on the non-agricultural host plant species, we estimated only σ for each species (Table 3). Adult aging rates were positively related to temperature for all host plant species. Only adult females reared on *E. cicutarium* showed a higher aging rate than those reared on *B. vulgaris*.

Table 3. Estimated parameters of the aging rate models for beet leafhoppers on five host plant species.

Host plant	Parameter	Estimate (SE)	Adjusted r^2
<i>Beta vulgaris</i>	a	-4.70 (0.121)	0.997
	b	4.506e-04 (1.016e-04)	
	c	-3.837e-05 (1.412e-05)	
<i>Erodium cicutarium</i>	σ	1.108 (0.0318)	0.975
<i>Kochia scoparia</i>	σ	0.700 (0.0460)	0.795
<i>Plantago ovata</i>	σ	0.796 (0.0619)	0.661
<i>Salsola tragus</i>	σ	0.904 (0.0435)	0.912

Age-specific oviposition rate and survival

The relationship between cumulative oviposition rate and normalized age was described well by the Weibull function ($F[57,1] = 1571.5$, $p < 0.001$, $adj-r^2 = 0.965$) (Fig 2A, Table 4). Most eggs were laid before beet leafhoppers reached the normalized age of 1 (Fig 2). Age-specific survival of beet leafhoppers was also described well by the Weibull function ($F[65,1] = 438.02$, $p < 0.001$, $adj-r^2 = 0.871$) (Fig 2B, Table 4). Approximately half of beet leafhoppers died at the normalized age of 1 (Fig 2).

Table 4. Estimated parameters of the age-specific oviposition rate and survival models for beet leafhopper.

Model	Parameter	Estimate (SE)	Adjusted r^2
Oviposition rate	α	0.501 (0.0124)	0.965
	β	1.957 (0.1368)	
Survival	γ	1.042 (0.0222)	0.871
	δ	-5.083 (0.6959)	

Simulation and validation

Oviposition models were used to simulate weekly oviposition of beet leafhoppers on *B. vulgaris* in relation to temperature (Fig 3). Oviposition period increased with decreasing temperature, and maximum weekly oviposition occurred at about 40 °C. Oviposition of beet leafhoppers on non-agricultural host plant species was monitored under fluctuating temperature conditions (S1 Table). Oviposition under semi-field and greenhouse conditions was compared with the oviposition model outputs (Fig 4). Predicted oviposition followed the semi-field and greenhouse observation data well (*E. cicutarium*: $F[1,20] = 24.5$, $p < 0.001$, $adj-r^2 = 0.53$; *K. scoparia*: $F[1,27] = 55.91$, $p < 0.001$, $adj-r^2 = 0.66$; *P. ovata*: $F[1,22] = 59.83$, $p < 0.001$, $adj-r^2 = 0.72$; *S. tragus*: $F[1,20] = 33.9$, $p < 0.001$, $adj-r^2 = 0.61$).

Discussion

Temperature effects on immature development and total fecundity of beet leafhoppers reared only on *B. vulgaris* have been assessed [19], and results were converted into predictive models to aid in beet leafhopper management [18]. However, the some of the most damaging beet leafhopper populations that spread BCTV to crops are believed to originate from non-agricultural areas adjacent to agricultural fields [20]. In this study, we described oviposition of beet leafhoppers on four common non-agricultural host plant species and constructed oviposition models which were validated under semi-realistic conditions. In addition, we offered an important parameter, the maximum total fecundity (R_m), for each non-agricultural host plant, which represents the host suitability for beet leafhopper oviposition. *S. tragus* has been considered an important non-agricultural host plant for population growth of beet leafhopper [26]. However, we found that *S. tragus* and *E. cicutarium* have a lower potential for beet leafhopper population growth compared to *K. scoparia* and *P. ovata*. In addition, *S. tragus* was

less suitable as a reservoir of BCTV [26]. Therefore *K. scoparia* and *P. ovata* may be a more important host plant to beet leafhopper population dynamics in non-agricultural areas.

We found that total fecundity of beet leafhoppers was positively correlated to temperature until the optimal temperature (30.6 °C). Because beet leafhoppers are well-adapted to high-temperature conditions, the temperature for optimal fecundity is higher compared to other insect species [10, 12, 25]. Increasing temperature due to climate change is likely to favor population growth in non-agricultural areas. In addition, changes in rainfall patterns may affect phenology and density of non-agricultural host plants, which influence oviposition and survival of beet leafhoppers [27, 28]. Therefore, it is necessary to estimate the pest status of beet leafhopper in connection with changing environmental conditions and corresponding responses of host plants.

Spatio-temporal modeling of population dynamics as a function of abiotic conditions and vegetation composition will be needed in order to develop and implement precision-guided and sustainable management practices of beet leafhoppers. Furthermore, such modeling efforts will likely require inclusion of beet leafhoppers population dynamics in non-agricultural areas. As an example, the California Department of Food and Agriculture runs the Curly Top Virus Control program, which includes extensive and continuous sweep net sampling in the coastal and central foothills of California and airplane-based sprays with malathion in non-agricultural areas, when beet leafhopper densities reach certain thresholds. Due to the large geographical scale, region-wide decision support tools are needed to predict and visualize spatio-temporal dynamics of beet leafhopper densities in non-agricultural areas so that emerging hotspots can be detected.

Oviposition models as a function of temperature and host plant species presented in this study may be used to develop such decision support tools by predicting beet leafhopper hotspots based on host species distribution and temperature conditions in the non-agricultural areas. However,

such decision support tools should also include survival of nymphs on non-agricultural host plants and migration propensity of adults.

Conclusions

Both temperature and host plant species significantly affected oviposition of beet leafhoppers. Maximum total fecundity was estimated to be 30.6 °C, and beet leafhoppers reared on *K. scoparia* showed the highest total fecundity followed by those on *P. ovata*, *E. cicutarium*, and *S. tragus*. Effects of temperature and host plant species on oviposition were modeled and successfully validated under semi-field and greenhouse conditions. This information is critical with regard to characterization of the relative importance of host plant species for beet leafhopper population growth under fluctuating temperature conditions. Modeling approaches presented here will also be valuable for the development of spatio-temporal decision support tools for beet leafhoppers in non-agricultural areas.

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Figure legends

Figure 1. Temperature-dependent (A) total fecundity and (B) adult aging rate (1/mean longevity) models for beet leafhoppers on five host plant species. The data points for *Beta vulgaris* were obtained from the published paper, Harries and Douglass (1948) [24].

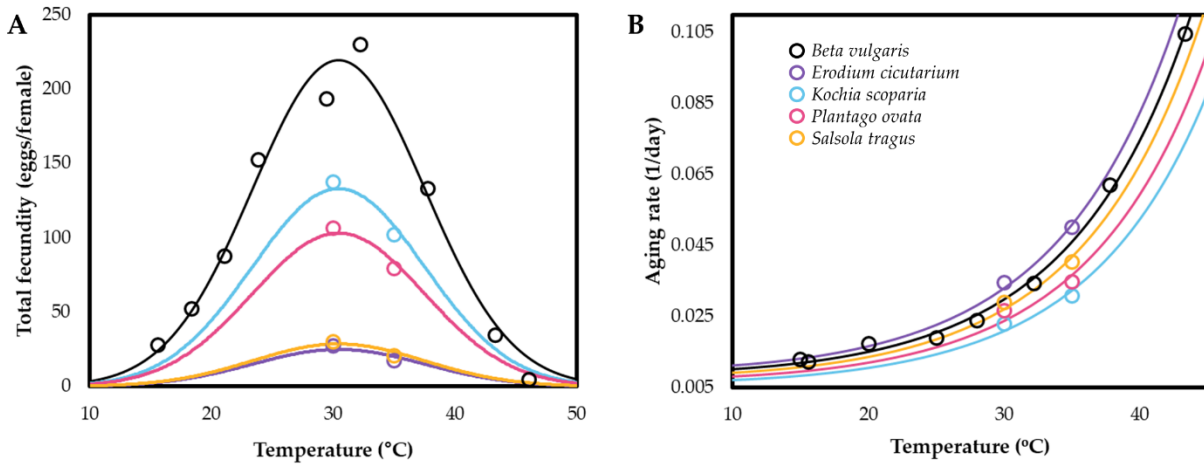


Figure 2. Age-specific (A) oviposition rate model and (B) survival model for beet leafhoppers on four non-agricultural host plant species at two temperature conditions.

