THRIPS TABACI (THYSANOPTERA: THRIPIDAE) AND IRIS YELLOW SPOT VIRUS
(GENUS: TOSPOVIRUS, FAMILY: BUNYAVIRIDAE) IN ONIONS: BIOLOGY AND
TRANSMISSION STUDIES

by

ANITHA CHITTURI

(Under the Direction of David G. Riley)

ABSTRACT

Thrips vectors of tospoviruses were surveyed in onions and other vegetable crops in India and in onions in Georgia, USA. The only known vector species of Iris yellow spot virus (IYSV) collected both in the USA and India was *Thrips tabaci* (Thysanoptera: Thripidae). In onions in Georgia, the two dominant species of thrips during this survey were *Thrips tabaci* and *Frankliniella fusca*. Competition studies between these two thrips species revealed that *T. tabaci* reproductively outcompeted *F. fusca* in onions. Distribution studies on *T. tabaci* in onions indicated that adult settling and oviposition are skewed towards the base of the plant. Field studies on the distribution of thrips and IYSV in onions demonstrated similar distributions of eggs and virus skewed towards the base of the plant. Transmission studies of IYSV indicate that *T. tabaci* can inoculate onion plants very efficiently, achieving 64% successful transmission in 15 minutes. *Thrips tabaci* appeared to be the main thrips vector of IYSV in both Georgia and India.
INDEX WORDS:  Thrips vectors, Onion thrips, India, Georgia, USA, Thrips competition, 

*Thrips tabaci, Frankliniella fusca*, Distribution, Settling, Oviposition, 

Within-leaf distribution of eggs, Distribution of IYSV, IYSV transmission bioassays, Transmission efficiency, Transmission time, Transmission number, DAS ELISA, NSs ELISA.
THRIPS TABACI (THYSANOPTERA: THRIPIDAE) AND IRIS YELLOW SPOT VIRUS
(GENUS: TOSPOVIRUS, FAMILY: BUNYAVIRIDAE) IN ONIONS: BIOLOGY AND
TRANSMISSION STUDIES

by

ANITHA CHITTURI

B.S (Ag)., Acharya N. G. Ranga Agricultural University, Hyderabad, India, 1999
M.S., The University of Georgia, 2005

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2010
DEDICATION

To my mom, Vijaya Lakshmi and my dad, Charly Milanktha Rao for instilling the confidence and determination when I needed it the most. I am grateful for their unconditional love, encouragement and moral support throughout this study.

To my beautiful daughter, Prashamsa, whose arrival brought me immense joy, and the motivation and strength to complete what I had started.

To my dearest brother, Joseph Christopher for his support throughout my career and for always being there for me.
ACKNOWLEDGEMENTS

First and foremost, I thank the Lord Almighty for his abundant grace who guarded me and blessed me to pursue this doctoral program in spite of so many difficult situations especially while conducting my field research in India.

I am highly indebted to my major advisor Dr. David Riley for his meticulous guidance and constant encouragement throughout this study. His patience and continued help both professionally and personally have contributed greatly for the completion and success of this research. I sincerely thank my committee members Dr. Joseph McHugh, Dr. Alton Sparks and Dr. John Sherwood for their insights, guidance and support throughout my research.

I wish to express my heartfelt thanks to the administrative assistants Ms. Carol Ireland and Jenny Granberry for their professional and personal help during my stay at the experiment station. Thank you so much for your friendship. I will miss you a lot!

I would like to thank the technical staff at the Vegetable Entomology lab, Jackie Davis, Donnie Cook and Sophia Kimbrel for their support and help in conducting my field research. I express my gratitude to the student workers Sarah Williford, Ronnie Smith and Jessica Kalina who helped me in setting up of my experiments, maintaining the thrips colonies and onion seedlings in the growth chambers throughout my research. I thank Steve Mullis from Plant Pathology for his time and help in running the ELISA samples and answering my numerous questions while I was conducting my experiments and Dr. Babu Srinivasan for giving me the lab space to run ELISA samples. I extend my sincere thanks to Stan Diffie, for his technical guidance and help in thrips identification and for running all the NSs samples.
I convey my special thanks to Dr. Hanu Pappu, Washington State University, for his guidance and encouragement in carrying out my experiments during the course of my studies. I also extend my sincere thanks to his post-doc Sudeep Bag for running NS’s ELISA on thrips samples and spending hours over the phone in clarifying my doubts about the techniques and procedures.

I owe my warmest thanks to my husband Kiran without whose support, encouragement and many compromises I could not have achieved this success. My heartfelt gratitude to my extended family, Mari Lou and John Joyce in Tifton and Lynn Faust in Athens for their support and unconditional love. A special thanks goes to my best friends Venu Margam, Swapna Margam and Shyam Sunder Singh. I would like to thank my family Karuna Sree, Jay Prakash, Noel, Rosita and Sudhakar in India for cheering me up and showering me with their love and affection. Finally, I thank the IPM CRSP pilot project and the Department of Entomology for funding this research.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LIST OF FIGURES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>xii</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Objectives</td>
<td>5</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>6</td>
</tr>
<tr>
<td>References Cited</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>13</td>
</tr>
<tr>
<td>Tospoviruses</td>
<td>13</td>
</tr>
<tr>
<td>Tospovirus species in USA</td>
<td>14</td>
</tr>
<tr>
<td>Tospovirus species in India</td>
<td>17</td>
</tr>
<tr>
<td>Thrips vectors of tospoviruses</td>
<td>18</td>
</tr>
<tr>
<td>Thrips vectors in USA</td>
<td>21</td>
</tr>
<tr>
<td>Thrips vectors in India</td>
<td>22</td>
</tr>
<tr>
<td>Thrips biology</td>
<td>23</td>
</tr>
<tr>
<td>Thrips transmission of tospoviruses</td>
<td>25</td>
</tr>
<tr>
<td>References cited</td>
<td>28</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>SURVEY OF THRIPS VECTORS OF TOSPOVIRUS IN ONIONS AND OTHER VEGETABLE CROPS</td>
<td>44</td>
</tr>
<tr>
<td>Abstract</td>
<td>45</td>
</tr>
<tr>
<td>Introduction</td>
<td>46</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>47</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>49</td>
</tr>
<tr>
<td>References Cited</td>
<td>52</td>
</tr>
<tr>
<td>THRIPS (THYSANOPTERA: THRIPIDAE) COMPETITION IN ONIONS</td>
<td>73</td>
</tr>
<tr>
<td>Abstract</td>
<td>74</td>
</tr>
<tr>
<td>Introduction</td>
<td>75</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>76</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>79</td>
</tr>
<tr>
<td>References Cited</td>
<td>84</td>
</tr>
<tr>
<td>DISTRIBUTION, SETTLING AND OVIPOSITION OF THRIPS TABACI (THYSANOPTERA: THRIPIDAE) ON ONION FOLIAGE</td>
<td>102</td>
</tr>
<tr>
<td>Abstract</td>
<td>103</td>
</tr>
<tr>
<td>Introduction</td>
<td>104</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>105</td>
</tr>
<tr>
<td>Results</td>
<td>106</td>
</tr>
<tr>
<td>Discussion</td>
<td>109</td>
</tr>
<tr>
<td>References Cited</td>
<td>111</td>
</tr>
<tr>
<td>WITHIN LEAF DISTRIBUTION OF THRIPS EGGS AND IRIS YELLOW SPOT VIRUS ON ONION FOLIAGE IN THE FIELD</td>
<td>129</td>
</tr>
</tbody>
</table>
Abstract ................................................................. 130
Introduction .................................................................. 131
Materials and Methods................................................. 132
Results ....................................................................... 133
Discussion .................................................................. 137
References Cited .......................................................... 139

7 THRIPS TRANSMISSION OF IRIS YELLOW SPOT VIRUS TO ONIONS ....... 158

Abstract ..................................................................... 159
Introduction .................................................................. 160
Materials and Methods................................................. 162
Results ....................................................................... 165
Discussion .................................................................. 168
References Cited .......................................................... 169

8 SUMMARY ................................................................ 177
REFERENCES ................................................................ 180

APPENDIX

APPENDIX 1: FIELD DATA ON THRIPS SURVEY CONDUCTED DURING
2006-2008 IN INDIA .......................................................... 202
LIST OF TABLES

