

**THE TEMPERATURE DEPENDENT DEVELOPMENT OF *BACTERICERA*
COCKERELLI (SULC) FROM SOUTH TEXAS (HEMIPTERA: TRIOZIDAE)**

A Thesis

by

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ABSTRACT

Bactericera cockerelli (Sulc) (Hemiptera: Triozidae) is a pest of potato (*Solanum tuberosum* L.) that vectors the bacterium that putatively causes zebra chip disease in potatoes, ‘*Candidatus Liberibacter solanacearum*.’ The economic risk of zebra chip disease is mitigated by controlling populations of *B. cockerelli* in commercial potato fields. Lacking an integrated pest management (IPM) strategy, growers have resorted to an intensive chemical control program that may be leading to insecticide-resistant *B. cockerelli* populations in south Texas and Mexico. To initiate the development of an integrated approach of controlling *B. cockerelli*, we used constant temperature studies and non-linear and linear modeling to determine degree day parameters for development of *B. cockerelli* infesting potato. We field validated the parameters by making degree day model predictions for three different *B. cockerelli* life stages tested against population data collected from 49 pesticide-free fields.

The models estimated the lower and upper threshold for overall (egg plus nymph) development of *B. cockerelli* as 6.5 and 29.3°C, respectively, with a thermal constant, *K*, of 354.6 degree days. In the field validation, the model accurately predicted within the normal sampling frequency of 7 days 73% of the egg-to-egg peaks, 80% of the nymph-to-nymph peaks, and 58% of the peaks for the highly mobile adults. It is impractical to predict first occurrence of *B. cockerelli* in potato plantings as adults are present as soon cotyledons break through the soil. Therefore, we suggest integrating the degree day model into current *B. cockerelli* management practices using a two-phase method. Phase one occurs from potato planting through the first peak of a *B. cockerelli*

field population and are managed using current practices. Once the *B. cockerelli* population peaks, phase two begins and the degree day model is initiated to predict the subsequent population peaks, thus providing growers a tool to proactively manage *B. cockerelli*.

DEDICATION

To my family and friends, who are everything to me.

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CHAPTER I

INTRODUCTION

The potato psyllid (also known as the tomato psyllid), *Bactericera cockerelli*, Šulc, (Hemiptera: Triozidae) is a phytophagous, polyphagous, piercing/sucking insect (Knowlton and Janes 1931) that is a pest of many solanaceous crops including potato (*Solanum tuberosum* L.). In 2008, *B. cockerelli* was discovered to vector the bacterium ‘*Candidatus Liberibacter solanacearum*’ (Lso), the putative causal agent of zebra chip disease in potato (Hansen et al. 2008, Liefting et al. 2008). Zebra chip disease’s characteristic symptom is dark stripping in the tuber when fried for human consumption as french fries or potato chips, and the disease renders infected potatoes unmarketable. Since its discovery in Saltillo, Mexico in 1994, the disease has spread into most of the potato producing states in the central and western U.S., as well as Guatemala, Honduras, Nicaragua, and New Zealand (Munyaneza 2012). Lacking an integrated management strategy, *B. cockerelli* is currently controlled using approximately weekly insecticide applications. In an effort to begin the development of an integrated solution of managing *B. cockerelli*, we develop a degree day model that predicts within season, within field populations peaks of *B. cockerelli*. The intent of this chapter is to 1) provide a review of the literature pertaining to management of zebra chip disease by controlling *B. cockerelli*, 2) outline the development of degree day models, and 3) lay out the development of methods that were used to create the degree day model for *B. cockerelli* that follows in chapter II.

To comprehensively search for information on *B. cockerelli*, one must first be familiar with its history and must be aware of the changes in its classification *B. cockerelli* has undergone. *B. cockerelli* was first described in 1909 as *Trioza cockerelli* by Karel Šulc, who received specimens from a garden of T.D.A. Cockerell in Boulder, CO (Šulc 1909). Šulc presented morphological details of the adult specimens he received and indicated that the biology of the insect was completely unknown. He noted they were found on *Capsicum annuum* L. and that since they were found in such large numbers, one could assume they could become a destructive pest. Crawford (1911) subsequently reassigned *Trioza* to a new genus, *Paratrioza*. Burckhardt and Lauterer (1997) determined *Trioza* to be a paraphyletic group and therefore moved *B. cockerelli* to the genus *Bactericera*.

For management, identification, and research of *B. cockerelli*, one is required to be knowledgeable of their morphology at each life stage and their life history. Pletsch (1947) describes *Bactericera cockerelli* eggs as pale white in color when first oviposited, turning yellow to orange as they age, with two red eyes of the nymph appearing in the final hours of egg development. The eggs are approximately 0.304 mm in height, 0.146 mm in width and they sit atop a stalk, approximately 0.194 mm in length (Pletsch 1947). On potato grown in Hidalgo county, Texas, the egg stage averaged 6.5 ± 0.01 (1 standard error, SE) days as reported by Yang et al. (2010), and an average of 5.0 ± 0.01 and 5.9 ± 0.01 (SE) days under laboratory conditions on eggplant and bell pepper, respectively.

Nymphs are dorsoventrally flattened, oval, and scale-like. They are white to pale yellow in color with two red eyes. Late in nymphal development, nymphs become green with wing pads readily visible to the naked eye (Pletsch 1947). Although some disagreement occurred in the early 20th century about the number of nymphal instars (Compere 1916, Essig 1917, Lehman 1930, Rowe and Knowlton 1935), the consensus is that *B. cockerelli* nymphs have five instars (Pletsch 1947, Wallis 1955, Butler and Trumble 2012). The width of nymphs range in size from 0.23 – 1.32 mm, depending on instar (Pletsch 1947). Pletsch (1947) measured the development of 362 individual *B. cockerelli* nymphs and reported no overlap in width between any of the five instars. Nymphal characteristics can be found in Burckhardt and Lauterer (1997). With potato as the host grown in Hidalgo county, Texas, Yang et al. (2010) reported the time of development through all of the nymphal stages averaged 15.9 ± 0.2 (SE) days. In the lab, (Yang and Liu 2009) reported the nymphal stages lasting and average of 19.1 ± 0.04 (SE) and 20.2 ± 0.07 (SE) days on eggplant and bell pepper, respectively.

Upon eclosion from the nymphal stage, adult *B. cockerelli* are pale green to yellow in color and become dark brown to black within two-three days corresponding with sexual maturity (Knowlton and Janes 1931). Adults typically hold their broad wings in a roof-like fashion over their abdomen and measure 1.33 to 1.66 mm from the anterior tip of the head to the posterior tip of the abdomen. They possess saltatorial hind legs and readily jump when disturbed (Lehman 1930). *B. cockerelli* are sexually dimorphic with males having five abdominal segments plus the genital segment whereas females have six abdominal segments plus the genital segment. Under laboratory conditions on

eggplant and bell pepper, the preoviposition period lasted an average 8.8 ± 0.7 (SE) and 8.0 ± 0.05 (SE) days, respectively (Yang and Liu 2009). In the field, however, the preoviposition period was only 3.8 ± 0.3 (SE) days on potato (Yang et al. 2010). In addition, adult longevity in the field was reported to be 17.4 ± 1.0 (SE) days for females and 16.5 ± 0.7 (SE) days for males on potato but 62.1 ± 11.3 (SE) and 35.3 ± 5.8 (SE) days for females and males on potato in the laboratory (Yang et al. 2010).

Wallis (1955) reported finding *B. cockerelli* adults on 20 plant families. Geographically, Wallis' findings from U.S. locations include North and South Dakota, Nebraska, Kansas, Oklahoma, and all states west – except Oregon and Washington. Outside of the USA, he reported they occur in Mexico and Canada. Since 1955, *B. cockerelli* has been reported in Oregon, Washington, Central America, and New Zealand on various agricultural, forest, and landscape plants (Jackson et al. 2009, Tran et al. 2012). While these records suggest *B. cockerelli* are polyphagous, a search of the literature revealed no reports of *B. cockerelli* reproducing on plants other than those from Solanaceae, except *Convolvulus arvensis* L. (field bindweed) and *Micromeria chamissonis* Benth. (a perennial evergreen shrub) (List 1939a).

Economically important hosts for *B. cockerelli* are tomato (*Solanum lycopersicum* L.), peppers (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.). Feeding by *B. cockerelli* reduces fruit production and causes leaf chlorosis, a condition known as psyllid yellows (Pletsch 1947). Although the scientific literature prior to 1950's suggested the culprit of psyllid yellows to be a toxin injected into the host during nymphal feeding by *B. cockerelli*, there are no

published experiments substantiating this claim by isolation, identification, or characterization of this putative toxin. In tomatoes, *B. cockerelli* has been reported to cause in excess of 50% total yield loss in California and Baja, Mexico in recent years (Liu and Trumble 2006).