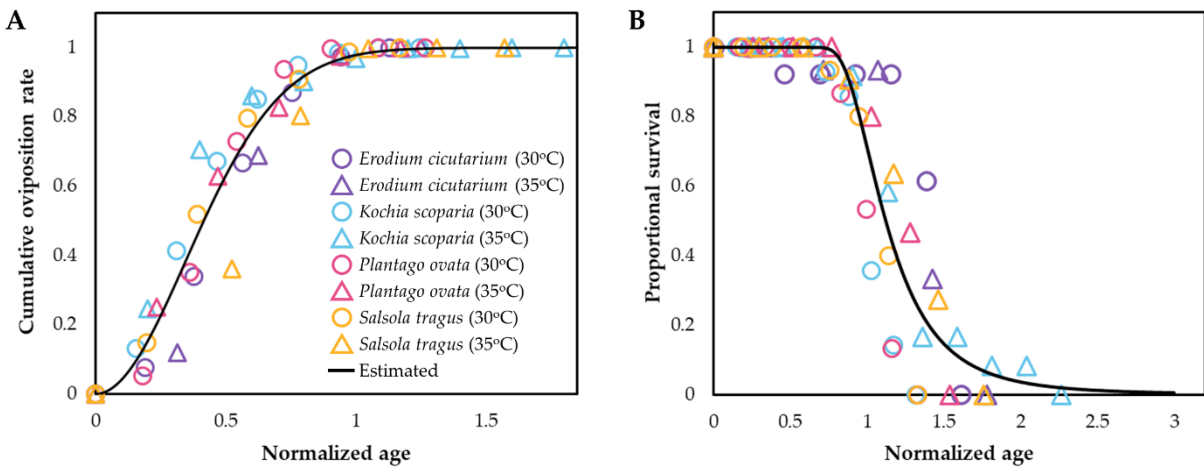


Figure 3. Estimated weekly oviposition curve of beet leafhoppers on *Beta vulgaris* in relation to temperature.

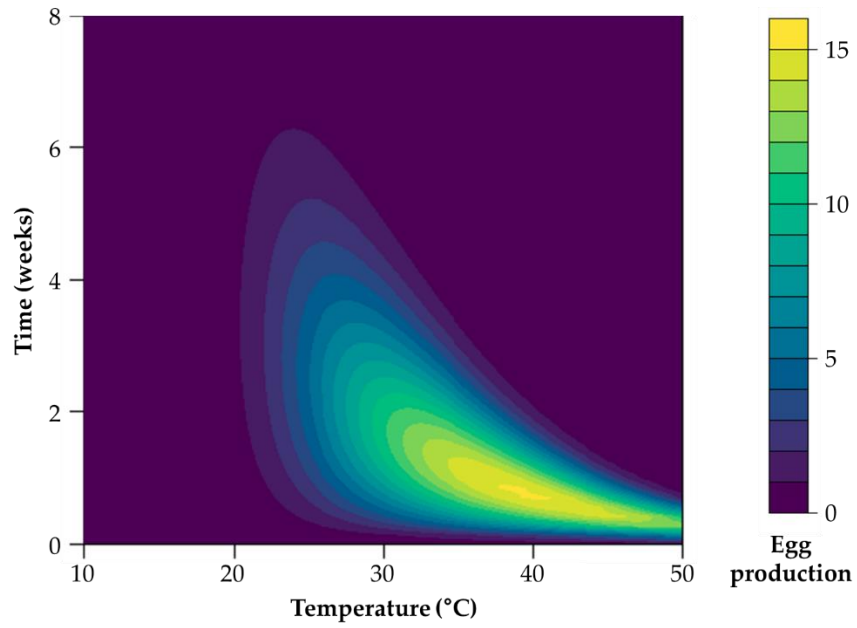
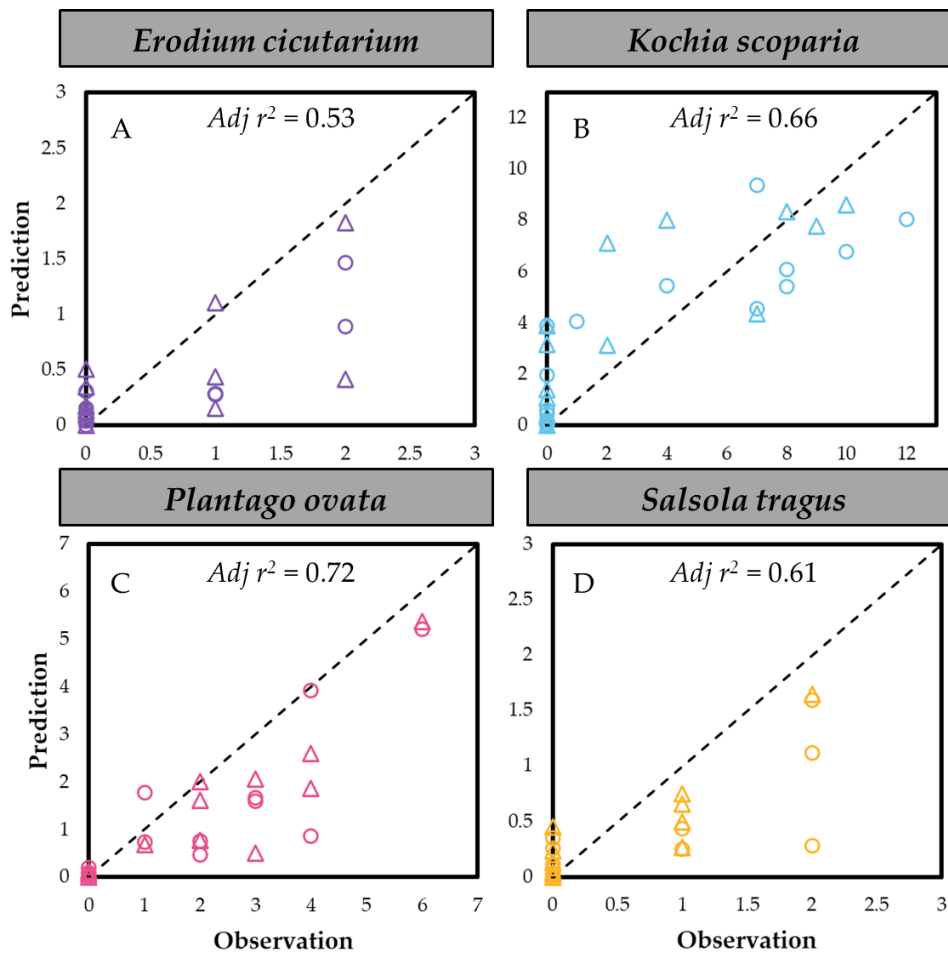


Figure 4. Model validation for oviposition of beet leafhoppers on four non-agricultural host plant species, (A) *Erodium cicutarium*, (B) *Kochia scoparia*, (C) *Plantago ovata*, and (D) *Salsola tragus*, under fluctuating temperature conditions (see Table S1). Open dots and triangles represent the results under fluctuating temperature conditions in the first and second replicate of the experiment, respectively. Correlation coefficients were calculated using the predicted oviposition at a certain week paired with the oviposition observed for that specific week.