Table 2.1: Thrips species on the genera Frankliniella, Thrips, Scirtothrips and Ceratothripoides and associated tospoviruses .......................................................... 19

Table 3.1: Sample locations in the two year thrips survey and sampling by state, district and crop in India .......................................................................................... 58

Table 3.2: Thrips vector species found in vegetable crops in India .................................................. 59

Table 4.1: Total adult and immature thrips emerged over all weeks on onion transplants infested with 10 immatures per plant during 2004 .......................................................................... 86

Table 4.2: Total adult and immature thrips collected at 5 day intervals on onion seedlings infested with 4 adult females per plant during 2008 ........................................................................ 87

Table 4.3: Distribution of mean number of Thrips tabaci and Frankliniella fusca adults on onion plants relative to time .......................................................................................... 88

Table 5.1: Mean settling of Thrips tabaci adults, immatures and oviposition by leaf length ...................................................................................................................... 114

Table 7.1: Transmission of Iris yellow spot virus (IYSV) to healthy onion plants with different number treatments and check treatments of adult Thrips tabaci as detected by DAS ELISA ........................................................................................................ 173

Table 7.2: Detection of Iris yellow spot virus (IYSV) in thrips using the nonstructural protein enzyme-linked immunosorbent assay (NSs ELISA) with different numbers of thrips transmitted to healthy onion plants conducted during mid March 2009 ............... 174
Table 7.3: Detection of *Iris yellow spot virus* (IYSV) in thrips using the nonstructural protein enzyme-linked immunosorbent assay (NSs ELISA) with different numbers of thrips transmitted to healthy onion plants conducted during early May 2009.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Structure of tospovirus particle</td>
<td>14</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Thrips life cycle</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Tospovirus transmission cycle</td>
<td>26</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Map of India showing thrips samples collected in three states</td>
<td>60</td>
</tr>
<tr>
<td>Figure 3.2a</td>
<td>Ventral view of <em>Thrips tabaci</em></td>
<td>61</td>
</tr>
<tr>
<td>Figure 3.2b</td>
<td>Ventral view of <em>Scirtothrips dorsalis</em></td>
<td>62</td>
</tr>
<tr>
<td>Figure 3.2c</td>
<td>Ventral view of <em>Frankliniella schultzei</em></td>
<td>63</td>
</tr>
<tr>
<td>Figure 3.2d</td>
<td>Ventral view of <em>Thrips palmi</em></td>
<td>64</td>
</tr>
<tr>
<td>Figure 3.2e</td>
<td>Ventral view of <em>Thrips hawaiiensis</em></td>
<td>65</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Total number of thrips sampled by month for the survey during 2006-2008 in India</td>
<td>66</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Mean number of thrips by crop and genus 2006-2008 in India</td>
<td>67</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Mean number of thrips in onions by state and genus 2006-2008 in India</td>
<td>68</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Mean number of thrips in tomato by state and genus 2006-2008 in India</td>
<td>69</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Mean number of thrips in chili peppers by state and genus 2006-2008 in India</td>
<td>70</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>Mean number of thrips sampled by month for 2008 and 2009 in Georgia, USA</td>
<td>71</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>Total number of thrips sampled by location and thrips species for 2008-2009 in Georgia, USA</td>
<td>72</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Onion transplants placed in 1 quart styrofoam cups to test for possible exclusion of <em>Thrips tabaci</em> by <em>Frankliniella fusca</em> during 2004</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 4.2: Onion seedlings placed into 13 × 100 mm glass test tubes to test for possible exclusion of *Thrips tabaci* by *Frankliniella fusca* during 2008 ........................................90

Figure 4.3a: Thrips numbers by species for week 1 during 2004 ........................................91

Figure 4.3b: Thrips numbers by species for week 2 during 2004 ........................................92

Figure 4.3c: Thrips numbers by species for week 3 during 2004 ........................................93

Figure 4.3d: Thrips numbers by species for week 4 during 2004 ........................................94

Figure 4.4a: Thrips numbers by species for 0-5 days during 2008 ........................................95

Figure 4.4b: Thrips numbers by species for 6-10 days during 2008 ....................................96

Figure 4.4c: Thrips numbers by species for 11-15 days during 2008 ....................................97

Figure 4.4d: Thrips numbers by species for 16-20 days during 2008 .....................................98

Figure 4.5: Distribution of *Thrips tabaci* and *Frankliniella fusca* on onion plants in the field relative to leaf position over all .................................................................99

Figure 4.6: Distribution of *Thrips tabaci* and *Franklinella fusca* on onion plants in the field relative to leaf position during late April 2009 ....................................................100

Figure 4.7: Distribution of *Thrips tabaci* and *Frankliniella fusca* on onion plants in the field relative to leaf position during late May 2009 .........................................................101

Figure 5.1: Leaf positions for the onion seedling to determine settling by *Thrips tabaci* adults and immatures .................................................................115

Figure 5.2: Potted 3-leaf onion plants placed in an insect cage measuring 47.5 × 47.5 × 47.5 cm .................................................................116

Figure 5.3a: Settling of *Thrips tabaci* adults and immatures on L1 (innermost leaf) at 3-leaf stage onion plant .................................................................117
Figure 5.3b: Settling of *Thrips tabaci* adults and immatures on L 2 (inside leaf) at 3-leaf stage onion plant .................................................................118

Figure 5.3c: Settling of *Thrips tabaci* adults and immatures on L3 (outermost leaf) at 3-leaf stage onion plant .................................................................119

Figure 5.4a: Oviposition of *Thrips tabaci* on L1 (innermost leaf) at 3-leaf stage onion plant ........................................................................................................120

Figure 5.4b: Oviposition of *Thrips tabaci* on L 2 (inside leaf) at 3-leaf stage onion plant ........................................................................................................121

Figure 5.4c: Oviposition of *Thrips tabaci* on L3 (outermost leaf) at 3-leaf stage onion plant ........................................................................................................122

Figure 5.5a: Settling of *Thrips tabaci* adults and immatures on L1 (innermost leaf) at 6-8 leaf stage onion plant .................................................................123

Figure 5.5b: Settling of *Thrips tabaci* adults and immatures on L 2 (inside leaf) at 6-8 leaf stage onion plant .................................................................124

Figure 5.5c: Settling of *Thrips tabaci* adults and immatures on L3 (outer leaf) at 6-8 leaf stage onion plant .................................................................125

Figure 5.6a: Oviposition of *Thrips tabaci* on L1 (innermost leaf) at 6-8 leaf stage onion plant ........................................................................................................126

Figure 5.6b: Oviposition of *Thrips tabaci* on L 2 (inside leaf) at 6-8 leaf stage onion plant ........................................................................................................127

Figure 5.6c: Oviposition of *Thrips tabaci* on L3 (outer leaf) at 6-8 leaf stage onion plant........128

Figure 6.1: Total number of thrips eggs sampled in the field relative to leaf length in 2009 .....................................................................................................................141
Figure 6.2: Mean number of thrips eggs sampled in the field during late February 2009............142
Figure 6.3: Mean number of thrips eggs sampled in the field during early March 2009 ............143
Figure 6.4: Mean number of thrips eggs sampled in the field during mid March 2009 .............144
Figure 6.5: Mean number of thrips eggs sampled in the field during late March 2009.............145
Figure 6.6: Mean number of thrips eggs sampled in the field during mid April 2009.............146
Figure 6.7: Mean number of thrips eggs sampled in the field during late April 2009.......... 147
Figure 6.8: Mean number of thrips eggs sampled in the field during early May 2009 .......... 148
Figure 6.9: Mean number of thrips eggs sampled in the field relative to leaf position L1........ 149
Figure 6.10: Mean number of thrips eggs sampled in the field relative to leaf position L2...... 150
Figure 6.11: Mean number of thrips eggs sampled in the field relative to leaf position L3...... 151
Figure 6.12: Total IYSV positive leaf segments per length of onion leaf sampled in the field
during mid March 2007 ..................................................................................................152
Figure 6.13: Total IYSV positive leaf segments per length of onion leaf sampled in field during
mid March 2009 .............................................................................................................153
Figure 6.14: Total IYSV positive leaf segments per length of onion leaf sampled in the field
during mid April 2009 .................................................................................................154
Figure 6.15: Total IYSV positive leaf segments per length of onion leaf sampled over both dates
in the field .......................................................................................................................155
Figure 6.16: Total IYSV positive leaf segments per length of onion leaf sampled in the
 greenhouse 2009 ...........................................................................................................156
Figure 6.17: IYSV incidence on onion leaf in the field relative to leaf length from the base of the
onion plant collected in 2007 .......................................................................................157
Figure 7.1: Transmission of IYSV by adult *Thrips tabaci* to healthy onion plants at different time intervals.
CHAPTER 1