In 1938, *B. cockerelli* was first recognized as a serious pest of potato when it caused yield losses of 25-50% in Wyoming and Montana and minor yield losses in Colorado, Idaho, and North Dakota (Pletsch 1947). In 1994 a disease known as “zebra chip” appeared in Saltillo, Mexico and in 2000 appeared in Pearsall, Texas. Infected potatoes produce black stripes when fried as chips rendering them unmarketable (Munyaneza et al. 2007). In addition, the disease was also found to reduce yield and kill the potato plant. The disease was intermittent and of minimal economic importance until 2004-2006 when the disease caused millions of dollars in yield losses to potato growers in Texas (Munyaneza et al. 2007). Since 2000, zebra chip disease has spread from Mexico, and Texas to Colorado, California, Oregon, Idaho, Nebraska, New Mexico, Arizona, New Zealand and Central America. Munyaneza et al. (2007) discovered the association of *B. cockerelli* with zebra chip disease. Hansen et al. (2008) discovered that the pathogen causing zebra chip disease was related to the bacterium that causes citrus greening disease, “*Candidatus Liberibacter asiaticus*’. He named the newly discovered bacterium that is vectored by *B. cockerelli*, ‘*Candidatus Liberibacter psyllaurosus*.’ Concurrently, New Zealand scientists identified the same bacterium as ‘*Candidatus Liberibacter solanacearum*’ (Lso) (Liefing et al. 2008, Butler and Trumble 2012).

At the present time, zebra chip disease management depends solely on the control of *B. cockerelli* the vector of the bacterium, Lso, which putatively causes zebra chip, using calendar-timed insecticide applications. Goolsby et al. (2007) developed a zebra chip disease management strategy which included the use of imidacloprid as an in-furrow treatment at planting time followed by weekly applications of foliar insecticides. While the management strategy was found to be very effective at reducing zebra chip disease incidence (Goolsby et al. 2007), and growers quickly adopted the management strategy (Guenthner et al. 2012), heavy insecticide use has led to concerns of insecticide resistance in *B. cockerelli*.

The heavy use of neonicotinoids in Mexico to control *B. cockerelli* could be a factor in the reduced susceptibility to imidacloprid of invading *B. cockerelli* when compared to native populations in California (Liu and Trumble 2007). More recently, it was found that field-collected *B. cockerelli* and reared for approximately two generations from south Texas were less susceptible to imidacloprid than laboratory colonies originating from the same area and maintained for six years (Prager et al. 2013). This reduced susceptibility to imidacloprid could be due to its high use among growers, who reportedly applied it to 92% of surveyed fields in Texas in 2011 (Guenthner et al. 2012). As concerns of insecticide resistance grow, methods are needed that can reduce insecticide usage while maintaining *B. cockerelli* populations below economic thresholds and thus, low zebra chip disease incidence.

One of the disadvantages of the zebra chip disease management strategy proposed by Goolsby et al. (2007) is that it depends on prophylactic, calendar-timed

insecticide applications with no regard to actual *B. cockerelli* populations. However, no other options are currently available to growers since the temporal occurrence of *B. cockerelli* populations are not consistent. Timing insecticide applications based on sampling is not appropriate because this a reactive approach. In this reactive approach, *B. cockerelli* are already present at high populations and potentially transmitting Lso to the potato plants before management decision are made. One method pest managers often use to time proactive control measures is degree day accumulation. Degree day accumulation to predict pest occurrence is used in an array of crops including *Lygus* spp. and sweetpotato whitefly (*Bemisia tabaci* Gennadius) in cotton (*Gossypium hirsutum* L.) (Sevacherian et al. 1977, Zalom et al. 1985), codling moth (*Cydia pomonella* L.) in apples (*Malus domestica* Borkh) (Brunner et al. 2005), oriental fruit moth (*Grapholita molesta* Busck) in peaches (*Prunus persica* L.) (Rice et al. 1984), and grape berry moth (*Paralobesia viteana* Ciemens) in grapes (*Vitis vinifera* L.) (Teixeira et al. 2009). Since insects are poikilotherms whose body temperature is largely determined by ambient temperature, their development rate can be estimated from the ambient temperature to which they are exposed during development.

Reaumur (1735) performed the first known studies relating poikilotherm development to ambient temperature. Reaumur created what would become known as the Reaumur unit, now known as the heat unit, which forms the foundations for degree-day modeling. The heat unit is a measure of the amount of heat experienced by an insect between its developmental thresholds. The lower developmental threshold (T_0) for an organism is the temperature below which development stops. The upper developmental

threshold (T_L) is the temperature above which the rate of growth or development stops. The total amount of heat required, between the lower and upper thresholds, for the development of an insect from a life stage to another is constant. This constant is termed the thermal constant, K . The thermal constant is most often in units of degree days, however, any time interval can theoretically be used, such as degree hours. Once the number of heat units accumulated between T_0 and T_L reaches K , one would expect the insect to have developed to the next life stage.

Predicting development of a poikilotherm depends on accurate parameters for development of the organism. That is, for each species, the developmental parameters of T_0 , T_L and K need to be determined. One of the most common methods of estimating the developmental parameters of an organism is by using constant temperature studies (Diaz et al. 2007, Chong et al. 2008, Ranjbar Aghdam et al. 2009, Lu et al. 2010, Damos and Savopoulou-Soultani 2011, Tran et al. 2012). In these papers, insects are reared under various constant temperatures while recording the duration of discrete life stages such as the egg and nymph/larva stage. The developmental rate at each constant temperature is calculated by taking the reciprocal of development time ($1/\text{development time in days}$). A linear equation ($y = mx + b$) is fit to the resulting data with development rate as the dependent variable on the y-axis and constant temperature as the independent variable on the x-axis. The T_0 of the insect can be estimated by solving the linear equation for x with y equal to zero. The thermal constant, K , is determined by the reciprocal of slope ($1/m$) (Campbell et al. 1974). Using this method, however, there is no estimation for T_L .

Indeed, many degree day predictions are adequate without the use of T_L . That is, all heat units accumulated above T_0 count towards the accumulated K and are not constrained by an upper temperature limit. However, Wang (1960) postulated that degree day models which predict poikilotherm development in warm climates may be more accurate by including T_L . Three papers address the difficulty of estimating the upper developmental threshold, T_L .

Logan et al. (1976), Lactin et al. (1995), and Briere et al. (1999) developed non-linear models that allow for the estimation of T_L , as well as T_0 . These methods for determining T_0 and T_L are similar to the linear estimation method above for estimating T_0 and K . Development rate at constant temperatures are used to fit the non-linear model and the best fit of the line is found using iterative processes minimizing root mean squared error, resulting in a non-linear equation that intersects the x-axis twice. The intercepts of the x-axis represent T_0 and T_L . While these models are often inadequate for estimating T_0 , they usually provide adequate estimations of T_L . However, none of the non-linear models provide an estimation of K . Therefore, linear and non-linear models are often used together where the linear model provides the T_0 and K and the non-linear model provides T_L .

B. cockerelli development may be especially limited at high temperatures. It has been observed by several authors that *B. cockerelli* tend to be largely absent during the warmest part of the year when many other pestiferous Hemipterans thrive (Hill 1947, Wallis 1955, Munyaneza et al. 2009). List (1939b) found that oviposition and life span was greatly reduced when exposed to a constant temperature of 32.2°C. In fact, eggs

exposed to 32.2°C for 9 hours and 26.7°C for 15 hours per day hatched, but no individuals survived to adult. This failure of nymphs to develop would suggest a T_L below 32.2°C.

Tran et al. (2012) and List (1939b) performed constant temperature experiments on *B. cockerelli* using populations from New Zealand and Colorado, respectively. They found that New Zealand populations seemed to be more heat tolerant than U.S. populations. Tran et al. (2012) estimated T_L to be 34°C, and List (1939b), who did not estimate T_L , found that *B. cockerelli* failed to develop at 32.2°C. This finding combined with the need for a predictive model that predicts *B. cockerelli* population dynamics, prompted us to develop a degree day model for Texas *B. cockerelli*.

In annual crops, it is convention that degree day models are used to predict the first appearance of an insect within a crop growing season. However, the value of such a prediction is dependent on the insect being largely absent before and during planting of the host crop. This is not the case with *B. cockerelli*, which is documented to be present in native vegetation well before conventional potato planting dates (Goolsby et al. 2012). Thus, the conventional methodology would be impractical.

Rather, our objective was to predict within season spikes in potato fields of *B. cockerelli* using the first peak to initiate the degree day model. A sub-objective was to determine if Lso-positive and Lso-negative *B. cockerelli* had different developmental parameters. Nachappa et al. (2012) discovered that Lso affected the fitness of *B. cockerelli* and therefore, could be an important consideration. If the developmental

parameters are different for Lso-positive and Lso-negative *B. cockerelli*, it may be necessary to develop separate degree day models.

Methods Development

In determining the developmental parameters for *B. cockerelli*, we sought to develop methods to perform constant temperature experiments on Lso-positive and Lso-negative *B. cockerelli*. A straight forward method might be to maintain two colonies of *B. cockerelli*, one colony Lso-positive and one colony Lso-negative. However, this approach could lead to the wrong conclusions. A difference in the developmental parameters between Lso-negative and Lso-positive colonies may not only be due to the effect of Lso, but also due to indirect effects of the pathogen on host plant quality. Lso-positive plants typically decline in quality after inoculation (Munyaneza et al. 2007) and psyllid eggs oviposited on these plants would be forced to develop on these poor quality plants. By contrast, Lso-negative colonies would not experience the possible deleterious effects of carrying the pathogen, but the psyllids would also develop on relatively high quality plants. As a result, differences in development time could be directly influenced by the insects scoring Lso-positive and indirectly affected by the differential quality of plants on which the insects were reared. Therefore, we pursued a method that would allow us to obtain Lso-positive and Lso-negative eggs from the same colony.