Supporting information

Table S1. Summary of fluctuating temperature conditions for model validation.

Host plant	Replicate	Mean temperature ± SE (°C)	Minimum temperature (°C)	Maximum temperature (°C)
<i>Erodium cicutarium</i>	1	14.12 ± 0.13	2.24	31.71
	2	14.59 ± 0.14	3.69	31.71
<i>Kochia scoparia</i>	1	24.13 ± 0.20	19.97	29.27
	2	24.06 ± 0.21	19.97	29.27
<i>Plantago ovata</i>	1	14.12 ± 0.13	2.24	31.71
	2	14.05 ± 0.13	2.24	31.71
<i>Salsola tragus</i>	1	14.12 ± 0.13	2.24	31.71
	2	14.05 ± 0.13	2.24	31.71

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Chapter 3: Timing matters: Model to estimate spring migration of an insect vector as a predictor of a crop disease outbreak

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Significance

Agricultural plant diseases globally cause yield losses in major food crops. Since most plant viruses causing plant diseases are transmitted by insect vectors, understanding vector migration is fundamental to avoiding plant virus infection. Here we report that spring migration time of an insect vector, beet leafhopper (*Circulifer tenellus*) (Hemiptera: Cicadellidae), is associated with vegetation greenness in its overwintering areas. Additionally, early spring migration is one of the key factors driving outbreaks of beet curly top virus (BCTV) in agricultural fields. We further developed a web-based mapping system for estimating spring migration time of beet leafhopper, which has potential use as a decision support tool for its management.

Abstract

Most plant viruses causing significant yield losses in crops are transmitted by insect vectors. One of key factors affecting a plant virus outbreak is population density of its insect vector. However, the importance of correlating migration time of insect vectors to plant virus disease outbreaks has not been fully understood for many insect-vectored plant viruses. Here, we tested plant greenness (i.e., plant quality as a food source) effects on flight propensity of beet leafhoppers (*Circulifer tenellus*) (Hemiptera: Cicadellidae) using two host plants; an inferior, host redstem filaree (*Erodium cicutarium*), and a superior host, sugar beet (*Beta vulgaris*). Plant greenness was negatively related to flight propensity of beet leafhoppers regardless of host species suitability. In addition, we built a model to estimate spring beet leafhopper migration times based on remotely sensed temporal changes in vegetation greenness in their overwintering areas. We investigated winter environmental conditions (precipitation, temperature, and vegetation greenness) and spring migration times of beet leafhoppers in the years of beet curly top virus (BCTV) outbreaks in California. We found that early spring migrations co-occurred with BCTV outbreak years

during the last 21 years, although there was no distinctive trend in winter environmental conditions that could be clearly correlated with this pattern. The combination of understanding key factors causing plant virus outbreaks and developing a model as a decision support tool is considered widely relevant and applicable to developing effective management strategies for other insect-vectorized plant pathogens.

Introduction

Global environmental changes (e.g., climate change and biodiversity loss) are increasing the uncertainty of spatiotemporal patterns of outbreaks of agricultural pests (1, 2) and diseases (3). Moreover, effective management of agricultural pests and diseases is needed to meet the demand of a need for a 60% increase in food production to feed 10 billion people by 2050 (4). Agricultural pests and diseases globally cause yield losses in major food crops: wheat (21.5%), rice (30.0%), maize (22.5%), potato (17.2%), and soybean (21.4%) (5). Plant viruses in particular account for 47% of the plant pathogens causing epidemics worldwide (6). Since the majority of plant viruses require insect vectors for their transmission to non-infected host plants (7), plant virus outbreak patterns are closely associated with movement of their insect vectors. Therefore, plant virus management mainly relies on controlling insect vectors to prevent infections.

High density of viruliferous insect vectors has been considered a major driver of plant virus outbreaks (6). Most studies link plant virus outbreaks with environmental conditions including temperature and resource availability because these factors influence the population density of migrant insect vectors (8); however, migration of insect vectors is one of the key

movement behaviors leading to transmission of plant viruses from external sources to crops (9). Importance of migration time of insect vectors on plant virus outbreaks remains poorly known, although plant virus resistance is strongly dependent on plant age at inoculation (10). Spatiotemporal variation in vegetation conditions has been understood as a major driver of insect migration because insects migrate to obtain a habitat with better resources (11). In addition, temperature acts as a threshold below which flight activity is inhibited (12). Therefore, vegetation and temperature conditions should be useful to estimate migration time of insect vectors and determine the relationship between outbreaks of plant viruses and migration time of their insect vectors.

This study examines the beet leafhopper, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae), the only known vector of *Beet curly top virus* (BCTV) (family Geminiviridae) in North America. BCTV is an economically important plant virus infecting many crops including sugar beet (*Beta vulgaris* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), common bean (*Phaseolus vulgaris* L.), and potato (*Solanum tuberosum* L.). In California, a BCTV outbreak caused ~\$100 million losses in tomato production in the San Joaquin Valley in 2013 (13, 14) and an unusual BCTV outbreak occurred in the Sacramento Valley in 2021 (*SI Appendix*, Fig. S1). In California, viruliferous (carrying BCTV) beet leafhoppers migrate from their overwintering areas (foothills of mountain ranges located on the western side of the Sacramento and San Joaquin Valleys of California) to agricultural fields in spring (15). Spring migration of beet leafhoppers has been studied over decades, and this has led to a long-held belief that plant senescence and increased temperature conditions in the foothills trigger their migration (16, 17). It has been widely believed that BCTV outbreaks are associated with a high density of migrant beet leafhoppers carrying high levels of BCTV (18, 19). Therefore, to manage

BCTV, the California Department of Food and Agriculture (CDFA) runs the Curly Top Virus Control Program (CTVCP) which surveys for beet leafhoppers with sweep nets each spring and sprays insecticide in the foothills when beet leafhopper densities reach certain thresholds (20). However, severe BCTV outbreaks periodically occur while the CTVCP was operational and its efficacy in reducing BCTV incidence remains unclear. Understanding of key factors causing BCTV outbreaks and more effective management strategies are needed to prevent future crop losses.

We investigated the effects of spring migration time of beet leafhoppers on BCTV outbreaks in tomato fields. First, the relationship between plant greenness (i.e., quality as a food source) and flight propensity was determined at a laboratory scale using a superior host (sugar beet), and an inferior host (redstem filaree, *Erodium cicutarium* [L.] L'Hér) (see Chapter 2). Spring migration time of beet leafhoppers from their overwintering areas was monitored for two years and timing of spring migrations was modeled based on remotely sensed vegetation greenness (i.e., EVI values) with a minimum flight-threshold temperature. Subsequently, the spring migration model was implemented in a web-based mapping system at a landscape scale as a decision support tool for beet leafhopper management. Estimated spring migration times and environmental factors (precipitation, temperature, and vegetation greenness) in the BCTV outbreak years were compared with those from 2001 to 2021, and possible factors causing BCTV outbreaks were evaluated.