INTRODUCTION

Thrips-borne tospoviruses are emerging as a significant limiting factor in sustainable vegetable production and other economically important agricultural crops worldwide. Currently, eighteen tospoviruses (Whitfield et al. 2005) have been identified worldwide and eleven thrips species (Thysanoptera: Thripidae) have been confirmed as vectors in the transmission of tospoviruses (Jones 2005). The broad plant host range and widespread distribution of thrips vectors with efficient virus transmitting ability contribute to the challenge in managing the thrips-borne viruses in the vegetable production systems worldwide (Pappu et al. 2009). This research focused on *Thrips tabaci* (Lindeman) as a main vector of *Iris yellow spot virus* (genus *Tospovirus*, family *Bunyaviridae*) in onions (Nagata et al. 1999). Studies were conducted mainly in Georgia, USA, however a study of other potential thrips vectors in onions and other vegetables was conducted in India.

*Iris yellow spot virus* (IYSV), a member of the genus *Tospovirus*, is less studied than *Tomato spotted wilt virus* (TSWV) (Jones 2005), and is an important virus of onion that is vectored by thrips (Thysanoptera: Thripidae). IYSV was first reported in 1998 from the Netherlands as a new tospovirus naturally infecting iris (*Iris hollandica* Tub.) in the field and leek (*Allium porrum*) in the greenhouse (Cortês et al. 1998). By early 2000, IYSV was reported infecting onion (*Allium cepa*) in all major onion growing regions worldwide (Smith et al. 2006). Currently, only one vector species of thrips, *Thrips tabaci* (onion thrips) Lindeman is confirmed to be the vector of IYSV (Nagata et al. 1999, Kitzman et al. 2001). Currently, IYSV infects at
least 47 species worldwide (Gent et al. 2006) naturally under field conditions including onions (Gera et al. 1998, Pozzer et al. 1999), garlic (Robèn-Soustrade, 2005) and ornamental crops such as lisianthus (Kirtzman et al. 2001), alstroemeria (Okuda et al. 2001) and certain weed species (Pappu et al. 2006, Sampangi et al. 2007, Nischwitz et al. 2007). Based on recent accounts IYSV occurs across 6 continents in 24 countries (CABI/EPPO 2009). The causes for the sudden spread of IYSV in onions and other cultivated plant species are unclear.

Economic damage due to IYSV in onion is variable, but it has been reported to cause up to 100% loss in 1999 (Pozzer et al. 1999) and 50-60% losses in onion bulb production in 2001 (Kirtzman et al. 2001). In recent years, the estimated economic loss due to outbreaks of IYSV in bulb and seed onion crops in Oregon, Idaho and Washington are reported to be $480,000 (Pappu et al. 2009). Symptoms of IYSV infection vary with host susceptibility, plant stress, cultivar and thrips fecundity levels (Gent et al. 2006). Characteristic symptoms of IYSV infection include distinct diamond-shaped chlorotic or necrotic lesions, as well as indistinct circular to irregular, chlorotic or necrotic lesions of various sizes (du Toit et al. 2004). Eventually, the diamond shaped chlorotic lesions coalesce leading to withering of the leaves and flowering stalks and, in severe cases, size and yield is reduced (Gent et al. 2006).

In Georgia, IYSV was first observed in 2003 in the onion seed beds of the Vidalia onion region (Mullis et al. 2004). In Georgia onions, primarily three thrips species, *Frankliniella fusca* (tobacco thrips), *Frankliniella occidentalis* (western flower thrips) and *Thrips tabaci* (onion thrips) occur (Riley and Sparks 2004, Sparks et al. 2005). *Frankliniella fusca* and *F. occidentalis* are the major vector species of TSWV in multiple cropping systems of the state (Riley and Pappu 2000, 2004).
Studies conducted to determine the transmissibility of IYSV by *F. occidentalis*, an efficient vector of TSWV and other tospoviruses, indicated that IYSV has only been transmitted by *T. tabaci* and not by *F. occidentalis* (Cortês et al. 1998, Kirtzman et al. 2001). *Thrips tabaci* is the only known vector of IYSV worldwide. Due to the presence of different vector species in onions in Georgia, the role of *T. tabaci* in vectoring IYSV in the Vidalia region needs to be further investigated. Under field conditions, IYSV has been reported infecting only two plant species in Georgia, onions (*Allium cepa*) (Mullis et al. 2004) and the weed species Spiny sow thistle (*Sonchus asper*) (Nischwitz et al. 2007), with no significant economic losses due to the virus. Based on recent surveys, there may be many more plant species infected with IYSV (Ron Gitaitis and Steve Mullis personal communication).

The tospovirus species known to occur in India to date, include *Groundnut bud necrosis virus* (GBNV) (Reddy et al. 1992, Satyanarayana et al. 1996), *Groundnut yellow spot virus* (GYSV) (Satyanarayana et al. 1998), *Watermelon bud necrosis virus* (WBNV) (Jain et al. 1998), *Iris yellow spot virus* (IYSV) (Ravi et al. 2006) and *Capsicum chlorosis virus* (CaCV) (Kunkalikar et al. 2007). GBNV and WBNV are the most economically important viruses affecting crops like peanut, tomato, potato and cucurbitaceous crops (Jain et al. 2007, Singh et al. 1997, Reddy et al. 1995, Singh and Srivastava 1995). The estimated annual losses for GBNV are over US $89 million (Reddy et al. 1995). For WBNV, 100% yield losses have been reported (Mandal et al. 2003, Jain et al. 2007). In India, IYSV occurs in onions in the Jalna and Nasik regions of Maharashtra, the major onion producing state in the country. Since the first report of the IYSV in 2006 (Ravi et al. 2006) economic losses have not been reported. The potential thrips vector species of tospoviruses reported in India include *Thrips palmi, Thrips tabaci, Thrips hawaiiensis, Scirtothrips dorsalis*, and *Frankliniella schultzei*. Although different thrips vector
species are reported in different vegetable cropping systems in India, only *T. tabaci* is reported to transmit IYSV in India.

*Thrips tabaci* reproduce sexually or parthenogenetically by oviparity in as little as two weeks resulting in many generations per year (Lewis 1973, Nault et al. 2006). The primary means of IYSV maintenance is through infected plants and viruliferous thrips. Like other tospoviruses (Ullman et al. 2002, Whitfield et al. 2005), it is thought that acquisition of IYSV is dependent on the oviposition of the female thrips, because only early instars can acquire the virus (Wijkamp et al. 1995) and larvae develop on the host on which they hatch. The infection cycle is initiated only when a female adult thrips lays eggs on a virus infected host plant that favors egg and larval development (Maris 2004). Recent studies conducted in Australia, provided information on where onion thrips choose to settle and oviposit in the field (Mo et al. 2008), but further studies are needed to determine the distribution of adults and oviposition sites on the onion plant since this could impact the distribution of virus acquisition and transmission to the onion plant. Multiple thrips species both vector and non-vector species, can occur on onions (Mound 1996, 2005, Riley and Batal 1998). Competition studies conducted with *F. occidentalis* and *F. tritici* indicated that thrips competition can result in competitive exclusion in the flowers of pepper, *Capsicum annuum* (Paini et al. 2008). Since multiple thrips vectors species are present in Southeast Georgia, studies for competitive exclusion of *T. tabaci* and other species is important to determine the efficiency of the vector species.
Objectives

This research project was conducted in India and in the USA focusing on *T. tabaci* as a major thrips vector species of IYSV in onions and other vegetable crops. To determine the status of thrips vector species, compared to other potential vector species of tospoviruses in the vegetable cropping systems in India, field research under the IPM-CRSP project was initiated in June, 2006. In India, research was conducted for two years by surveying and collecting potential thrips vector species in onions and other vegetable cropping systems. In Georgia, USA, onions were also surveyed for potential thrips vectors of IYSV. In addition, at the Coastal Plain Experiment Station, Tifton, GA, different biological parameters of *T. tabaci* in onions were investigated including competition, adult settling and thrips oviposition. Through this research project, the following objectives were addressed:

1) To survey, collect and taxonomically identify the potential thrips vector species infesting onions and other major vegetable crops in India and onions in GA, USA (Chapter 3).