The method used in this research depended on the 47% transovarial transmission rate of Lso from mother to offspring (Hansen et al. 2008). Therefore, approximately half of *B. cockerelli* individuals possess Lso who are progeny from Lso-positive parents, forbearing horizontal transmission. By using only one insect per plant, we could ensure

there would be no horizontal transmission of Lso once the eggs hatched. Upon maturation to adult, individual insects would be subjected to diagnostic polymerase chain reaction (PCR) to determine post-hoc if they were Lso-positive or Lso-negative. To begin testing this method, approximately 30 adult *B. cockerelli* were allowed to oviposit eggs on an excised terminal potato leaflet (cv 'FL1867') that was inserted into a vial of water for 24 hours. Following removal of the adult *B. cockerelli*, the stalk of the eggs were carefully grasped with a forceps and the eggs were transferred to separate plants. However, after ten days none of the eggs hatched. Rather, the eggs were flat, shriveled, and desiccated. Assuming the eggs had sustained damage during their transfer to the new leaf, we tried a slight variation of the method that did not require touching the egg stalk with forceps. Eggs were harvested using a 3 mm leaf disk borer centered over the egg and pressed down to cut out a leaf disk. The leaf disk was then transferred to a potato leaf and left to hatch. The leaf disks quickly desiccated, but the egg was still attached to the leaf disk by the stalk. As before, however, eggs desiccated and none hatched after 10 days as before. After a search of the literature, it became obvious that *B. cockerelli* eggs may uptake water from the leaf.

White (1968) showed that another Psylloidean species with stalked eggs, *Cardiaspina densitexta* Taylor (Hemiptera: Aphalaridae), actively take up water from the leaf during egg development. White (1968) mentioned that the egg's stalk penetrates through the leaf epidermis and is rooted in the mesophyll where the stalk takes up water and hydrates the egg. Being in the same superfamily (Psylloidea) as *C. densitexta*, it is conceivable the eggs of *B. cockerelli* may also take up water from the leaf. Other

Hemipterans such as *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) (Byrne et al. 1990) and *Deraeocoris ruber* L. (Hemiptera: Miridae) were also found to take up water through the egg stalk. It is possible the reason *B. cockerelli* eggs desiccated during our previous two experiments may be due to the fact that we removed the water source from the egg stalk and they were unable to remain hydrated.

Returning to our previous leaf disk method, eggs were individually extracted using a 3 mm leaf disk borer centered over the egg and pressed down to cut out a leaf disk. Rather than placing the leaf disks on a plant where they would desiccate, we placed them on tap-water - saturated quilt batting. Quilt batting is a padding material used between the two layers of fabric in quilts, which was used here for its ability to soak-up and hold water, which kept the leaf disks moist and turgid during egg development. Of the 22 eggs that were on leaf disks, 16 (73%) hatched into nymphs. The leaf disks containing the newly-hatched nymphs were then transferred to a terminal leaflet of a potato plant using a drop of 5% Elmer's Glue-All® (Elmer's Products, Columbus, OH) to adhere the leaf disk to the surface of the potato leaf. Of the 16 nymphs on leaf disks that were placed on the potato leaflets, 14 moved from the leaf disk to the potato leaf within 24 hours. Of the two remaining nymphs, one went missing and the other was found desiccated on the leaf disk.

Eggs readily hatching on the hydrated leaf disks but failing to hatch on the desiccated leaf disks, provided evidence that *B. cockerelli* eggs probably take up water from the leaf. This possible water uptake of the egg from the leaf could have implications for Lso transfer. It is unknown into which potato leaf tissues the egg stalk

penetrates. If the egg stalk penetrates through the epidermis and mesophyll into the phloem, it is possible the egg could take up phloem limited bacteria, such as Lso.

However, more studies are needed to determine if the egg actually takes up water from the leaf, where in the leaf the egg stalk originates, and whether or not Lso is taken up by the egg.

Utilizing the leaf disk method, constant temperature experiments were carried out to determine the developmental parameters of *B. cockerelli* eggs and nymphs. Thirty adult *B. cockerelli* from an Lso-positive colony were placed in an arena with a terminal potato leaflet and allowed to oviposit for 24 hours. The resulting eggs were removed from the leaf using a 3mm leaf disk borer. Leaf disks with eggs were then placed on a 10 cm x 10 cm piece of quilt batting contained within a 150 x 15 mm (diameter x depth) Petri dish with no lid. The quilt batting was saturated with tap water to keep the leaf disks moist and turgid. The Petri dishes were placed in incubators (Model E-30B, Percival Scientific Inc., Perry, IA) with a 12:12 photoperiod at constant temperatures of 10, 14, 20, 24, 27, and 28°C. Upon egg hatch, the nymphs on the leaf disks were placed on a terminal leaflet of a potato plant using a drop of 5% Elmer's Glue-All® (Elmer's Products, Columbus, OH) to adhere the leaf disk to the leaf. The potato plants, each containing one nymph, were placed in the incubator from which the egg came. Incubators were checked every 24 hours for egg hatch or nymphs molting to adults. Once adults were observed, they were removed prior to their wings filling with hemolymph, which ensured they did not move to a different plant within the incubator. Adults were killed with a cotton ball soaked in 70% ethanol within a capped 10 dram

plastic vial. Once dead, the adults were transferred to and held dry in 1.5 ml microcentrifuge tubes in a refrigerator set at 3°C for up to six months, awaiting PCR. The preserved adults were subjected to diagnostic PCR using the DNA extraction, amplification, and visualization methods from Lévy et al. (2013) and the LsoF/OI2c forward/reverse primer pair from Li et al. (2009). To ensure viable DNA was present in each sample, *B. cockerelli* 28s rDNA primers (Nachappa et al. 2011) were used in separate reactions as an internal control. Of the 144 *B. cockerelli* that developed to adults, only three (2%) tested positive for Lso.

This was an unexpected result since the source colony for eggs remained Lso-positive throughout the constant temperature experiment and Hansen et al. (2008) reports a 47% transovarial transmission rate of Lso. This discrepancy could be explained by a higher mortality of Lso-positive eggs and/or nymphs than Lso-negative eggs and/or nymphs. Nachappa et al. (2012) demonstrated that Lso-positive *B. cockerelli* eggs were no more likely to survive on tomato than Lso-negative *B. cockerelli*, yet, Lso-negative nymphs were 1.5 times more likely to survive than Lso-positive nymphs. This taken with the fact that we eliminated horizontal Lso transmission by using on *B. cockerelli* per plant, could be a factor in us only receiving 2% Lso-positive *B. cockerelli* in our constant temperature studies. However, since we did not test the Lso infection status of individuals that did not survive to adult, we cannot make a conclusion based solely on our study.

Our objective of the introduction was to review the literature as it relates to pest management of *B. cockerelli* and to develop the methods needed to create a degree day

model for *B. cockerelli*. In the literature review, we determined that *B. cockerelli* management lacks an integrated approach and methods are needed to begin the development of an integrated approach. This led us to develop the methodology of using constant temperature studies, linear and non-linear modeling to determine the developmental parameters needed for degree day modeling of *B. cockerelli*. The following chapter is an implementation of the methods developed here.

CHAPTER II

"VJ G FGI REE DAY RCTCO GVGTU FOR THE DEVELOPMENT OF *BACTERICERA COCKERELLI* (SULC.) (HEMIPTERA: TRIOZIDAE)

Introduction

Bactericera cockerelli (Sulc) (Hemiptera: Triozidae), the potato psyllid (also known as tomato psyllid), is a phloem feeding insect that is a pest of many solanaceous crops including potato (*Solanum tuberosum* L.). In 2008, *B. cockerelli* was discovered to vector the bacterium 'Candidatus Liberibacter solanacearum' (Lso), the putative causal agent of zebra chip disease in potato (Hansen et al. 2008, Liefting et al. 2008). Although zebra chip disease also causes swollen and shortened internodes, chlorotic foliage, and aerial tubers in potato plants, the characteristic symptom is dark striping in the tuber when fried as french fries or potato chips, rendering the infected potatoes unmarketable (Secor et al. 2009). Since its discovery in Saltillo, Mexico in 1994, the disease has spread into most of the potato-producing states in the central and western U.S., as well as Guatemala, Honduras, Nicaragua, and New Zealand (Munyaneza 2012). Currently, the only way to mitigate the threat of zebra chip disease is to control field populations of *B. cockerelli*.