Results

Flight propensity of beet leafhopper

Redstem filaree (inferior host) and sugar beet (superior host) plants showed decreased EVI values as they were exposed to drought for longer time periods (Fig. 1B). Flight propensity (i.e., proportion of beet leafhoppers on sticky cards placed alongside the plants) of beet leafhoppers on each plant species was negatively correlated with EVI values (Redstem filaree: $F[1,58] = 48.34$, $P < 0.001$, $adj-R^2 = 0.45$, $slope = -1.34$; Sugar beet: $F[1,57] = 25.32$ $P < 0.001$, $adj-R^2 = 0.30$, $slope = -1.33$) (Fig. 1C). The relationships between plant greenness and beet leafhopper flight propensity on both plant species were not significantly different ($F[1,115] = 2.70$, $P = 0.105$), suggesting plant greenness may play a similar role in initiating flight of beet leafhoppers regardless of host species suitability.

Model for estimating spring migration time

Spring migrant beet leafhoppers were observed at three study sites (Fresno, Kern, and Kings counties) in the foothills in 2019 and 2020. In 2019, one simultaneous peak of migrating beet leafhoppers was observed at the study sites, but in 2020, a single peak, two peaks, and indistinct peaks were observed for Kern, Fresno, and Kings, respectively (Fig. 2A and B). In 2019, enhanced vegetation index (EVI) values showed a similar pattern among the study sites (Fig. 2C), but in the case of the Kern site in 2020, EVI values were remained relatively low (Fig. 2D). To compare spring migration times among the study sites and years, numbers of migrating beet leafhoppers were transformed into cumulative proportions. Spring migration was began in the range of 0.2-0.3 EVI values at all study sites, and the cumulative proportion of migrating beet leafhoppers increased with decreasing EVI values (Fig 2E). The relationship between EVI value and cumulative proportion of migrating beet leafhoppers was modeled using a Weibull function ($F[46,1] = 234.1$, $P < 0.001$, $adj-R^2 = 0.76$). The estimated parameter values for a and b were 0.1667 ($SE = 0.0035$) and -5.5953 ($SE = 0.8926$), respectively. The spring migration model was

implemented in a web-based mapping system on Google Earth Engine

(<https://hyslee.users.earthengine.app/view/beet-leafhopper-migration-in-ca>).

Environmental conditions and spring migration time effects on BCTV outbreaks

Annual trends in winter environmental conditions (i.e., temperature, precipitation, and EVI value) in the two foothill regions, adjacent to the Sacramento and San Joaquin Valleys, were compared over a 21-year period from 2001 to 2021. During the BCTV outbreak years (2021 for the Sacramento Valley and 2013 for the San Joaquin Valley), mean winter temperatures were higher than the 21-year averages in both foothill regions (Fig. 3A and B). However, mean winter rainfall and EVI values were lower than the 21-year averages (Fig. 3C-F). The mean values of winter environmental conditions in the BCTV outbreak years were within ± 2 SD of the 21-year averages. The results suggest that these environmental factors may not be directly associated with BCTV outbreaks.

Annual trends in spring migration time were compared by estimating the increase in geographic areas of foothills where migration has been documented and times at which spring migration occurred in 50% of the foothill regions from 2001 to 2021 (*SI Appendix*, Fig. S2 and Table S1 for the foothills to the west of the Sacramento Valley and Fig. S3 and Table S2 for those to the west of the San Joaquin Valley). In the foothills to the west of the Sacramento Valley, the earliest spring migration occurred in the BCTV outbreak year (Fig. 4A). In the foothills to the west of the San Joaquin Valley, spring migration occurred earlier in 2002 and 2013 compared to those in other years (Fig. 4B). The years during which spring migration occurred in 50% of the foothills occurred earliest in the BCTV outbreak years in both foothill regions (Fig. 4C).

Discussion

Drought stress causes morphological and physiological changes such as reduced leaf size (21), decreased chlorophyll content (22), and increased levels of secondary metabolites (23). Species-specific drought tolerance also affects response levels to drought stress (24, 25), and plant communities in the foothills possess varying degrees of diversity (26). Redstem filaree and sugar beet are host plants for beet leafhopper, although there is a significant difference in their host suitability for reproduction of beet leafhopper (see Chapter 2). However, we found no significant difference between redstem filaree and sugar beet in stimulating flight behavior of beet leafhoppers depending on changes in their greenness. As a result, we propose plant greenness as a comprehensive predictor (regardless of host plant species) of flight propensity of beet leafhoppers. However, abiotic conditions such as soil composition, solar angle, and temperature affect remotely measured vegetation greenness. Therefore, field observed vegetation greenness data are required to develop models for estimating migration of insects under field conditions.

In many studies, insect population densities have been estimated from vegetation conditions quantified by various vegetation greenness indices (27-30). It has been reported that BCTV outbreaks in tomato fields are associated with a high number of viruliferous beet leafhoppers in spring (20). However, we found that BCTV outbreaks were not significantly associated with EVI values themselves, although high EVI values may represent high population density of beet leafhoppers as well as better vegetation quality. In addition, precipitation (31) and temperature (32) conditions were considered important factors in beet leafhopper reproduction, although we found no association between precipitation and temperature conditions and BCTV outbreaks. Since the western foothills of the San Joaquin and Sacramento Valleys are

geographically wide, sufficient number of beet leafhoppers to cause BCTV outbreaks may be produced even under low EVI, precipitation, and temperature conditions. Instead, a strong correlation was found between spring migration times and the occurrence of BCTV outbreaks. We suspect that early spring migration may be an important factor contributing to BCTV outbreaks and more severe symptoms in tomato plants because it would result in virus transmission to young plants which are more susceptible to infection and development of symptoms than older plants (33, 34).

Using remote sensing techniques, we investigated plant greenness as an indicator of flight propensity of beet leafhopper at a laboratory scale and developed a web-based mapping system on Google Earth Engine at a landscape scale. The mapping system can be used as a decision support tool for scheduling beet leafhopper management based on estimated spring migration time. Because remote sensing data can cover large geographic areas, it can be used to develop decision support tools for management of insect pests and diseases in forest systems as well as in agricultural systems (35-38). In addition, accumulated diverse remote sensing data sets could be used not only for measuring vegetation quality (39) but also for plant species classification (40). In polyphagous insect pests, distribution and population density are associated with vegetation quality and plant species composition. Future research will require a remote sensing-based mapping system for estimating population density and locating potential hotspots of insect pests as a decision support tool for pest management.

Materials & Methods

Plant greenness effect on flight propensity of beet leafhoppers

Two host plants, redstem filaree and sugar beet, were selected as an inferior and superior host plant species, respectively (see Chapter 2). Redstem filaree seeds were collected from the foothill in Kings County, California (36.038 °N, -120.115 °W), and sugar beet seeds were obtained from commercial seed supplies. Plants were grown in a greenhouse (25 ± 5 °C and $80 \pm 10\%$ RH) for four weeks. All plants were watered daily and fertilized with 0.5% soluble N-P-K fertilizer (6:1:4) in 200 ml of water. Variations in plant greenness of inferior host redstem filaree ($n = 60$) and superior host sugar beet ($n = 59$) was generated by not watering plants for various durations (0, 3, 6, and 9 days) at 35 °C and 50% RH in a Conviron E7 growth chamber (Conviron, Winnipeg, Canada). EVI value of individual plants was measured by a hyperspectral camera (PIKA L; www.resonon.com) as described in Nguyen and Nansen (41) (Fig. 1A). EVI values were calculated using the bands positioned at 850-880 nm (near-infrared), 640-670 nm (red), and 450-510 nm (blue). The equation and parameters for EVI values were the same as the Moderate Resolution Imaging Spectroradiometer (MODIS) EVI algorithm (42). In a mesh dorm cage (61 x 61 x 61 cm, Megaview Science, Taichung, Taiwan), four yellow sticky cards (15.2 x 20.3 cm) were placed around individual plants and then 10 beet leafhoppers were released on the plants. After 24 h, beet leafhoppers on yellow sticky cards were counted to measure their flight propensity. Regression analysis was performed to examine the relationship between plant greenness and flight propensity for each plant species using a general linear model (GLM). An analysis of covariance (ANCOVA) model was used to determine host plant species-specific effects on the relationship with flight propensity. All statistical analyses were performed in R 4.1.2 (43) with $\alpha = 0.05$.