2) To investigate competition between the two main thrips species in Georgia onions, *T. tabaci* (onion thrips) and *F. fusca* (tobacco thrips) (Chapter 4).

3) To determine the distribution of settling and oviposition of *T. tabaci* on onion foliage in controlled laboratory studies (Chapter 5).

4) To determine the within leaf distribution of *T. tabaci* eggs and *Iris yellow spot virus* (IYSV) on onion plants in the field (Chapter 6).

5) To develop a simple bioassay for conducting *T. tabaci* transmission studies of *Iris yellow spot virus* (IYSV) using onion seedlings in order to assess transmission efficiency (Chapter 7).
Hypothesis

The following hypotheses were addressed in this study:

1) *Thrips tabaci* is the main vector species of *Iris yellow spot virus* in onions in GA, USA and India.

2) *Thrips tabaci* is a better insect competitor than *Frankliniella fusca*.

3) Distribution of thrips is skewed towards the base of onion plants.

4) Distribution of IYSV is skewed towards the base of onion plants.

5) *Thrips tabaci* is a highly efficient vector of *Iris yellow spot virus*. 
References Cited


Phytopathology 94: 706-711.


Serological relationships and purification of bud necrosis virus, a tospovirus occurring in

necrosis virus disease: an overview. pp. 3-7. In: Buiel, A. A. M., Parlevliet, J. E. and
Lenne, J. M. [ed.], Recent Studies on Peanut Bud Necrosis Disease: Proceedings of a
Meeting. March 20, 1995, ICRISAT Asia Centre.

Research-Extension Report. University of Georgia, Cooperative Research-Extension

Tomato spotted wilt tospovirus in tomato. Plant Dis. 84: 847-852.

and Tomato spotted wilt virus in tomato. J. Econ. Entomol. 97: 648-1658.

viruses in vidalia onions. Georgia Onion Cooperative Research-Extension Publication
No. 3-2004. pp. 54-56.

Robène-Soustrade, I., Hostachy, B., Roux-Cuvelier, M., Minatchy, J., Hédont, M., Pallas, R.,
Couteau, A., Cassam, N., and Wuster, G. 2005. First report of Iris yellow spot virus in
onion bulb and seed production fields in Reunion Island. New Dis. Rep., 11,


CHAPTER 2

LITERATURE REVIEW

Tospoviruses

Tospoviruses are an unusual and economically important genus of plant viruses in the family Bunyaviridae that are distributed worldwide. One or more tospoviruses have been recorded from over 50 countries representing six continents (Mumford et al. 1996) and have become the subject of intense investigation in the last few decades. Based on the serological properties and amino acid sequence identity of the nucleocapsid protein (NP) gene, currently 18 Tospovirus species have been identified (Whitfield et al. 2005). The family Bunyaviridae includes animal-infecting viruses of the genera Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus, and the Tospovirus genus which is the only plant-infecting virus of the family (Whitfield et al. 2005). Members of the Tospoviruses genus are naturally transmitted by thrips in a circulative and propagative manner (Mound, 1996, Ullman et al. 1997, Jones 2005, Whitfield et al. 2005). The genus Tospovirus is derived from the type species, Tomato spotted wilt virus (TSWV). The disease tomato spotted wilt was first described in Australia in 1915 (Brittlebank 1919), and in 1930 it was determined that the causal agent was viral (Samuel et al. 1930).

Tospoviruses are quasi-spherical in shape (80-110 nm in diameter) with a characteristic lipid envelope. The membranous envelope consists of two viral coded surface glycolproteins G1 (78 kd) and G2 (58 kd) enclosing three nucleocapsids. The nucleocapsids contain three single stranded RNA molecules of varying size, small S RNA (2.9 kb), medium M RNA (~ 5 kb) and
large L RNA (8.9 kb) (German et al. 1992, Pappu et al. 2000) (Fig. 2.1). Each RNA segment is associated with several copies of virus encoded N (nucleocapsid) protein and few copies of RNA-dependant RNA polymerase (RdRp) (Mumford et al. 1996). The L RNA is negative sense encoding RdRp and, M RNA and S RNA are ambisense coding. M and S RNAs encode the two envelope glycoproteins and a nonstructural protein (NSm) and NSs and N protein. Infected plants and thrips have the NSm protein which is proposed as the possible virus movement protein in plants (Ullman et al. 1995, Soellick et al. 2000).

![Figure 2.1 Structure of Tospovirus particle](http://www.cals.ncsu.edu/plantpath/people/faculty/moyer/moyer_jw/posters/tswv/evolution_tswv.html)

**Figure 2.1 Structure of Tospovirus particle** (Source: NCSU Website).

Tospovirus species in USA

In the USA, three tospoviruses have been reported, including TSWV (Culbreath et al. 1991), *Impatiens necrotic spot virus* (INSV) (Daughtrey et al. 1997), and *Iris yellow spot virus* (IYSV) (Moyer et al. 2003). The host range for each tospovirus species varies but at least 848 plant species within 106 families have been reported (Csinos et al. 2009). TSWV and INSV are reported to be the most damaging plant viruses in the USA (Sherwood et al. 2001). In Georgia,
all the three viruses TSWV, INSV and IYSV are reportedly vectored by at least one of three thrips species *Frankliniella occidentalis* (Pergande), *Frankliniella fusca* (Hinds) and *Thrips tabaci*, Lindeman (Thysanoptera: Thripidae) that occur in vegetable crops, (Riley and Pappu 2000, 2004, Sparks et al. 2005).

TSWV is the most destructive tospovirus reported worldwide and is vectored by thrips in the genera *Frankliniella* and *Thrips* (Parrella et al. 2003, Peters, 2004). Pitman (1927) and Dickson (1929) described the first successful transmission of TSWV with *T. tabaci*. In 1930, *T. tabaci* was again shown to be a vector of TSWV (Samuel and Bald 1930). In Georgia, TSWV has become epidemic in peppers, tomatoes, peanuts, tobacco, vegetables and ornamentals (Sherwood 2009). TSWV was first reported in Georgia in 1986, but not considered a problem until 1989 (Culbreath et al. 1991). TSWV causes significant damage to major field crops in Georgia with estimated annual losses of $12.3 million in peanut, $11.3 million in tobacco and $9 million in tomato and pepper for a 10-year total of $326 million (Williams-Woodward J. L. 1998, 1999, 2000, 2001, 2002, 2003, Martinez, 2005, 2006, 2007).

A serotype of TSWV was reported in 1989 in *Impatiens* sp. which was initially designated as TSWV-1. However, based on the amino acid sequence similarities between nucleocapsid protein (NP) genes of the members in the tospovirus genus, TSWV-1 was designated as INSV (Law and Moyer 1990). INSV is most commonly associated with ornamental and green house production crops (Daughtrey et al. 1997). INSV was first detected in 1998 on peanut crops from Georgia and Texas (Pappu et al. 1999). Recently, the virus was detected in tobacco (Martinez-Ochoa et al. 2003), two weed species, yellow and purple nut sedge, (Martinez-Ochoa et al. 2004) and sweet pepper (Naidu et al. 2005). The two weed species are a serious problem in Georgia and incidence of the virus in these weeds might be a source for
spreading the virus. The distribution of INSV has increased in the state, but the impact of the virus has not been documented.