Goolsby et al. (2007) tested and published a *B. cockerelli* management program that reduced zebra chip disease incidence to below economic thresholds. The program relied on a neonicotinoid systemic insecticide, imidacloprid, applied in-furrow at planting time followed by a regimen of foliar insecticides of various active ingredients

applied at approximately seven day intervals. With no alternative means to control *B. cockerelli*, growers readily adopted the chemical control program. Surveyed potato fields (10 fields in 2009 and 12 fields each in 2011 and 2012) normally received 8 to 9 insecticide applications per growing season with imidacloprid applied in 92% of surveyed fields in 2011 (Guenther et al. 2012). The heavy use of imidacloprid has led to concerns that insecticide resistance could form in *B. cockerelli*. Prager et al. (2013) revealed a change in the susceptibility of south Texas *B. cockerelli* populations to the insecticide. In these studies, a *B. cockerelli* colony originating from *B. cockerelli* sampled from south Texas and reared for two generations was less susceptible to imidacloprid than laboratory colonies of *B. cockerelli* originally collected from south Texas, held in culture for six years and not exposed to imidacloprid. With a threat of insecticide resistance mounting, methods are needed to minimize the prophylactic use of insecticides while maintaining low *B. cockerelli* populations and low zebra chip disease incidence.

Degree day prediction of insect populations is a tool widely used in integrated pest management (IPM) to time management decisions. Several authors have developed and field tested degree day models to predict the arrival of pest insects or their subsequent generations in crops with a few examples including *Lygus* spp. and sweetpotato whitefly (*Bemisia tabaci* Gennadius) in cotton (*Gossypium hirsutum* L.) (Sevacherian et al. 1977, Zalom et al. 1985), codling moth (*Cydia pomonella* L.) in apples (*Malus domestica* Borkh) (Brunner et al. 2005), oriental fruit moth (*Grapholita molesta* Busck) in peaches (*Prunus persica* L.) (Rice et al. 1984), and grape berry moth

(*Paralobesia viteana* Ciemens) in grapes (*Vitis vinifera* L.) (Teixeira et al. 2009). These predictive degree day models provide pest managers the knowledge of when to expect pest occurrence and thus, ultimately reduce insecticide inputs by timing insecticide applications to the presence of the target life stage(s) of the pest.

The overarching goal of this project was to improve *B. cockerelli* IPM through the development of a degree day model to assist pest managers in timing insecticide applications to maximize their efficacy against *B. cockerelli* while reducing unneeded insecticide applications. The specific objectives were to: 1) generate *B. cockerelli* constant temperature data to determine the degree day parameters for the development of *B. cockerelli* and; 2) determine the accuracy of a model derived from the estimated parameters when predicting within season population peaks of *B. cockerelli* using sampling data from untreated potato plots across the southern High Plains from south Texas to Nebraska.

Materials and Methods

Degree days are based on the rate of an insect's development at temperatures between upper and lower limits for development. One common approach to modeling temperature effects on insect development is to convert the duration of development to their reciprocals. This simple transformation is used to reveal the relationship between temperature and rate of development and permits the determination of the thermal constant (K). The thermal constant is expressed as the number of degree-days (DD) and provides an alternative measure of the physiological time required for the completion of a process or a particular developmental event (Damos and Savopoulou-Soultani 2011).

The following methods in this manuscript were used to determine the development time of *B. cockerelli* at several constant temperatures. Nonlinear and linear models were subsequently fit to the development rate data to estimate the lower (T_0) and upper (T_L) temperature threshold, and the thermal constant (K) for development. Once the model was developed, we tested its reliability against *B. cockerelli* phenological events observed in datasets obtained from field surveys. Lastly, we suggest a two-phase method for the potential use of the estimated parameters (T_0 , T_L , and K) in a degree day model for controlling *B. cockerelli* in commercial potato fields.

Insect Colony. An Lso-positive *B. cockerelli* colony was established in a greenhouse in College Station, TX with evaporative cooling and no supplemental light. Temperature and humidity were not monitored in the greenhouse. The colony was established using *B. cockerelli* descendants originally collected in Weslaco, TX, from zebra-chip-diseased fields in 2008. *B. cockerelli* were maintained on potato (*cv* ‘FL1867’) in BugDorm 2® Insect Tents with thrips proof netting (Model no. BD2120F, MegaView Science Co, LTD, Taichung, Taiwan). Potato plants used in the colony were grown from tubers (either from Black Gold Farms, Grand Forks, ND, or CSS Potato Farms, Colorado Springs, CO) planted in 15.5 cm wide at the top tapering to 11 cm wide at the bottom – by 15 cm high pots in Sunshine® #1 Complete Mix soil (Sun Gro Horticulture, Agawam, MA). Plants were added to the insect colony between three- and six-weeks after plant emergence from the soil and watered when soil became dry with tap water until complete soil saturation, determined by water running out of the bottom of the pot. To verify insects remained Lso-positive, 10-20 unsexed, arbitrarily chosen, *B.*

cockerelli were sampled from the colony every 4-to-8 weeks and subjected to diagnostic polymerase chain reaction (PCR). The presence of Lso was determined using the direct PCR and visualization methods described by Lévy et al. (2013) and the LsoF/OI2c forward/reverse primer pair described by Li et al. (2009). To ensure viable DNA was present in each sample, *B. cockerelli* 28s rDNA primers (Nachappa et al. 2011) were used in separate reactions as an internal control.

Constant Temperature Experiments. Potato plants used in the constant temperature experiments were minitubers (*cv* ‘FL1867’, CSS Potato Farms, Colorado Springs, CO) planted in 7.6 cm at the top tapering to 5 cm at the bottom – by 7.6 cm high pots filled with Sunshine® #1 Complete Mix soil (Sun Gro Horticulture, Agawam, MA) and grown under high output fluorescent lighting at $\approx 27^{\circ}\text{C}$ with a 12:12 (L:D) photoperiod and watered to saturation as the soil dried using reverse osmosis water with no fertilization. Plants were used in the experiments between two- and six-weeks after plants emerged from the soil. As part of the process to harvest *B. cockerelli* eggs, leaves from the middle-to-top portion of potato plants were excised and stripped of all but the terminal leaflets. The excised terminal leaflets were inserted into a 20 ml vial of water and added to a 1 L cylindrical plastic arena measuring approximately 11 x 16 cm (height x width). Thirty, unsexed, sexually-mature (using the dark coloration described by Pletsch (1947) as the indicator) *B. cockerelli* were arbitrarily selected from the insect colony and introduced into each arena and allowed to oviposit on the plants for 24 hours. After removing the adults, a 3 mm diameter cork borer was used to excise leaf disks containing a single egg. Twenty-five or 35 leaf disks were placed on top of a 10 x 10 x 1

cm (length x width x height) piece of Poly-fil® quilt batting (Fairfield Processing, Danbury, CT) and placed within a 150 x 15 mm (diameter x depth) Petri dish with no lid. Quilt batting is a padding material used between the two layers of fabric in quilts, which was used here for its ability to absorb and hold water, which kept the leaf disks moist. The quilt batting was saturated with tap water and inserted into an incubator (Model E-30B, Percival Scientific Inc., Perry, IA) set at one of the target constant temperatures listed in Table 1 with a 12:12 (L:D) photoperiod and high output fluorescent lighting supplemented with a 25 watt incandescent bulb. Based on a similar study on *B. cockerelli* populations from New Zealand (Tran et al. 2012), constant temperatures were chosen to be most concentrated around the T_0 , T_L and the optimal development temperature (T_{opt}), the temperature at which development rate is the highest. A WatchDog® (Spectrum Technologies, Inc., Aurora, IL) temperature logger

Table 1. Mortality of *B. cockerelli* at target and actual constant temperatures

Target temp (°C)	Actual mean temp (°C \pm 1 SEM) ^b	n (no. of incubators)	No. of eggs	Mean mortality (%) \pm 1 SEM ^a		
				Egg	Nymph	Overall
10	10.1 \pm 0.49	3	95	36.8 \pm 0.05	76.7 \pm 0.04	85.3 \pm 3.51
14	13.8 \pm 0.18	3	85	22.3 \pm 0.08	62.1 \pm 0.07	70.6 \pm 0.07
20	19.2 \pm 0.49	2	50	16.0 \pm 0.00	47.6 \pm 0.10	56.0 \pm 0.08
24	24.2 \pm 0.34	3	85	11.6 \pm 0.04	35.9 \pm 0.11	43.5 \pm 0.11
27	27.3 \pm 0.20	2	60	18.3 \pm 0.04	51.0 \pm 0.18	60.0 \pm 0.26
28	27.8 \pm 0.22	2	50	22.0 \pm 0.01	69.2 \pm 0.04	76.0 \pm 0.00
31	31.4 \pm 0.18	3	85	21.2 \pm 0.03	100.0 \pm 0.00	100.0 \pm 0.00

^aMean and SEM (standard error of the mean) are weighted based on the number of insects per incubator.

^bMean temperature and SEM were calculated by averaging the unweighted mean temperature, which was logged every 30 min, from each incubator.

set to record the temperature every 30 min was placed in the center of each incubator to determine the actual constant temperature at which *B. cockerelli* were exposed during development. Since temperatures in the incubators deviated from the targeted constant temperatures, the actual mean temperature (Table 1) recorded over the course of development was used in the modeling described below.