Beet leafhopper spring migration modeling

Spring migration of beet leafhoppers was monitored from January 2019 to June 2020 in the western foothills of the San Joaquin Valley in Fresno, Kings, and Kern counties (36.629 °N, -120.641 °W; 36.038 °N, -120.115 °W; 35.124 °N, -119.509 °W). At each site, 10 yellow sticky cards (15.2 x 20.3 cm) were deployed 1 m above the ground. Yellow sticky cards were replaced biweekly (every two weeks), and beet leafhoppers on the cards were counted under a binocular stereomicroscope (Olympus SZ51; Olympus, Tokyo, Japan). The number of spring migrant beet leafhoppers was transformed into a cumulative proportion to compare across the study sites. EVI values of the study sites were extracted from the 16-day composites MODIS EVI (MOD13Q1 from Terra) at a spatial resolution of 250 m (44). The relationship between cumulative proportion of migrating beet leafhoppers and vegetation greenness was modeled using a Weibull function:

$$f(t) = 1 - \exp \left[- \left(\frac{EVI(t)}{a} \right)^b \right]$$

where $f(t)$ is the cumulative proportion of migrating beet leafhoppers, $EVI(t)$ is the EVI values at Julian day t , and a and b are estimated parameters. In addition, 15.56 °C was applied as the minimum threshold temperature for spring migration (45), and temperature data was obtained from the 8-day composites MODIS (MOD11A2 from Terra) at a spatial resolution of 1 km (46). All parameters were estimated with a least-squares method and iterative process of Gauss-Newton using R 4.1.2 (43).

Annual trends in winter environmental conditions and spring migration time

Level III ecoregion classification from the US Environmental Protection Agency (USEPA) was used to geographically select the foothills in California (<https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states>). We evaluated mean winter environmental conditions over the areas in the foothills categorized as shrubland, grass/pasture, and fallow/idle

lands by the National Agricultural Statistics Service (NASS) (47). Temperature, precipitation, and EVI data were obtained from MODIS product MOD11A1 (Terra daily 1 km) (48), Daly, *et al.* (49), and MOD13Q1 (Terra 16-day 250 m) (44), respectively. Mean winter environmental conditions were calculated from January to March from 2001 to 2021. In addition, annual trends in the proportion of areas showing spring migration were evaluated using a logistic function (*SI Appendix*, Supplemental Methods). Midpoint of individual logistic curves (i.e., spring migration-occurred time in 50% of the foothills) were compared to evaluate variations in spring migration time. Trends in spring migration times were evaluated separately for two foothill regions, those west of the Sacramento and San Joaquin Valleys (see *SI Appendix*, Fig. S4). Winter environmental conditions and spring migration times were examined in the BCTV outbreak years, 2021 and 2013 across the foothills to the west of the Sacramento and San Joaquin Valleys, respectively, to detect differences from other years when BCTV infection prevalence was mild or moderate.

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Figures

Figure 1. Hyperspectral imaging system for measuring plant greenness (A). Enhanced vegetation index (EVI) (mean \pm SE) changes in redstem filaree (n = 60) and sugar beet (n = 59) plants according to drying period (B). Relationships between EVI value and flight propensity of beet leafhoppers on redstem filaree (gray dots) and sugar beet (open dots) (C). Linear regressions for redstem filaree (dashed line) and sugar beet (solid line).

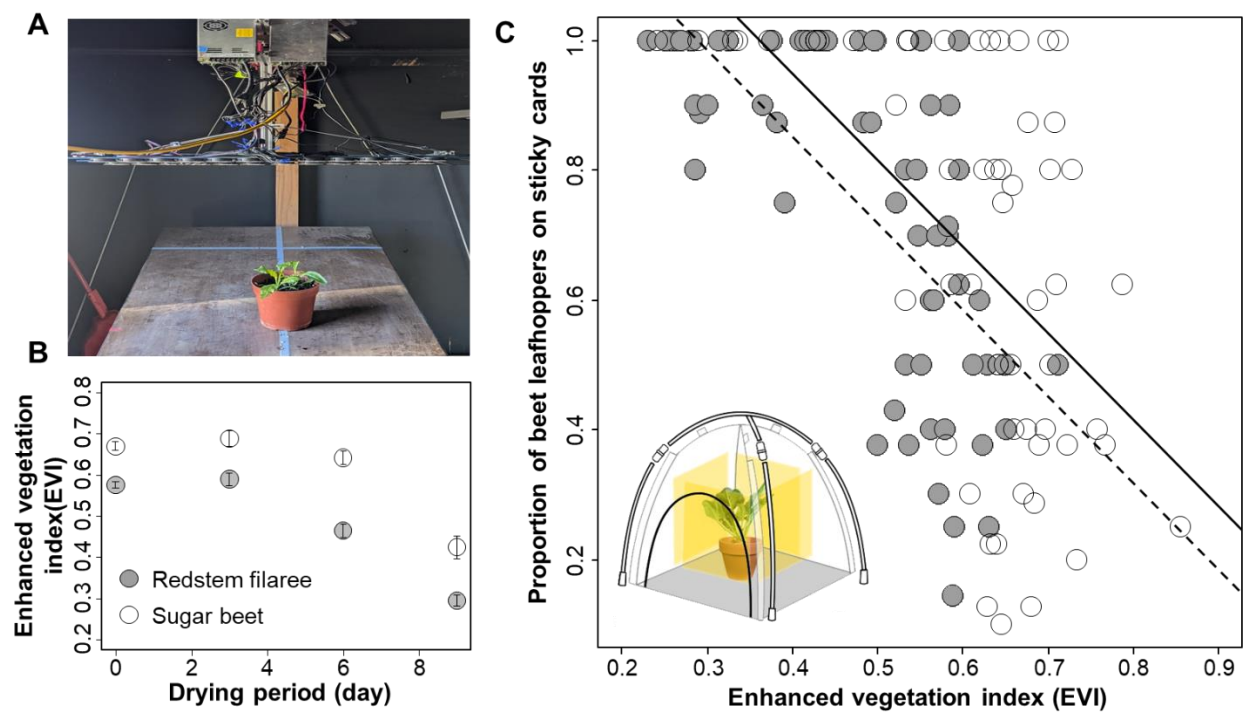


Figure 2. Field observation (mean \pm SD) of migrating beet leafhoppers in the foothills in California in 2019 (A) and 2020 (B).

Remotely sensed enhanced vegetation index (EVI) at the study sites in 2019 (C) and 2020 (D). The relationship between EVI values and cumulative proportion of migrating beet leafhoppers (E). The observation data was fitted to a Weibull function (adjusted $R^2 = 0.76$) (dashed line).