The focus of this dissertation is IYSV. IYSV was first reported in 1998 from the Netherlands as a new tospovirus naturally infecting iris and leek (Cortês et al. 1998). Later, the virus was identified infecting onion, in major onion growing regions worldwide (Smith et al. 2006). Based on the recent accounts, IYSV is reported in 6 continents in 24 countries (CABI/EPPO 2009) and the cause for the sudden widespread distribution of IYSV in onion bulb crops is not known. Currently, IYSV infects at least 47 plant species worldwide (Gent et al. 2006) under field conditions including onions (Gera et al. 1998, Pozzer et al. 1999), garlic (Robène-Soustrade, 2005) and ornamental crops such as lisianthus (Kirtzman et al. 2001), alstroemeria (Okuda et al. 2001) and certain weed species (Pappu et al. 2006, Sampangi et al. 2007, Nischwitz et al. 2007). Currently, only one vector species *Thrips tabaci* (onion thrips) Lindeman is confirmed to be the vector of IYSV (Nagata et al. 1999, Kitzman et al. 2001).

Economic damage due to IYSV in onion is variable, but it has been reported to cause up to 100 % loss in 1999 (Pozzer et al. 1999) and 50-60 % losses in onion bulb production (Kirtzman et al. 2001). In Colorado in 2003, IYSV spread rapidly as an epidemic in onions with an estimated $50 million annual loss (Gent et al. 2006). Subsequently in recent years, the estimated economic loss due to outbreaks of IYSV in bulb and seed onion crops in Oregon, Idaho and Washington were reported to be $480,000 (Pappu et al. 2009). In Georgia, Vidalia sweet onions are a high value specialty crop cultivated as a short day winter crop generating an estimated farm gate value of $139 million annually (Boatright and McKissick, 2009). IYSV was first observed in Vidalia onion growing region of the state in 2003 (Mullis et al. 2004). Since the report of IYSV in 2003, no economic losses have been reported, however the virus has been found every year in all of the
onion growing regions in the state (Nischwitz et al. 2007). Under field conditions, IYSV has been reported infecting only two plant species in Georgia, onions (*Allium cepa*) (Mullis et al. 2004) and the weed species Spiny sow thistle (*Sonchus asper*) (Nischwitz et al. 2007). Based on recent surveys, there may be many more plant species infected with IYSV (Ron Gitaitis and Steve Mullis personal communication).

**Tospovirus species in India**

Tospoviruses are emerging as widespread and devastating pathogens in the Indian subcontinent affecting vegetables and other economically cultivated crops (Varma et al. 2002). Based on the nucleocapsid protein (NP) gene characteristics, currently, five tospoviruses have been reported: *Groundnut bud necrosis virus* (GBNV) (Reddy et al. 1992, Satyanarayana et al. 1996), *Watermelon bud necrosis virus* (WBNV) (Jain et al. 1998), *Groundnut yellow spot virus* (GYSV) (Satyanarayana et al. 1998), IYSV (Ravi et al. 2006) and *Capsicum chlorosis virus* (CaCV) (Kunkalikar et al. 2007).

*Groundnut/Peanut bud necrosis virus* (GBNV/PBNV) was first described as a disease causing necrosis in peanut in 1968 (Reddy et al. 1968), causing up to 80% yield losses (Ghanekar et al. 1979). Subsequently, the disease was serologically tested and named as Groundnut /Peanut bud necrosis virus (Reddy et al. 1992). In recent years, GBNV has been reported infecting crops such as soybean (Bhat et al. 2002), mung bean (Thien et al. 2003), tomato (Umamaheswaran et al. 2003) and potato (Jain et al. 2004). The estimated annual losses for GBNV were reported to be over US $89 million (Reddy et al. 1995).

*Watermelon bud necrosis virus* (WBNV) was first described as a virus infecting watermelons in 1991-92 from the states of Karnataka, Andhra Pradesh and Maharashtra, India with the virus causing 39-100% disease incidence and an estimated 60-100% yield loss
Based on the host range, symptoms and serological testing, the casual agent was identified as a new tospovirus and named as WBNV (Singh and Krishnareddy 1996). WBNV has been reported infecting watermelon, *Citrullus lanatus* Thunb. (Singh and Krishnareddy 1996), *Luffa acutangula* (ridge gourd) (Mandal et al. 2003), three cucurbits and three fabaceous crops (cowpea, frenchbeans and sem) (Jain et al. 2007) with estimated up to 100% yield losses. GBNV and WBNV are the most economically important viruses reported affecting crops like peanut, tomato, potato and cucurbitaceous crops (Singh and Srivastava 1995, Reddy et al. 1995, Singh et al. 1997, Jain et al. 2007). Based on the NP gene, GBNV and WBNV are currently assigned to the *Watermelon silver mottle virus* (WSMoV) serogroup (Fauquet et al. 2005).

*Groundnut/Peanut yellow spot virus* (GYSV/ PYSV) was first reported in peanut and was described as a disease that causes necrosis due to chlorotic/yellow leaf spots infecting 90% of the fields (Anon 1980). The disease was again reported in 1991 as a distinct tospovirus causing systemic infections in peas, mung bean and cow pea (Reddy et al. 1991), and subsequently the disease was serologically tested and confirmed as GYSV (Satyanarayana et al. 1998).

IYSV has been reported in onions in Jalna and Nasik regions of Maharashtra, the major onion producing state in the country, since the first report of virus in 2006 (Ravi et al. 2006) no economic losses have been reported. Recently, *Capsicum chlorosis virus* (CaCV) has been reported infecting tomato (Kunkalikar et al. 2007) and chili pepper (Krishnareddy et al. 2008).

**Thrips vectors of Tospoviruses**

Thrips (Thysanoptera) affect plants by directly feeding and indirectly by transmission of viruses (Mound 2005). Of the ~5500 thrips species described, only few species are efficient
vectors feeding on broad range of crops (Ullman et al. 2002). Only six species of *Frankliniella*,
three species of *Thrips*, and one species of *Scirtothrips* and *Ceratothripoides* are known to be
vectors of tospoviruses (Jones 2005). In addition to the thrips vector species listed by Jones
(2005), there are some additional thrips vectors of tospoviruses reported by Pappu et al. (2009)
(Table 2.1).

Table 2.1 Thrips species in the genera *Frankliniella*, *Thrips*, *Scirtothrips* and
*Ceratothripoides* and associated tospoviruses (Compiled from Persley et al. 2006, Pappu et al.
2009).