At the same time daily, beginning 24 hours after initially placing the petri dish with the newly laid eggs in the incubator, eggs were checked for eclosion to nymphs. As eggs on the leaf disks eclosed, the resulting nymphs on the leaf disks were transferred to a terminal leaflet on the top half portion of the potato plant. A small drop of 5% Elmer's Glue-All® (Elmer's Products, Columbus, OH) was used to adhere the leaf disk to the upper surface of the terminal leaflet. Nymphs on the plants were returned to the incubator from which they originated. Only one nymph was placed on each plant and nymphs readily moved from the leaf disk to the leaflet of the potato plant. Once adults emerged, they were removed from the plant and killed with a cotton ball soaked in 70% ethanol within a capped 10 dram plastic vial. Once dead, the adults were transferred to and held dry in 1.5 ml microcentrifuge tubes in a refrigerator set at 3°C for up to six months, awaiting PCR. The stored *B. cockerelli* were individually subjected to diagnostic PCR to detect Lso using the methods described previously. Since only three of the 144 (2%) *B. cockerelli* surviving to adult tested positive for Lso, we were unable to test for an effect of Lso on development times. This low percentage (2%) of Lso-positive *B. cockerelli* that survived to adult in our experiment, even though we used an Lso-positive colony as an egg source, could be due to the incomplete transovarial rate of

Lso (Hansen et al. 2008) and the fact that we eliminated horizontal transmission of Lso by using only one nymph per plant. The three *B. cockerelli* that scored positive for Lso remained in the dataset because their development times were within the range of those that tested negative for Lso.

Non-linear and Linear Modeling. To estimate the three parameters (T_0 , T_L , K) needed for our degree day model, non-linear and linear models were fit to the egg, nymph, and overall (egg plus nymph) development rate data generated from the constant temperature experiments.

Non-linear Modeling. Although many non-linear models have been developed to estimate the parameters used in degree day estimation, Tran et al. (2012) tested the three most common non-linear models (Logan et al. 1976, Lactin et al. 1995, Briere et al. 1999) using constant temperature-development rate data from New Zealand *B. cockerelli* populations. Tran et al. (2012) concluded that the non-linear model from Briere et al. (1999) provided the best fit to *B. cockerelli* development. In fact, the Briere et al. (1999) model has generally provided a superior fit to Hemipteran development rate data when compared to the three aforementioned nonlinear models (Nielsen et al. 2008, Tazerouni et al. 2013). Accordingly, the Briere et al. (1999) non-linear model was fit to the data to estimate T_0 , T_{opt} , and T_L ; where;

$$1/d = ax(x-T_0)(T_L-x)^{1/2}$$

and $1/d$ is the development rate (the reciprocal of development time in days), x is the ambient rearing temperature and a is a fitted parameter that is an arbitrary empirical

constant approximating the development rate. T_0 and T_L are fitted parameters and T_{opt} is estimated using the method described by Briere et al. (1999):

$$T_{opt} = [4T_L + 3T_0 + (16T_L^2 + 9T_0^2 - 16T_0T_L)^{1/2}] / 10$$

Linear Modeling. A linear model was also fit to the data because: 1) the Briere et al. (1999) non-linear model does not provide an estimate of the thermal constant, K , for insect development, and 2) to provide another estimate of T_0 , in addition to the non-linear estimate of T_0 because of the linear method's wide use in insect phenology prediction. It has been shown that due to non-linearity at high temperatures, fitting a line to unconstrained insect development rate data can result in unrealistically low predictions of the x-intercept (Lamb 1992, Bergant and Trdan 2006, Walgama and Zalucki 2006) and thus, result in an inaccurate estimation of T_0 and K . Since development was clearly not linear at high temperatures in our experiment (Fig. 1), only development rates (1/d), of constant temperatures below T_{opt} from the non-linear model above were used. The linear model was used to estimate T_0 and K (Campbell et al. 1974, Worner 1988); where;

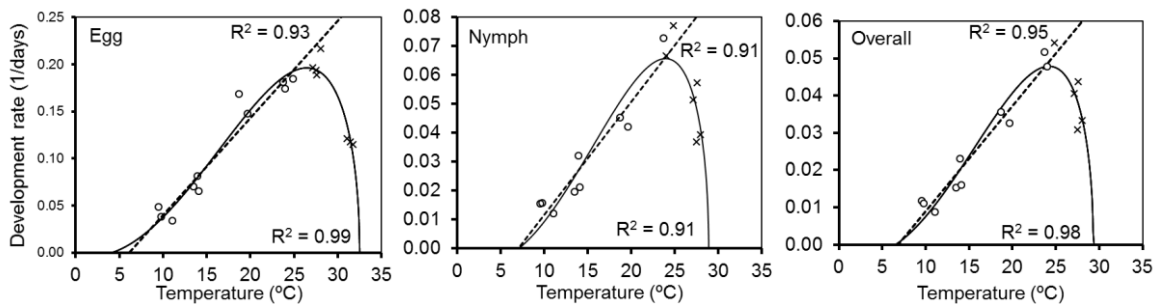


Figure 1. *B. cockerelli* development rate fit to non-linear (solid) and linear (dotted) models. **o** and **x** indicate data points that were included in the non-linear model. Only **o** data points were included in the linear model, which was constrained to data points below the optimum development temperature.

$$1/d = b + (1/K)$$

and x is the ambient rearing temperature and T_0 is estimated by setting $1/d$ to zero.

Line fitting was performed in SAS Web Editor® 2.5 (SAS Institute Inc., Cary, NC). Nonlinear models were fit using the SAS NLIN Procedure. The SAS NLIN procedure fits the model beginning with user-supplied starting parameters followed by an iterative process which minimizes the residual sum of squares. Our starting parameters were 5°C for T_0 , 30°C for T_L , and 0.0001 for a . The linear model was fit using the SAS REG Procedure. To minimize incubator effects, fifteen incubators were used in the experiment and replicates of a target temperature never occurred in the same incubator. Incubators were identified as statistical replicates with individual insects within an incubator identified as samples within a replicate.

Field Validation. Most often, degree day models predict the first occurrence of an insect during the growing season of the crop for which it is a pest. Validating and using the developmental parameters in a degree day model for *B. cockerelli* in this way is impractical as *B. cockerelli* are usually present throughout Texas and the High Plains prior to the potato growing season (Goolsby et al. 2007, Goolsby et al. 2012). Given these limitations, we chose to validate the developmental parameters for *B. cockerelli* by predicting the time between population peaks, and comparing the predictions with observed time between peaks.

The estimated parameters for *B. cockerelli* overall development were validated using data obtained from a *B. cockerelli* sampling program performed throughout the

Table 2. First sample date and number of predicted peaks for *B. cockerelli* eggs, nymphs, and adults at eight locations over six years.

Year	Location (plot no. ^a)	First sample date ^b	No. of peaks predicted		
			Egg	Nymph	Adult
2009					
	Dalhart, TX (1)	25 Feb.	NS	NS	4
	Weslaco, TX (1)	12 Jan.	NS	NS	1
2010					
	Dalhart, TX (1)	14 June	NS	NS	1
	Halfway, TX (1)	26 Apr.	NS	NS	1
	Imperial, NE (1)	10 June	NS	NS	1
	Garden City, KS (1)	10 Apr.	NS	NS	2
	Scottsbluff, NE (1)	10 June	NS	NS	1
	Weslaco, TX (1)	10 Dec.	NS	NS	3
	Weslaco, TX (2)	10 Jan.	NS	NS	1
	Weslaco, TX (3)	10 Jan.	NS	NS	1
	Weslaco, TX (4)	10 Feb.	NS	NS	1
2011					
	Dalhart, KS (1)	11 June	NS	NS	2
	Garden City, KS (1)	11 Mar.	NS	NS	2
	Weslaco, TX (2)	11 Jan.	1	1	1
	Weslaco, TX (3)	11 Feb.	0	2	1
2012					
	Alamosa, CO (1)	12 June	1	1	1
	Pearsall, TX (1)	12 Feb.	2	0	0
	Pearsall, TX (2)	12 Feb.	1	1	0
	Pearsall, TX (3)	12 Feb.	1	0	0
	Weslaco, TX (1)	12 Jan.	1	1	2
	Weslaco, TX (2)	12 Jan.	1	1	2
	Weslaco, TX (3)	12 Jan.	1	1	1
2013					
	Weslaco, TX (1)	13 Jan.	1	0	1
	Weslaco, TX (2)	13 Feb.	1	2	2
	Weslaco, TX (3)	13 Feb.	1	1	1
2014					
	Weslaco, TX (1)	14 Jan.	1	2	2
	Weslaco, TX (2)	14 Feb.	1	1	2
	Weslaco, TX (3)	14 Mar.	1	1	1
Total number of peaks predicted			15	15	38

NS, not sampled.

^aSimilar plot and location numbers do not necessarily indicate the same plot between years.

^bExact planting date is unknown. The month of planting can be approximated by subtracting 20 days, the mean emergence time of 50% of field potatoes from Sale (1979), from the first sampling date, since sampling began at plant emergence from the soil.