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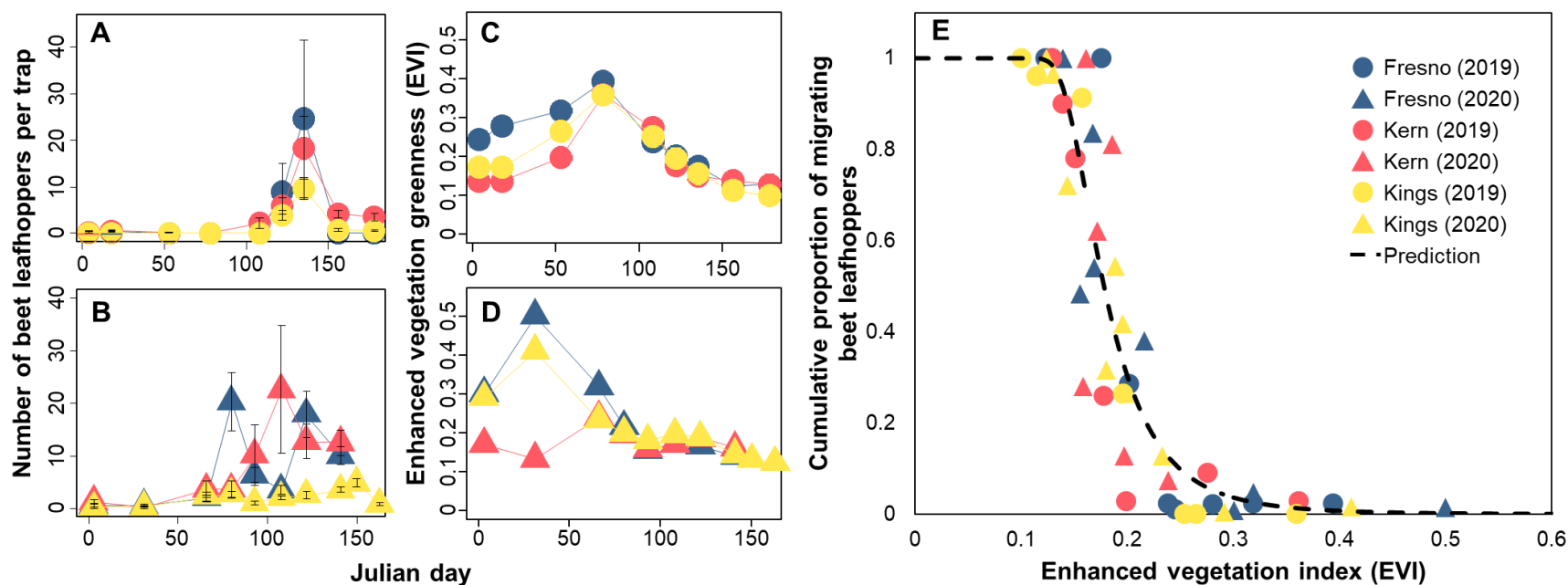


Figure 3. Trends in mean winter enhanced vegetation index (EVI) (A and B), winter temperature (C and D), and winter precipitation (E and F) in the foothills to the west of the Sacramento and San Joaquin Valleys of California from 2001 to 2021. Dashed lines are the 21-year average values. Red dots represent the values when severe beet curly top virus (BCTV) outbreaks occurred (i.e., 2021 for the Sacramento Valley and 2013 for San Joaquin Valley).

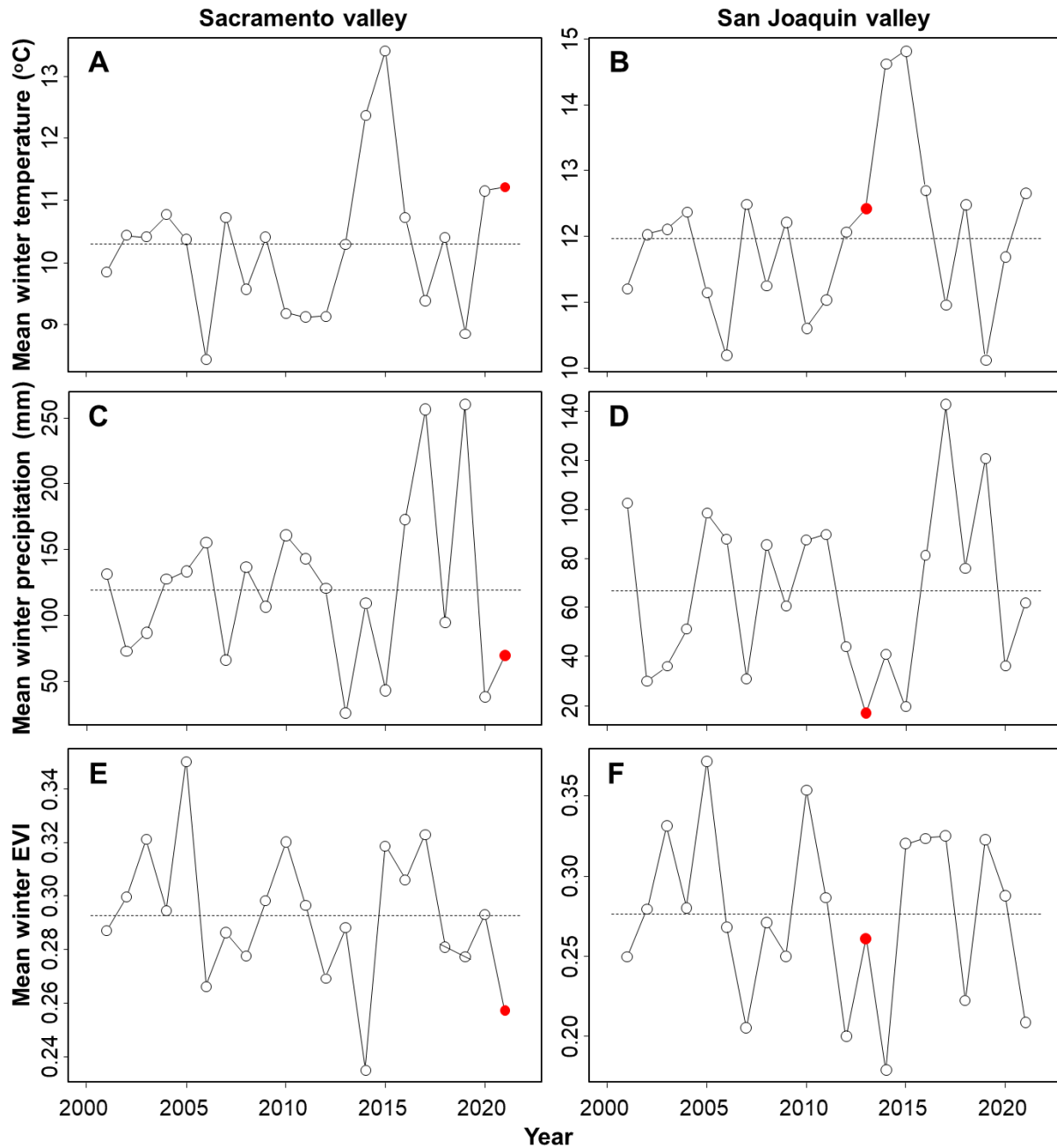
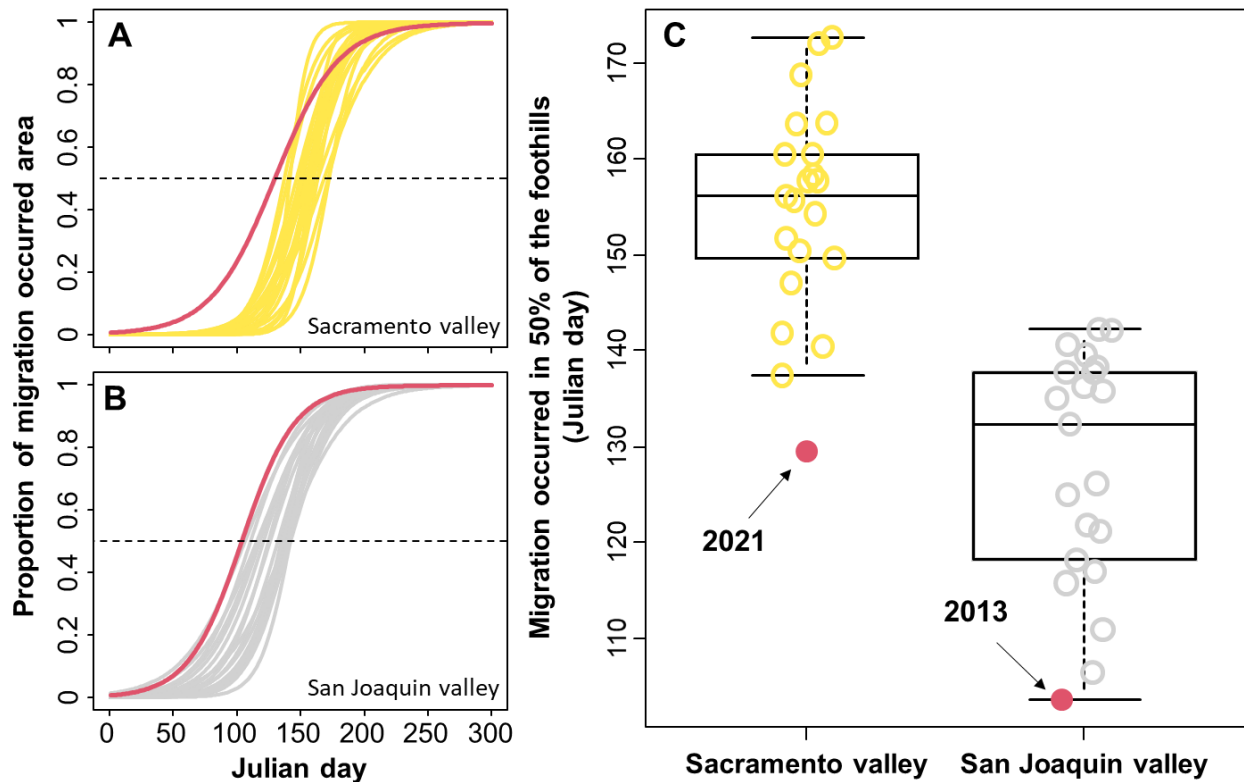