<table>
<thead>
<tr>
<th>Thrips species</th>
<th>Tospovirus transmitted</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Frankliniella occidentalis</em></td>
<td><em>Tomato spotted wilt virus</em> (TSWV)</td>
<td>Sakimura 1962</td>
</tr>
<tr>
<td></td>
<td><em>Impatiens necrotic spot virus</em> (INSV)</td>
<td>de Angelis et al. 1993</td>
</tr>
<tr>
<td></td>
<td><em>Groundnut ring spot virus</em> (GRSV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Tomato chlorotic spot virus</em> (TCSV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Chrysanthemum stem necrosis virus</em> (CSNV)</td>
<td>Bezzara et al. 1999</td>
</tr>
<tr>
<td><em>Frankliniella fusca</em></td>
<td><em>Tomato spotted wilt virus</em> (TSWV)</td>
<td>Sakimura 1963</td>
</tr>
<tr>
<td></td>
<td><em>Impatiens necrotic spot virus</em> (INSV)</td>
<td>Naidu et al. 2001</td>
</tr>
<tr>
<td><em>Frankliniella bispinosa</em></td>
<td><em>Tomato spotted wilt virus</em> (TSWV)</td>
<td>Webb et al. 1998</td>
</tr>
<tr>
<td><em>Frankliniella intonsa</em></td>
<td><em>Tomato spotted wilt virus</em> (TSWV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Tomato chlorotic spot virus</em> (TCSV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Impatiens necrotic spot virus</em> (INSV)</td>
<td>Sakurai et al. 2004</td>
</tr>
<tr>
<td><em>Frankliniella schultzei</em></td>
<td><em>Tomato spotted wilt virus</em> (TSWV)</td>
<td>Samuel et al. 1930</td>
</tr>
<tr>
<td></td>
<td><em>Groundnut bud necrosis virus</em> (GBNV)</td>
<td>Lakshmi et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Groundnut ring spot virus</em> (GRSV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Tomato chlorotic spot virus</em> (TCSV)</td>
<td>Wijkamp et al. 1995</td>
</tr>
<tr>
<td></td>
<td><em>Chrysanthemum stem necrosis virus</em> (CSNV)</td>
<td>Bezzara et al. 1999</td>
</tr>
<tr>
<td><em>Frankliniella zucchini</em></td>
<td><em>Zucchini lethal chlorosis virus</em> (ZLCV)</td>
<td>Nakahara and Monteiro 1999</td>
</tr>
<tr>
<td>Thrips species</td>
<td>Virus species and Abbreviations</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><em>Frankliniella cephalica</em></td>
<td>Tomato spotted wilt virus (TSWV)</td>
<td>Ohnishi et al. 2006</td>
</tr>
<tr>
<td><em>Frankliniella gemina</em></td>
<td>Groundnut ring spot virus (GRSV)</td>
<td>de Bourbon et al. 2006</td>
</tr>
<tr>
<td>Thrips palmi</td>
<td>Groundnut bud necrosis virus (GBNV)</td>
<td>Vijayalakshmi 1994</td>
</tr>
<tr>
<td></td>
<td>Water melon bud necrosis virus</td>
<td>Singh and Krishnareddy 1995</td>
</tr>
<tr>
<td></td>
<td>(WBNV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water melon silver mottle virus</td>
<td>Chen et al. 1995</td>
</tr>
<tr>
<td></td>
<td>(WSMoV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tomato spotted wilt virus (TSWV)</td>
<td>Fujisawa et al. 1988</td>
</tr>
<tr>
<td></td>
<td>Melon yellow spot virus (MYSV)</td>
<td>Kato et al. 1999</td>
</tr>
<tr>
<td></td>
<td>Capsicum chlorosis virus (CaCV)</td>
<td>Persley et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Calla lily chlorotic spot virus</td>
<td>Chen et al. 2005</td>
</tr>
<tr>
<td></td>
<td>(CCSV)</td>
<td></td>
</tr>
<tr>
<td>Thrips tabaci</td>
<td>Iris yellow spot virus (IYSV)</td>
<td>Nagata et al. 1999</td>
</tr>
<tr>
<td></td>
<td>Tomato spotted wilt virus (TSWV)</td>
<td>Pittman 1927</td>
</tr>
<tr>
<td></td>
<td>Tomato yellow fruit ring virus (TYFRV)</td>
<td>Ghotbi et al. 2005</td>
</tr>
<tr>
<td>Thrips setosus</td>
<td>Tomato spotted wilt virus (TSWV)</td>
<td>Fujisawa et al. 1988</td>
</tr>
<tr>
<td>Scirtothrips dorsalis</td>
<td>Capsicum chlorosis virus (CaCV)</td>
<td>Chiemomsombat et al. 2008</td>
</tr>
<tr>
<td></td>
<td>Groundnut chlorotic fanspot virus</td>
<td>Chen and Chiu 1996</td>
</tr>
<tr>
<td></td>
<td>(GCFV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Groundnut bud necrosis virus (GBNV)</td>
<td>Amin et al.1981</td>
</tr>
<tr>
<td></td>
<td>Groundnut yellow spot virus (GYSV)</td>
<td>Reddy et al. 1991</td>
</tr>
<tr>
<td>Ceratothripoides claratris</td>
<td>Capsicum chlorosis virus (CaCV)</td>
<td>Premchandra et al. 2005</td>
</tr>
</tbody>
</table>

*The thrips species reported as additional vectors transmitting tospoviruses (Pappu et al. 2009)*
Thrips vectors in USA

*Frankliniella* and *Thrips* (Thripidae), are the two genera of thrips that include vectors capable of transmitting TSWV, INSV and IYSV in the USA. Five vectors in the genus *Frankliniella*, (*Frankliniella occidentalis* (Pergande), *F. fusca* (Hinds), *F. schultzei* (Trybom), *F. intonsa* (Trybom), and *F. bispinosa* (Morgan)) and one vector species in the genus *Thrips* (*Thrips tabaci* (Lindeman)) are confirmed as vectors of TSWV in different crops (Ullman et al. 2002). INSV is primarily vectored by *F. occidentalis* (de Angelis et al. 1993) and IYSV is exclusively vectored by *T. tabaci* (Nagata et al. 1999).

*Thrips tabaci* was the first reported vector species of TSWV (Pittman 1927). Later, *F. schultzei* was also reported as a vector of the virus (Samuel et al. 1930). In southeastern USA, *F. occidentalis*, *F. fusca* and *F. bispinosa* are reported as the primary thrips species associated with TSWV epidemics (Riley and Pappu 2000, Salguero et al. 1991, Todd et al. 1996 and Webb et al. 1997). In Georgia, *F. fusca* is associated with TSWV incidence in peanut (Culbreath et al. 1991) and tobacco (McPherson et al. 1999) while *F. occidentalis* is associated with TSWV incidence in tomato (Riley and Pappu 2000). Even though, *F. bispinosa* transmits TSWV (Webb et al. 1997) its importance in vectoring the virus is not known.

For many years, *F. occidentalis* has been reported as the only vector transmitting INSV primarily on ornamentals and green house crops (de Angelis et al. 1993). Later the virus has been reported transmitted by other thrips species (Naidu et al. 2001). In Georgia, two vectors species *F. occidentalis* (Daughtrey et al. 1997) and *F. fusca* (Naidu et al. 2001) are reported as the vectors transmitting INSV. In Georgia, both vectors are reported infecting economically important crops such as peppers (Naidu et al. 2005), peanut and tobacco (Martý´nez-Ochoa et al.
2003) and two common weed species, yellow and purple nutsedge (Martínez-Ochoa et al. 2004) in Southeast USA.

*Thrips tabaci* is the only vector species reported transmitting IYSV (Nagata et al. 1999). In Georgia, concurrent with the detection of IYSV in 2003 (Mullis et al. 2004), *T. tabaci* was found in onions along with other vectors, *F. occidentalis* and *F. fusca* (Riley and Sparks 2004, Sparks et al. 2005). *Frankliniella occidentalis* is reported as a nonvectoring species (Nagata et al. 1999, Kitzman et al. 2001) and the ability of *F. fusca* to transmit IYSV is only recently being investigated (Srinivasan personal communication). The vector species, *T. tabaci*, and its role in vectoring IYSV in the Vidalia onion region has much to be investigated.

**Thrips vectors in India**

*Frankliniella, Thrips* and *Scirtothrips* are the three genera that include most of the thrips vector species transmitting tospoviruses. The vector species *F. schultzei* (Lakshmi et al. 1995) and *T. palmi* (Vijayalakshmi 1994) were reported transmitting GBNV/PBNV in peanut. The thrips vector *S. dorsalis* was reported vectoring GYSV/PYSV (Reddy et al. 1991) in peanut. *T. flavus* was reported as a vector for WBNV (Sigh and Krishna Reddy 1995), but it was assumed that this species might be mistaken for *T. palmi* (Mound 1996), an important thrips vector for most of the tospoviruses reported in India. A recent study from Karnataka state reported *T. palmi* as a vector of *Sunflower necrosis virus* (SNV) a new tospovirus on sunflower, an important oilseed crop in India (Lokesh et al. 2008). There are other tospovirus species such as WBNV, GYSV and IYSV reported on several cucurbitaceous, fabaceous and other economically important crops, but information is not available on the confirmed thrips vectors that transmit these tospoviruses in those crops.
**Thrips Biology**