High Plains from South Texas to Nebraska. The 2009 to 2011 sampling data (Table 2) comes from the sampling program supervised by Dr. John Goolsby, who at the time was employed by the USDA-ARS (2413 E Hwy 83, Weslaco, TX 78596). Upon closing of the USDA-ARS research facility, Dr. Don Henne, who at the time was employed by Texas A&M AgriLife Research (2415 W Business 83, Weslaco, TX 78596), carried on the sampling program from 2012 to 2014 using similar methods as those of Dr. Goolsby.

During the 2009 through 2014 growing seasons, 0.135 ha plots were established in several locations. The sampling locations and the life stage for which *B. cockerelli* were sampled varied from year-to-year and are summarized in Table 2. Although the potato varieties in the untreated plots were commercial varieties, the actual varieties planted in each plot were not known. The month of planting can be approximated by subtracting 20 days, the mean emergence time of 50% of field potatoes in Sale (1979), from the first sampling date listed in Table 2, since sampling began at plant emergence from the soil. After plant emergence from the soil, sampling occurred weekly through the end of the growing season following the procedures described in Goolsby et al. (2012). Briefly, adults were sampled using five yellow sticky traps placed 1 m from an edge of plot to the center of the plot in a transect. Eggs and nymphs were sampled by removing 10 lower potato leaves from randomly-chosen, fixed locations in the plot. Adults were counted on the yellow sticky traps and eggs and nymphs were counted on leaves using a stereomicroscope. The data were graphed with total number of egg, nymph, or adults trapped on the y-axis and sample date on the x-axis by plot and year.

The resulting graphs (not shown in this manuscript) were visually analyzed for peaks in the total number of *B. cockerelli* at each life stage, field, and year. At fields and locations having at least two peaks, the number of days between the first and second peak and, if present, the second and third peak was recorded.

Since our validation method relied on the assumption that population peaks were generated solely by temperature-dependent, within-field reproduction, we applied a rubric to the field data in an effort to exclude peaks due to other factors, such as immigration. In cases where peaks occurred within the theoretical minimum development time (MDT) of the last peak, the peak was excluded from the predictions and the prediction was made to the next peak. The MDT was calculated for each location by dividing K by the most degree days accumulated in one day for the previous eight to 16 years, depending on the availability of temperature data. For all locations, the MDT was between 14 and 17 days. Daily minimum and maximum temperature data were used from a National Oceanic and Atmospheric Administration (NOAA) weather station near each city. The distance from each city in which the plots were located to the weather station used was: Weslaco, TX, 2.85 km; Pearsall, TX, 0.58 km; Halfway, TX, 17.25 km; Dalhart, TX, 5.77 km; Alamosa, CO, 2.65 km; Garden City, KS, 5.74 km; Imperial, NE, 11.87 km; and Scottsbluff, NE, 6.10 km. The maximal altitudinal difference from each city to the weather station was 76.2 m. These data were used to determine predicted number of days between peaks by life stage and year using the overall developmental parameters of *B. cockerelli* estimated by the non-linear and linear models.

A preliminary comparison of T_0 from the non-linear model (6.5°C) and the T_0 from the linear model (6.8°C) resulted in no numerical difference in the predicted number of weeks between peaks, therefore, either T_0 can be used equivocally in the field validation. The developmental parameters used for estimating the predicted time between peaks were T_0 of 6.8°C or 6.5°C, T_L of 29.3°C, and K of 354.6. Degree days were calculated with an online tool (UC IPM 2014) using the single-sine estimation method, which estimates the daily temperature curve from the daily minimum and maximum temperatures, paired with a horizontal cut-off, which assumes development is constant above T_L . Roltsch et al. (1999) found this pair to provide the most accurate estimation of degree days across a wide range of climates.

The validation data were analyzed in JMP Pro 11 (SAS Institute Inc., Cary, NC) where the difference between observed and predicted time in days for each peak by life

Table 3. Development time in days (mean \pm 1 SEM) of *B. cockerelli* at target constant temperatures

Target temp (°C)	Mean development time (days) ^a		
	Egg	Nymph	Overall ^b
10	26.1 \pm 1.1	70.0 \pm 3.2	96.1 \pm 3.7
14	13.5 \pm 0.4	39.4 \pm 2.1	53.0 \pm 2.4
20	6.4 \pm 0.2	23.1 \pm 0.7	29.5 \pm 0.8
24	5.6 \pm 0.1	14.4 \pm 0.5	20.0 \pm 0.5
27	5.1 \pm 0.1	21.8 \pm 1.1	26.9 \pm 1.1
28	5.0 \pm 0.2	21.6 \pm 1.3	26.6 \pm 1.2
31	8.5 \pm 0.1	n/a	n/a

SEM, standard error of the mean.

^aMeans were calculated by averaging the mean development time of insects in each incubator.

^bEgg plus nymph development time.

stage was compared with a paired t-test and 95% confidence intervals (CI) were calculated for the difference in the Matched Pairs platform. The null hypothesis of the paired t-test was that there was no difference between the two means.

Results

Mortality (Table 1) and development time (Table 3) were determined at seven constant temperatures ranging from 10°C to 31°C. Egg mortality was highest (36.8% \pm 1 standard error of the mean (SEM) of 0.05%) at 10°C and lowest (11.6% \pm 1 SEM of 0.04%) at 24°C. Nymphal mortality was highest at 31°C where all insects died in the nymph stage and the lowest at 24°C (35.9% \pm 0.11%). With the exception of 10°C and

Table 4. Estimated parameters (\pm 1 SEM) for *B. cockerelli* development rate as determined by nonlinear and linear models.

	Egg	Nymph	Overall
Nonlinear			
<i>a</i>	0.000141 \pm 0.000013	0.000073 \pm 0.000011	0.000050 \pm 0.000007
T_0 (°C)	4.5 \pm 1.32	7.1 \pm 1.54	6.5 \pm 1.40
T_{opt} (°C)	26.3	23.9	24.2
T_L (°C)	32.2 \pm 0.34	28.9 \pm 0.38	29.3 \pm 0.42
R ²	0.99	0.97	0.98
Linear			
1/ <i>K</i>	0.01033 \pm 0.00091	0.00389 \pm 0.00046	0.00282 \pm 0.00023
<i>b</i>	-0.06332 \pm 0.01602	-0.02714 \pm 0.01602	-0.01924 \pm 0.0038
T_0 (°C)	6.1	7.0	6.8
<i>K</i> (degree days)	96.8	257.1	354.6
R ²	0.93	0.91	0.95

SEM, standard error of the mean. SEM for the parameters estimated in the nonlinear model were approximated by SAS PROC NLIN (SAS Institute Inc., Cary, NC), which uses asymptotic standard errors that are calculated as if the model is linear.

31°C, nymphal mortality was roughly three times higher than egg mortality at all temperatures used in the experiment. At 10°C and 31°C, nymphal mortality was two and five times higher than egg mortality, respectively. The shortest development time was 5.0 ± 0.2 (SEM) days at 28°C for eggs and 14.4 ± 0.5 (SEM) days at 24°C for nymphs and 20.0 ± 0.5 (SEM) days at 24°C for overall development (Table 3). Development time was slowest at 10°C for eggs, nymphs, and overall development. Non-linear and linear models were fitted to *B. cockerelli* development rate data generated by the constant temperature experiments to estimate the developmental parameters. Values for T_0 associated with egg, nymph, and overall development were estimated by the nonlinear model as 4.5, 7.1, and 6.5°C and by the linear model as 6.1, 7.0, and 6.8°C, respectively (Table 4). The nonlinear model estimated values for T_{opt} as 26.3, 23.9, and 24.2°C for egg, nymph, and overall development, respectively. Values for T_L were estimated by the nonlinear model as 32.2, 28.9, and 29.3°C for the egg, nymph, and overall development,

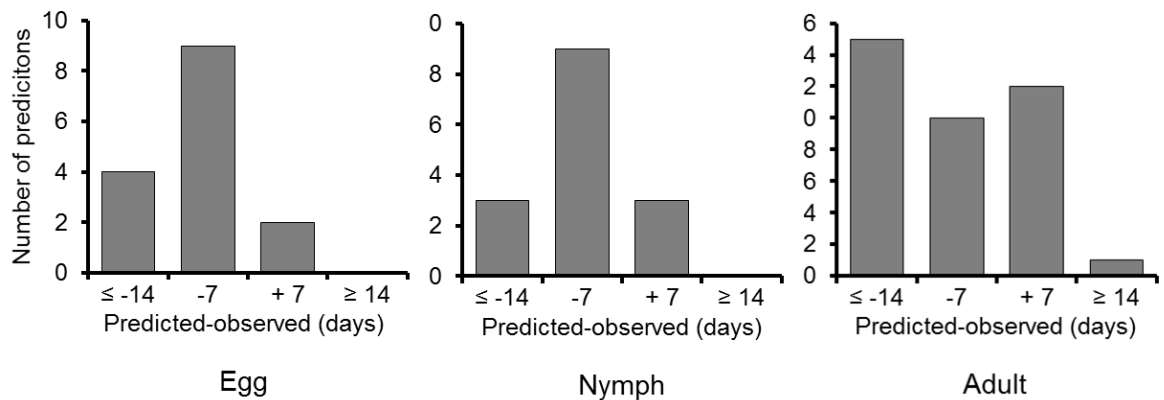


Figure 2. The number of predicted *B. cockerelli* population peaks and their deviations from observed weeks between peaks in the field validation. X-values < zero indicate an early prediction, x-values = zero indicate a coincident prediction, and x-values > zero indicate a late prediction.

respectively. The linear model estimated the values for K associated with *B. cockerelli* egg, nymph, and overall development as 96.8, 257.1, and 354.6 DD, respectively.