Figure 4. Annual trends in beet leafhopper spring migration-occurred area in the foothills to the west of the Sacramento (A) and San Joaquin Valleys (B) of California. A value of 0.5 (dashed lines) indicates that spring migration has occurred in 50% of the foothill region. Times at which spring migration occurred in 50% of the foothills to the west of the Sacramento and San Joaquin Valleys from 2001 to 2021 (C). Each point represents each year, jittered for visibility. Red lines and dots represent data for the severe beet curly top virus (BCTV) outbreak years for each region.



Supplementary Appendix

Figure S1. Tomato fields in the Sacramento Valley (California, USA) with high beet curly top virus (BCTV) incidence. BCTV infected tomato plants exhibit stunted growth, upward curling of leaves, yellow discoloration, and vein purpling.



Supplemental Methods

Migration probability of beet leafhoppers was calculated using the spring migration model in the main text. As a measurement of vegetation greenness, enhanced vegetation index (EVI) values across the foothills to the west of the Sacramento and San Joaquin Valleys were extracted from the 16-day composites MODIS EVI (MOD13Q1 from Terra) at a spatial resolution of 250 m from January 1st to July 30th (1). Spring migration times of beet leafhoppers in the foothills to the west of the Sacramento and San Joaquin Valleys were evaluated as proportion of spring migration-occurred area in each foothill region. Annual trends in proportion of spring migration-occurred area were modeled using a logistic function:

$$f(x) = \frac{1}{1 + e^{-k(x-x_0)}}$$

where $f(x)$ is the proportion of spring migration-occurred area, x is Julian day, and k and x_0 are estimated parameters representing the steepness and the midpoint of the curve (i.e., spring migration-occurred time in 50% of the foothills), respectively. x_0 values were used to compare migration trends from year to year in each foothill region.

Figure S2. Annual trends in spring migration time of beet leafhoppers in the foothills to the west of the Sacramento Valley (California, USA). Estimated proportion of areas showing spring migration-(dots) was fitted to a logistic function (solid line). A value of 0.5 (dashed line) indicates that spring migration has occurred in 50% of the foothill regions.

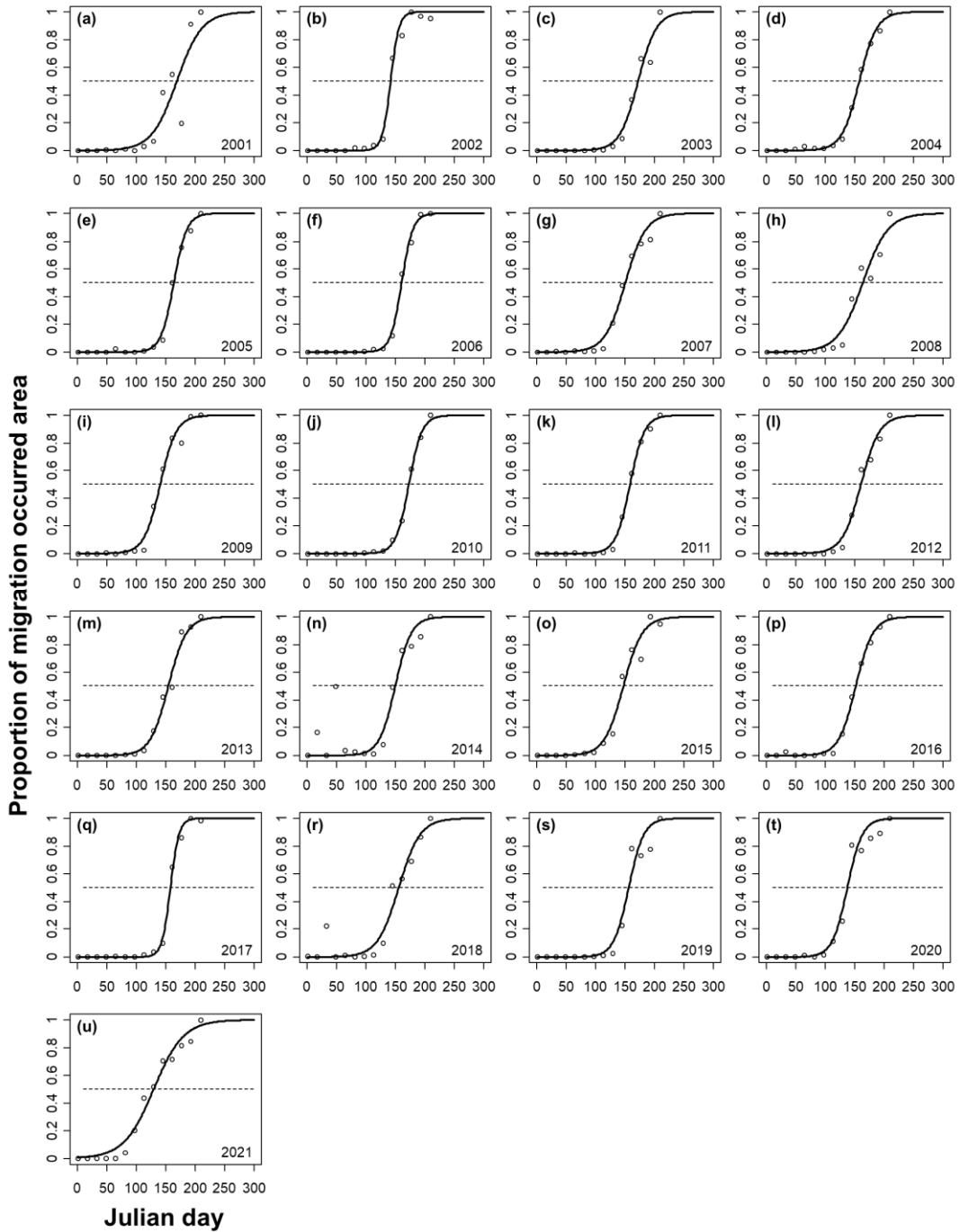


Figure S3. Annual trends in spring migration time of beet leafhoppers in the foothills to the west of the San Joaquin Valley (California, USA). Estimated proportion of areas showing spring migration-(dots) was fitted to a logistic function (solid line). A value of 0.5 (dashed line) indicates that spring migration has occurred in 50% of the foothill regions.