Thrips are minute (1-2 mm), slender, primarily phytophagous insects that affect plants by directly feeding and indirectly by transmitting viruses (Mound 2005). Feeding by adults and larvae on leaves results in the formation of silvery patches that turn brown as the tissues dry up beneath the epidermis and ultimately induces premature leaf fall (Ananthakrishnan 1971). Thrips are closely related to other members of the Hemipteroid assemblage (Psocoptera, Phthiraptera and Hemiptera) most of which have modified sucking mouthparts. Thrips have asymmetrical mouthparts which consist of two maxillary stylets and one mandibular stylet surrounded by a mouth cone forming a feeding apparatus (Cranston and Gullan 2003). Nine families are currently distinguished with 95% of the known species in Thripidae and Phlaeothripidae. The thrips vectors of tospoviruses are all members of the family Thripidae (Mound 1996, 1997). Most thrips vector species complete their life cycle from egg to adult in 2-3 weeks (Fig. 2.2), but the time varies with the host, temperature and humidity (Ananthakrishnan 1993). Temperature is the main factor that determines the rate at which thrips complete their life cycle. Examples of developmental degree days (dd) for specific vector species include: *F. fusca* on peanut which requires 234.1dd at a base temperature of 10.5 ºC, *F. occidentalis* on green beans which requires 253.9 dd at a base temperature of 6.5 ºC (Lowry et al. 1992), and *T. tabaci* on onion which requires 191.1(dd) at a base temperature of 11.5 ºC (Edelson and Magaro 1988). Females lay eggs singly, inserting them into leaf or plant tissue using a saw like ovipositor. Eggs usually hatch in 2-3 days. The insect undergoes two wingless larval stages in 7-10 days, feeding on leaves and flowering structures. The second larval stage stops feeding and undergoes a pre-pupal and pupal stage in the soil or leaf litter. Pupae emerge as winged/wingless adults and disperse widely (Terry 1997).
Reproduction in thrips can occur by both sexual and parthenogenetic methods. In parthenogenesis, thrips can reproduce by different methods such as thelytoky, where females are produced from unfertilized eggs, arrhenotoky, where males are produced from unfertilized eggs and females are produced from fertilized eggs and deuterotoky, where females and males produced from unfertilized eggs (Ananthakrishnan 1993, Mound 1996). All phytophagous thrips species reproduce primarily by thelytoky and arrhenotoky. In suborder Terebrantia, females lay slightly kidney shaped eggs with smooth, pale white or yellow shells within incisions made in plant tissue by the ovipositor. Eggs are usually laid on the underside of leaves. Oviposition and reproduction success in thrips is strongly influenced by the nature of the habitat and its
nutritional quality. Oviposition is influenced by the nutritional quality of the food, while egg laying is influenced by temperature and humidity. A female thrips can oviposit 30-60 eggs depending on its nutrition (Sakimura 1937, Watts 1936). Studies conducted by Riley et al. (2007), found that the addition of a nutritionally rich diet like pollen can significantly increase oviposition in western flower thrips (on tomato) and tobacco thrips (on peanut). The duration of development, size and fecundity of adults are determined partly by the nutritive value of food eaten by larvae (Lewis 1973).

**Thrips transmission of tospoviruses**

*Thrips tabaci* was reported as the first thrips vector species of tospoviruses in 1930, transmitting TSWV (Samuel et al. 1930). The biological transmission of tospoviruses involves a specific relationship between the virus and its vector. The transmission of tospoviruses by thrips is closely related with the feeding habits and structure of the mouthparts. Thrips have asymmetrical mouthparts that consist of two maxillary stylets and one mandibular stylet surrounded by a mouth cone forming a feeding apparatus (Cranston and Gullan 2003). During the feeding process, a thrips punctures the plant cells with its mandible and the cell contents are emptied by ingesting through a feeding tube formed by the maxillary stylets (Hunter and Ullman 1989). The close link between thrips development and tospovirus epidemiology is basic to understand the tospovirus infection cycle (Fig. 2.3). The infection cycle for the thrips is initiated only when female adult thrips lays eggs on the virus infected host plants that favor egg and larval development. The female adults select and oviposit on host plants on which larvae can develop and acquire the virus (Ullman et al. 1992a). Eggs are laid inside the plant tissue with a saw-like ovipositor that hatch within 2-3 days and emerge as wingless larvae.
Figure 2.3 Tospovirus transmission cycle. (Source: Whitfield et al. 2005).

Virus acquisition occurs only during the first and second larval stages. The virus once acquired, multiplies in the larvae and survives by transtodially passing through the later developmental stages (Mumford et al. 1996, Whitfield et al. 2005). The emerging adults are winged and only those that acquired the virus during larval stages can transmit tospoviruses to their progeny (Ullman et al. 1997, Moritz et al. 2004, Whitfield et al. 2005). Acquisition of tospovirus is dependent on oviposition by female thrips, since only early instars can acquire the virus and larvae develop on the host on which they hatch (Maris et al. 2004). Thrips feeding on a suitable host plant significantly influences the virus acquisition and transmission (German et al. 1992). Riley et al. (2007) demonstrated that the addition of pollen to the leaf surface of virus
infected-host plants can significantly increase the number of viruliferous thrips emerging. The efficiency of acquisition is greatly influenced by the feeding preferences of the thrips and the viral distribution within the plant material on which the thrips feed. When a plant is sensed as a suitable host, thrips larvae ingest larger quantities of cytoplasm than when a plant is less favorable (Broadbent et al. 1990). Increased ingestion of plant fluids may increase the number of virions entering the midgut and thus enhance the cellular kinetics that govern successful acquisition of virus (Cho et al. 1991, Ullman et al. 1991). Virus acquisition by feeding larvae can occur in about 30 minutes (Razvyzkina 1953, Wijkamp and Peters 1993). The efficiency of virus acquisition is also influenced by age of the larvae, where an increase in the age of the larvae results in decreasing their ability to acquire the virus (Van de wetering et al. 1996). Tospovirus, once acquired, can be retained in thrips through molting, pupation, and adult stage (Sakimura 1962).

Transmission of tospoviruses is primarily done by adult thrips that feed actively and disperse widely. Once a viruliferous thrips enters adulthood its potential to infect plants is very high and a single viruliferous adult can infect by feeding on different plants in its lifetime (Mau et al. 1991, Ullman et al. 1992b).


Website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-08/SB41-08.html


Website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-09/SB41-09.html

website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-10/SB41-10.html


Mullis, S. W., Langston, D. B., Jr., Gitaitis, R. D., Sherwood, J. L., and Csinos, A. C. 2004. First report of Vidalia onion (\textit{Allium cepa}) naturally infected with \textit{Tomato spotted wilt virus}
and Iris yellow spot virus (family Bunyaviridae, genus Tospovirus) in Georgia. Plant Dis. 88: 1285.


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-01/SB41-01.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-02/SB41-02.html
website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-03/SB41-03.html

website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-04/SB41-04.html

website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-05/SB41-05.html

website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-06/SB41-06.html

CHAPTER 3

SURVEY OF THRIPS VECTORS OF TOSPOVIRUS IN ONIONS AND OTHER VEGETABLE CROPS

1 Chitturi, A., Riley, D.G. and Diffie, S. To be submitted to *Environmental Entomology*
Abstract

Thrips (Thysanoptera: Thripidae) sampling was conducted in India for two years by surveying the common thrips vectors infesting major vegetable crops, onion, tomato, and chili peppers. Also, thrips on onions were sampled for two years in Georgia, USA to identify thrips species. In India the first year survey was done in three states Andhra Pradesh, Maharashtra and Karnataka and the second year survey was concentrated only in the states of Andhra Pradesh and Maharashtra. Different thrips vector species infesting the vegetable crops were collected mainly by the beat cup method for sampling plant foliage and blossoms. Five potential tospovirus vector species of thrips were identified in the survey and the tospovirus occurrence was listed for each of the states by crop to suggest possible associations. *Iris yellow spot virus* (IYSV), first reported from onions in the Maharashtra state in India, was only associated with *Thrips tabaci* in this survey. *Thrips tabaci* were collected only from onions in all the states surveyed. The distribution of thrips genera across the crops surveyed showed a dominance of *Thrips* sp. in onion, *Scirtothrips* sp. in chili pepper and *Frankliniella* sp. in tomato. In Georgia, this survey identified the onion thrips, *T. tabaci*, as the main vector species of IYSV, in onion. *Frankliniella fusca* was also found as a prevalent pest of onions in Georgia.