Sampling data from untreated field plots were gathered from a variety of locales and used to validate T_0 , T_L , and K for overall development of *B. cockerelli* generated from the modeling. Fifteen peaks fit the criteria for predicting each the egg-to-egg and nymph-to-nymph peaks. We predicted 38 peaks in total for the adult stage, more than double that of the egg and nymph due to the availability of more sampling data for the adults. Deviations were calculated as observed minus predicted number of days between peaks. Thus, negative deviations indicate predictions earlier than observed and positive indicates predictions later than observed. The range of deviations for egg-to-egg peaks were -17 to +8 days with a mean deviation of -4.5 ± 3.6 (95% CI) days. Nymph-to-nymph peak deviations ranged from -35 to +10 days with a mean deviation of -5.3 ± 4.9 (95% CI) days. The deviation range for adult-to-adult peaks was -5 to 2 weeks with a mean deviation of -7.0 ± 4.1 (95% CI) days. The difference between predicted and observed peak-to-peak time was significantly different than zero for all life stages (paired t-test, $p < 0.05$). However, this statistical result is influenced by predicted values having a resolution of days and the field data having a resolution of weeks (7 days). As a result, we examined histograms for each life stage showing the number of deviations at ≤ -14 , -7 , $+7$, and $\geq +14$ days as shown in Fig. 2. Eleven (73%) and 12 (80%) of the 15 egg-to-egg and nymph-to-nymph (respectively) peak predications were within the expected deviation range of ± 7 days due to the 7 day sampling interval of the field data.

However, only 22 (58%) of the 38 adult-to-adult peak predictions were within the ± 7 day expected deviation range due to the 7 day sampling interval of the field data.

Discussion

The *B. cockerelli* mortality rate measured as part of this research was similar to another laboratory study of *B. cockerelli* on potato where egg mortality was lowest in the egg stage and highest in the nymph stage (Yang et al. 2010). In addition, mortality was also measured to be the lowest at the egg stage when using tomato (*Lycopersicon lycopersicum* L.) (Abdullah 2008, Yang et al. 2013), eggplant (*Solanum melongena* L.), and bell pepper (*Capsicum annuum* L.) (Yang and Liu 2009) as the host plants. This observation would suggest that relative stage-specific mortality is consistent across most *B. cockerelli* hosts.

Tran et al. (2012) performed constant temperature development experiments and development rate modeling for *B. cockerelli* reared on potato. Tran et al. (2012) used *B. cockerelli* populations originating in New Zealand whereas the *B. cockerelli* used in our studies originated in south Texas. At 10°C, the overall mortality ($85.3\% \pm \text{SEM of } 3.51\%$) for the Texas populations was comparable to 81% mortality reported for the New Zealand population maintained at the same temperature. By contrast, we measured an overall mortality rate of $43.5 \pm 0.11\%$ (SEM) at 24°C for the Texas population whereas Tran et al. (2012) reported 63% overall mortality for his New Zealand population at 27°C. Another major difference was noted at 31°C where 100% of the Texas *B. cockerelli* died compared to only 87% of the New Zealand population dying at the same temperature. Both the magnitudes and consistency of these differences indicate

that the south Texas and New Zealand *B. cockerelli* populations respond differently to increasing temperatures.

Results from other temperature development studies conducted on *B. cockerelli* reared on potato and other hosts were different than those reported from this study. Tran et al. (2012), who used New Zealand populations, and Yang et al. (2010), who used Texas populations, reported the constant temperatures at which they reared *B. cockerelli* as 27 and 26.7°C with overall development times recorded at 21.1 ± 2.3 (standard deviation, SD) and 19.6 ± 0.3 (SEM) days, respectively. Our experiment found the development time at 27°C to be 26.9 ± 1.1 (SEM) days, 6 to 7 days longer than Tran et al. (2012) and Yang et al. (2010). In fact, overall development time in our study was longer than that in Tran et al. (2012) at all common temperatures (10, 20, 27°C). Differences in the methodology between this and the Tran et al. (2012) and Yang et al. (2010) studies could have led to these differences in development time. For instance, we used *B. cockerelli* from an Lso-positive colony as an egg source, while Tran et al. (2012) and Yang et al. (2010) used *B. cockerelli* from an Lso-negative colony as an egg source. It is also possible that genotypic variation between the *B. cockerelli* populations used in these studies could have led to these differences in development time. Liu et al. (2006) found that *B. cockerelli* populations invading California from Mexico were genetically distinct from the population native to Texas. Liu and Trumble (2007) found that the *B. cockerelli* from Texas had a faster development time when reared on tomato and pepper than *B. cockerelli* invading California from Mexico. However, since Yang et al. (2010), Tran et al. (2012), and ourselves did not determine the genotype of the *B. cockerelli* used

in our developmental studies, the source of these differences in development times remains unclear. Whereas the Yang et al. (2010) study did not estimate developmental parameters for *B. cockerelli*, the longer development times in our study led to a lower estimation of T_L than reported in the New Zealand study.

Although our estimation of T_0 and K for overall development of *B. cockerelli* are nearly identical to that of Tran et al. (2012), our estimation of T_L (29.3°C) is nearly 5°C lower. Indeed, the T_L for overall development estimated here is lower than other soft-bodied and pestiferous Hemipterans such as *Paracoccus marginatus* Williams and Granara de Willink (31°C) (Amarasekare et al. 2008); *Aphis gossypii* Glover (35°C) (Zamani et al. 2006); *Myzus persicae* Sulzer (37.3°C) (Davis et al. 2006); and *Bemisia tabaci* Bellows and Perring (B-biotype, 40°C; Q-biotype, 41°C) (Muñiz and Nombela 2001). However, our nonlinear model's estimation of T_L at 29.3°C is supported by our constant temperature development data where all nymphs failed to develop at 31°C, alluding to a T_L below 31°C. Additionally, our unusually low T_L is supported by the work of List (1939b) who conducted his studies with tomato as the host plant. List (1939b) found that *B. cockerelli* readily hatched at a constant temperature of 26.7°C and failed to hatch at the next highest constant temperature he tested of 32.2°C, suggesting a T_L between 26.7 and 32.2°C. The apparently low T_L provides evidence to support the hypothesis that *B. cockerelli* populations, at least in the U.S., are heat sensitive and thus, could explain the decline or absence of *B. cockerelli* populations during the warmer months and years (Hill 1947, Wallis 1955, Munyaneza et al. 2009). The heat sensitivity of *B. cockerelli* could be taken advantage of in IPM by growing heat tolerant potato

varieties that can be grown during the warmer months when *B. cockerelli* are unlikely to be present. Two varieties reported to be heat tolerant are cv 'Desiree' and 'Norchip' (Khedher and Ewing 1985, Ahn et al. 2004). However, it is unclear if these varieties could be satisfactorily produced during the warmer months when *B. cockerelli* is virtually absent.

Our field validation showed that deviation between the predicted and observed peak populations was largely equal to or less than the sampling frequency of ± 7 days, as field samples were collected weekly. However, the egg (73%) and nymph (80%) had a higher percentage of predictions that fell within single sample frequency of ± 7 day deviation than did the adults (58%). This observation may result from the sessile nature of the egg and nymph life stages compared to the highly mobile adults. It further indicates that egg and nymph peak predictions, as opposed to adult peak predictions, would be the most accurate when utilizing the degree day model in a *B. cockerelli* management program.

With the presence of *B. cockerelli* during the planting season precluding the use of the degree day model in a conventional manner by predicting its first occurrence, we suggest a two-phase method to integrate the degree day model into current *B. cockerelli* management programs. The first phase begins at planting and occurs through the first population peak of *B. cockerelli*. During the first phase, potatoes are monitored for *B. cockerelli* and, if present at above economic thresholds, *B. cockerelli* are managed by calendar- or sampling-timed chemical applications. Once the populations of *B. cockerelli* peak, phase two is initiated and degree day accumulations begin. When total degree day

accumulation equals K , an insecticide is applied to control *B. cockerelli*. These prediction-timed insecticide applications have the advantage of controlling *B. cockerelli* populations at the onset of infestation whereas the sampling-timed insecticide application has the disadvantage of controlling *B. cockerelli* populations after the onset of infestation. Waiting until after the onset of infestation, *B. cockerelli* has a greater amount of time to feed and thus, could increase the transmission of Lso to potatoes. This two-phase method could also help reduce insecticide inputs by only applying insecticides when *B. cockerelli* are likely to be present, rather than using weekly scheduled applications of insecticides that growers are currently using to control *B. cockerelli* in commercial fields.