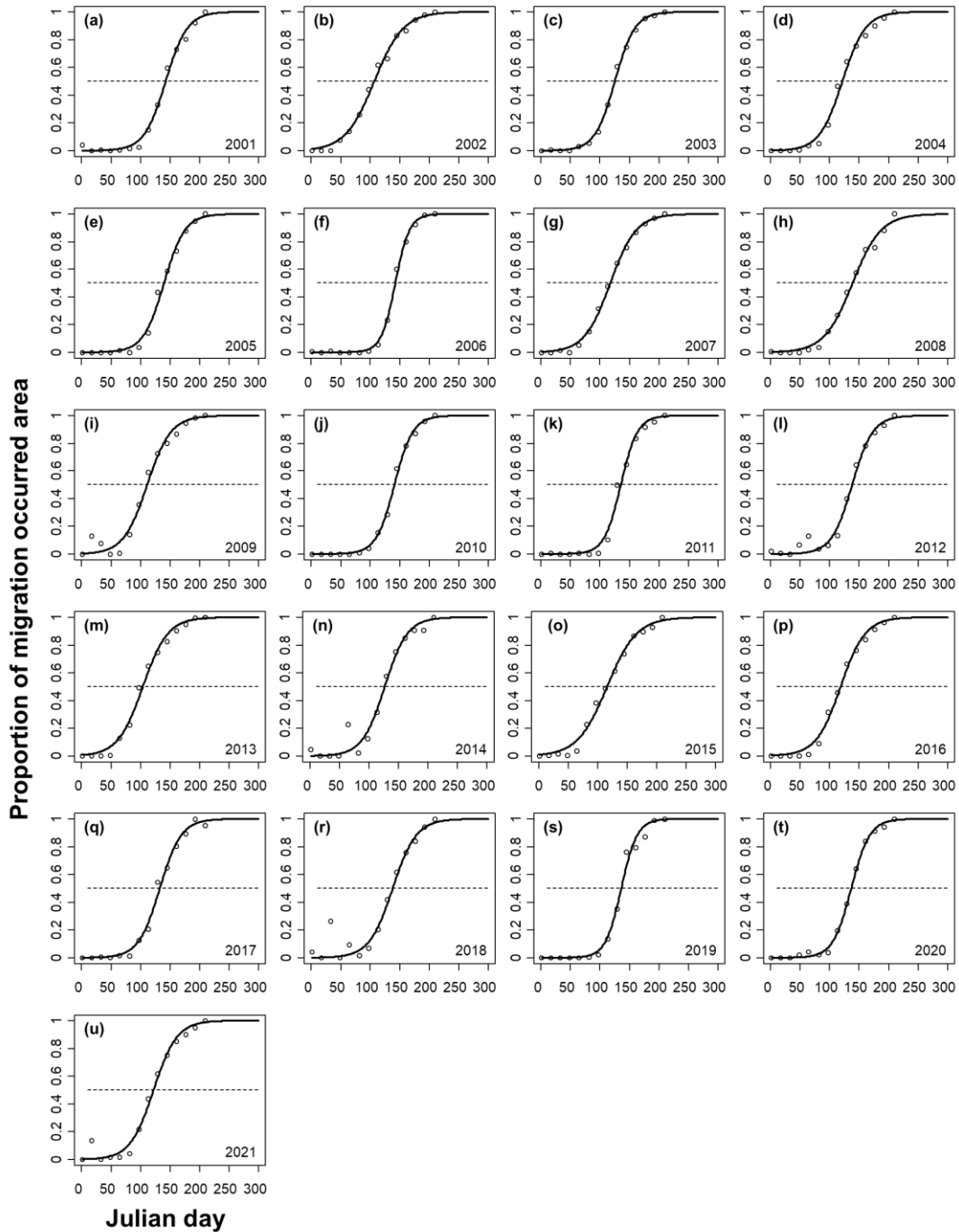


Table S1. Estimated parameters and Akaike’s Information Criterion (AIC) values for logistic models for annual trends in proportion of areas showing spring migration in the foothills to the west of the Sacramento Valley (California, USA).

Year	k	x_0	AIC
2001	0.047055	168.6685	-10.1398
2002	0.141453	141.8171	-44.3591
2003	0.064598	172.0452	-34.2935
2004	0.067434	157.7359	-59.6725
2005	0.091112	163.6432	-51.4214
2006	0.106275	160.5242	-57.1486
2007	0.058026	150.4527	-41.4764
2008	0.047374	163.7238	-26.6307
2009	0.07316	140.5261	-40.2554
2010	0.090492	172.7017	-71.1661
2011	0.082609	158.3757	-62.0297
2012	0.063671	160.4735	-42.785
2013	0.065648	154.2478	-42.5987
2014	0.074082	149.6848	-7.27729
2015	0.062345	147.0665	-31.5316
2016	0.06909	151.7869	-61.5777
2017	0.139369	157.8291	-55.1879
2018	0.055574	155.5829	-24.02
2019	0.078623	156.1487	-25.4633
2020	0.079049	137.4917	-29.7715
2021	0.039758	129.5399	-35.7053

Table S2. Estimated parameters and Akaike’s Information Criterion (AIC) values for logistic models for annual trends in proportion of areas showing spring migration in the foothills to the west of the San Joaquin Valley (California, USA).

Year	k	x_0	AIC
2001	0.054937	142.3296	-50.899
2002	0.040705	106.5279	-54.0006
2003	0.059221	124.958	-67.3259
2004	0.051296	121.1421	-46.7852
2005	0.056822	139.4996	-51.2084
2006	0.083832	142.1492	-66.5581
2007	0.045786	117.05	-63.2936
2008	0.041663	138.3649	-50.2559
2009	0.049752	110.9495	-36.0259
2010	0.064765	140.6767	-62.7088
2011	0.071211	135.0012	-44.4047
2012	0.05732	137.7311	-42.3467
2013	0.047524	103.5937	-47.0203
2014	0.052324	126.2452	-32.1935
2015	0.040136	115.8632	-47.4388
2016	0.048349	118.258	-48.873
2017	0.055461	132.3557	-48.2454
2018	0.050339	137.7185	-26.1515
2019	0.075025	135.8034	-43.3443
2020	0.064273	136.2403	-66.4085
2021	0.050629	121.7965	-40.8973

Figure S4. Foothills to the west of the Sacramento (yellow) and San Joaquin Valleys (gray). The Central California Foothills and Coastal Mountains Ecoregion in the Level III ecoregion classification (<https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states>), categorized as shrubland, grass/pasture, and fallow/idle cropland by the National Agricultural Statistics Service (NASS), were selected for analysis. This map was generated using Google Earth Engine.



Reference

1. Didan K (2015) MOD13Q1 MODIS/Terra Vegetation Indices 16-Day L3 Global 250m SIN Grid V006. (NASA EOSDIS Land Processes DAAC).