Although the advantage of our method to degree day prediction is its high predictive power relative to its simplicity, our method's simplicity does confer some limitations. Our method requires peaks due to in-field reproduction and discrete *B. cockerelli* population peaks. To deal with peaks due to other factors, such as immigration, we applied a rubric that removed peaks from the dataset that were within the MDT of the previous peak. To mitigate the risk of immigration in commercial situations, even if pest managers are not using our two-phase method, *B. cockerelli* in surrounding non-potato vegetation may need to be monitored and managed. Another limitation of our validation method is its inability to accurately predict non-discrete peaks. The non-discrete peaks were characterized by a multi-week increase of *B. cockerelli* populations before peaking. In such cases, our degree day model usually predicted the peak during the multi-week increase in *B. cockerelli* populations when in

fact, the actual peak did not occur until later. Since these non-discrete peaks were common in our field data, they were likely a major source of deviation in our validation. This may be less of a limitation in commercial field settings, since pest managers should, and likely will, quickly take measures to reduce *B. cockerelli* populations once they begin to rise.

Our overarching goal of this work was to improve *B. cockerelli* IPM by incorporating optimally time insecticide applications that could reduce insecticide inputs by maximizing their efficacy and minimizing unneeded insecticide applications. To accomplish this goal, the first objective of this research was to generate development rate data by studying development time at constant temperatures and to model development rate to determine the three key developmental parameters (T_0 , T_L , and K) used in degree day modeling. Our constant temperature studies confirmed that *B. cockerelli* populations in Texas are more heat sensitive than other pestiferous and soft-bodied Hemipterans as well as *B. cockerelli* from New Zealand. Our models estimated the parameters for overall development as $T_0 = 6.5$ or 6.8°C , $T_L = 29.3^\circ\text{C}$, and $K = 354.6$ DD. The second objective was to validate the three parameters to determine if they would be useful in *B. cockerelli* IPM by predicting within-field population peaks of *B. cockerelli*. Our field validation indicates that the three parameters for overall development are adequate for use in a degree day model that predicts *B. cockerelli* population egg and nymph peaks, but not adult peaks. Future objectives might focus on testing the ability of our proposed two-phase method to accurately predict *B. cockerelli* population dynamics across different regions that were not tested herein and if the two-

phase method can keep *B. cockerelli* and zebra chip disease levels below economic thresholds.

CHAPTER III

GENERAL CONCLUSIONS

Even though *B. cockerelli* has been a pest of potatoes for almost 100 years, a true IPM program has yet to be developed and implemented to control the pest. With the occurrence of zebra chip disease over the past 14 years, a control program relying almost exclusively on calendar-timed insecticide applications has been implemented to control the vector, which transmits the bacterium, Lso, that causes zebra chip disease. Currently, growers rely on weekly, calendar-timed insecticide applications to control *B. cockerelli*. Over-use of at least one neonicotinoid, imidacloprid, could be leading to imidacloprid resistant *B. cockerelli* populations in south Texas (Prager et al. 2013). *B. cockerelli* populations presumably invading from Mexico into California were also found to be less susceptible to imidacloprid, perhaps due to the over-use of this compound in Mexico (Liu and Trumble 2007).

In an effort to begin the development of an IPM program for *B. cockerelli* infesting potatoes, we developed and validated the degree day parameters for *B. cockerelli*. Our sub-objective was to determine if *B. cockerelli* that were Lso-positive and those that were Lso-negative had different developmental parameters. If such was the case, they would need to be monitored as separate populations. Therefore, we developed the leaf disk method that would allow us to take advantage of the roughly 50% transovarial transmission rate of Lso reported in Hansen et al. (2008). Using these methods, we could obtain some eggs that were Lso-positive and some that were Lso-negative from the Lso-positive colony, and thus avoid a rearing affect.

During development of this methodology, we discovered the stalk on which the egg rests may act as a conduit to take water to the egg from the plant. This was evidenced by the 100% failure of eggs to develop on desiccated leaf disks compared to 73% of eggs which hatched to nymphs if the leaf disks were kept moistened and turgid. It is conceivable this could be a possible route of Lso transmission from the leaf to the eggs. However, too little is known about physiological and morphological aspects of the egg stalk and its relation to the plant surface. The origin of the stalk within the leaf needs to be determined. If it is found that the egg takes up water and that the stalk originates in the phloem, it is possible that the egg could take-up the phloem limited Lso.

Following the development of the leaf disk method, we performed constant temperature studies of *B. cockerelli* to model its development rate and estimate its degree day parameters for development. With the intention of estimating developmental parameters of Lso-positive and Lso-negative populations, we performed diagnostic PCR on all individuals that survived to adult in the studies post-hoc. Expecting roughly half of the 144 adult *B. cockerelli* to be Lso-positive per their 47% transovarial transmission rate and the use of an Lso-positive colony as an egg source, only 2% were Lso-positive. A likely explanation for the unexpectedly low Lso-positive status is that Lso-positive *B. cockerelli* are less likely to survive than Lso-negative *B. cockerelli*, which has been

Results from Tran et al. (2012), who performed constant temperature experiments on New Zealand *B. cockerelli*, and Yang et al. (2010), who used Texas *B. cockerelli*, differed from our results. When reared at 27°C in Tran et al. (2012) and Tran et al. (2012) on potato, *B. cockerelli* overall development times were 21.1 ± 2.3

(standard deviation, SD) and 19.6 ± 0.3 (SEM) days, respectively. However, we found the overall development time at 27°C to be 26.9 ± 1.1 (SEM) days, 6 to 7 days longer than Tran et al. (2012) and Yang et al. (2010). Additionally, overall development time was longer in our experiment than that reported in Tran et al. (2012) at all common temperatures (10, 20, 27°C). Methodological differences between our experiment and the Tran et al. (2012) and Yang et al. (2010) experiments may explain the differences in development times. For example, *B. cockerelli* used in our experiment were from an Lso-positive colony, and Tran et al. (2012) and Yang et al. (2010) used *B. cockerelli* from an Lso-negative colony. Additionally, haplotypic variation could have led to these differences in development time.

While we did not test the haplotype of *B. cockerelli* used in our experiment, we may be able to confidently infer the haplotype of *B. cockerelli* based on location from which our *B. cockerelli* colony originated. The *B. cockerelli* colony used in our experiments were progeny of insects collected in Weslaco, TX in 2008. Swisher et al. (2013) found that all 48 *B. cockerelli* tested from Weslaco, TX from 2009-2011 were of the central haplotype. Therefore, it is reasonable to assume our *B. cockerelli* colony was of the central haplotype. Conversely, the *B. cockerelli* used in Tran et al. (2012) were from New Zealand, where *B. cockerelli* have been documented to be of the western haplotype (Thomas et al. 2011). Since Liu and Trumble (2007) found differences in the development time between the central and western haplotype, it is possible that this difference in haplotype could be the cause of the differing development times found between this study and the Tran et al. (2012) experiment. However, it is even more

difficult to pinpoint the cause of the longer development times in Yang et al. (2010) when compared to our study because both of our studies used colonies established from *B. cockerelli* collected from Weslaco, TX.

The shorter development times in our experiment when compared to the Tran et al. (2012) experiment resulted in our estimation of a lower T_L for overall development. Our T_L of 29.3°C was nearly 5°C lower than that found with New Zealand populations, suggesting the U.S. populations of *B. cockerelli* are more sensitive to heat. In fact, the T_L we estimated for *B. cockerelli* was lower than many other pestiferous Hemipterans such as *Paracoccus marginatus* Williams and Granara de Willink (31°C) (Amarasekare et al. 2008); *Aphis gossypii* Glover (35°C) (Zamani et al. 2006); *Myzus persicae* Sulzer (37.3°C) (Davis et al. 2006); and *Bemisia tabaci* Bellows and Perring (B-biotype, 40°C; Q-biotype, 41°C) (Muñiz and Nombela 2001).

Our estimation of T_L is supported by List (1939b) who published incomplete constant temperature studies of *B. cockerelli*. List's highest temperature tested was also 32.2°C, at which a percentage of eggs developed to nymph, but 100% of nymphs failed to complete development. In addition, he found daily exposures to 32.2°C for 9 hours and 27.7°C for 15 hours was also lethal to nymph. In validating the parameters (T_0 , T_L , K) for overall development of *B. cockerelli*, we found that they are adequate for use in an IPM program.

Because populations of the *B. cockerelli* are present prior to and during the potato growing season, we did not attempt to predict the first appearance of the potato psyllid. Rather, we attempted to predict within field population peaks of each life stage

using the prior peak to initiate the model. The parameters generated in our models for overall development predicted peaks 7 days or less early for all life stages. We discussed the possibility that this deviation could be due to the one week sampling interval of the data or our validation method's inability to predict broad peaks.

While the developmental parameters (T_0 , T_L , K) estimated in this research were found to be adequate for use in an IPM program in regions stretching from south Texas to Nebraska, the parameters should be tested in the west and pacific north west, regions where *B. cockerelli* is also a pest. Additionally, future objectives should focus on testing our two-phase method for its ability to keep *B. cockerelli*, and thus zebra chip disease, below economic thresholds.

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