**Keywords:** survey, sampling, thrips vectors, tospoviruses, *Iris yellow spot virus, Thrips tabaci, Scirtothrips dorsalis, Frankliniella schultzei, Thrips palmi*, chili peppers, tomatoes, onions
Introduction

Tospoviruses are emerging as widespread and devastating pathogens in the Indian-sub continent affecting vegetables and other economically cultivated crops (Varma et al. 2002). Vegetable production in South-Central India is diverse (Parthasarathy Rao et al. 2004) and includes many crops that are affected by thrips-vectored tospoviruses (Pappu et al. 2009). Based on nucleocapsid protein (NP) gene characteristics, in India there are currently five tospoviruses that have been reported: (1) *Groundnut/Peanut bud necrosis virus* (GBNV/PBNV) (Reddy et al. 1992, Satyanarayana et al. 1996) in soybean (Bhat et al. 2002), mung bean (Thien et al. 2003), tomato (Umamaheswaran et al. 2003) and potato (Jain et al. 2004), (2) *Watermelon bud necrosis virus* (WBNV) (Jain et al. 1998), in watermelon (Singh and Krishnareddy 1996), *Luffa acutangula* (ridge gourd) (Mandal et al. 2003), three cucurbits and three fabaceous (cowpea, frenchbeans and sem) (Jain et al. 2007), (3) *Groundnut/Peanut yellow spot virus* (GYSV/PYSV) (Satyanarayana et al. 1998), in peas, mung bean and cowpea (Reddy et al. 1991), (4) *Iris yellow spot virus* (IYSV) (Ravi et al. 2006) in onion and (5) *Capsicum chlorosis virus* (CaCV), in tomato (Kunkalikar et al. 2007) and chili pepper (Krishnareddy et al. 2008). Based on known thrips-virus associations (Pappu et al. 2009), potential thrips vector species can be identified through surveys that would explain the prevalence of these tospoviruses in India.

The thrips vectors of tospovirus that were known to occur in vegetables in India long before the current survey was initiated are *Scirtothrips dorsalis* and *Thrips tabaci* (Ananthakrishnan 1980, 1984). Both *S. dorsalis* (Ghanekar et al. 1979, Gahukar 1999, Duraimurgan and Jagadish 2002) *T. tabaci* (Mahal et al. 1992, Gupta et al. 1994, Kadri and Goud 2005) and *Frankliniella schultzei* (Kumar et al. 2004 a, b, Jagdeeshwar et al. 2005) have been reported in more recent surveys. More recently, *Thrips palmi* (Kendre et al. 2000) and *Thrips hawaiensis* (Patil et al. 1997, Jhala et al. 2004) have been recognized as tospovirus
vectors in India. Two other potential thrips tospovirus vectors that occur in vegetables in Asia are *Ceratothripoides claratris* and *Frankliniella cephalica* (Pappu et al. 2009). The thrips tospovirus vectors that have been reported in Georgia include *F. occidentalis, F. fusca, F. bispinosa* (Chamberlin et al. 1992, McPherson et al. 1999, Webb et al. 1997) and *T. tabaci* (Sparks and Riley 2007). Considering the diversity of the thrips vectors and their ability to transmit new tospoviruses, it is important to investigate the thrips vectors that occur specifically in vegetable cropping systems. Hence, the main objective of this study is to survey and identify potential thrips vectors species and determine which thrips species are likely vectors of tospovirus in tomato, pepper and onions in various locations in India, and in onions in two locations in Georgia, USA.

**Materials and Methods**

The thrips vector survey in India concentrated initially on three vegetable growing locations, namely Andhra Pradesh, Maharashtra and Karnataka (Fig. 3.1). During the initial survey in June, 2006, crops that were suspected of having tospovirus infections were collected from the three states (Table 3.1) using three sampling techniques: the beat cup method for plant foliage, blossom samples, and sticky traps. Of all three sampling techniques used, the beat cup was deemed the best method for collecting the thrips samples, therefore it was used throughout the entire survey.

The beat cup method for sampling thrips was described by Joost and Riley (2003). The selected plant is bent into a 946 ml (11.5 cm in diameter, 16.5 cm in depth) Styrofoam cup (Dart Container, Corp., Mason, MI) and shaken vigorously for 5 seconds. Plants are then removed and the thrips inside the cup are collected and counted. Static electricity holds the thrips against the inside of the cup so that they can be easily collected with a small damp, paint brush and placed
directly into alcohol. Compared to other collection methods, the beat cup is the most efficient for thrips in young plants (Joost and Riley 2004).

For season-long sampling of thrips in Georgia, both whole plant inspection and leaf top samples were used. Whole plant inspection involves counting dark and light colored adults and immature thrips for 10 plant samples, replicated in four blocks per sample date. The leaf top samples consist of 5 plant tops clipped at the soil surface, Ziplock bagged, and then frozen to kill thrips. The leaf top samples were taken from 4 replicates per sample date. The leaf tops were then thawed and inspected under 10X magnification and all thrips were collected into 70% EtOH. Thrips were identified to species using taxonomic keys (Moritz et al. 2004).

In India two different fluids were used for collection and preservation of thrips samples during summer and fall seasons. Initially during summer, thrips were collected into AGA, a mixture of 10 parts of 60% ethyl alcohol with 1 part glycerin and 1 part of acetic acid. In the fall, thrips were collected in 60% EtOH. After 2 weeks thrips collected in AGA solution were transferred to 60% EtOH and stored. Thrips collected in AGA solution were the best for identification as this mixture helped to distend the body and keep the body parts supple, which facilitated the mounting process (Palmer 1989). All the specimens collected in India were brought back to the USA and sorted to the genus level by using the morphological identification key developed by Moritz (2004), and preserved in 60% EtOH for slide mounting. Before slide mounting, maceration was used to remove the body contents as recommended by Moritz (2004). Then dehydration was used to remove water and render the specimens translucent with clove oil. In the dehydration process alcohols and clove oil were used to absorb water, a particularly useful technique in warm humid conditions. Different percentages of alcohol were used as indicated by Moritz (2004). For identification to species, slides were viewed under a compound microscope at
100X magnification and identified using the key morphological characteristics suggested by Moritz (2004).

Results and Discussion

There were five potential tospovirus vector species of thrips identified in the survey in India in three states (Fig. 3.2a, b, c, d, and e). Potential tospovirus occurrence was also listed for each of the states by crop to suggest possible associations (Table 3.2). One clear association from this survey was that *T. tabaci* were collected only from onions and IYSV was only reported to occur in onions in India. *Thrips palmi* has been reported associated with both tospoviruses on solanaceous crops in India, GBNV=PBNV and CaCV and was found in both chili pepper (Krishnareddy et al. 2008) and tomato (Kunkalikar et al. 2007). Thus, this thrips species is likely one that is important in these two cropping systems and perhaps other vegetable crops as well in India. *Scirtothrips dorsalis* has been associated with GBNV=PBNV, an important tospovirus in peanut, tomato and soybean (Pappu et al 2009). *Frankliniella schultzei* has not been associated with tospoviruses in India, but its status as a vector of at least six tospoviruses worldwide makes it a potential candidate for vectoring the tospoviruses (Pappu et al. 2009) in addition to *Chrysanthemum stem necrosis virus* (Bezzara et al. 1999), *Groundnut ring spot virus* (Wijkamp et al. 1995), *Groundnut bud necrosis virus* (Lakshmi et al. 1995), *Tomato chlorotic spot virus* (Wijkamp et al. 1995) and *Tomato spotted wilt virus* (Samuel et al. 1930) which have been reported vectored by *F. schultzei* (Jones 2005, Pappu et al. 2009). There was some sampling bias associated with this survey which is important to identify. Specifically, the distance between the three states and practical difficulty to travel all three states, thus, later thrips sample collections concentrated much of the sampling in Andhra Pradesh where the bulk of the thrips specimens were collected in the fall of 2006. The second year thrips survey for spring 2008 in India was
concentrated at two vegetable growing locations, namely, Maharashtra (onions) and Andhra Pradesh (chili pepper, onions and tomatoes). Based on this limited sampling, the months with the most thrips collected over all locations were November through January (Fig. 3.3). During this survey, six other host crops were sampled, namely watermelon in Maharashtra, and okra, eggplant, parthenium (weeds), chrysanthemum, and marigold in Andhra Pradesh. The thrips genera found in these other host plants were: *Thrips* sp. in watermelon, chrysanthemum, and marigold, and *Frankliniella* sp. in okra, eggplant, parthenium, chrysanthemum, and marigold. The distribution of genera across the main crops in the survey showed a dominance of *Thrips* sp. in onion, *Scirtothrips* sp. in chili pepper and *Frankliniella* sp. in tomato (Fig. 3.4). The proportion of thrips genera in onions was consistent between states, i.e., predominantly *Thrips* sp. (Fig. 3.5), however, due to different intensity of sampling done in each state there was less consistency with tomato (Fig. 3.6) and chili pepper (Fig. 3.7) across states.

In Georgia, this survey identified onion thrips, *T. tabaci* and tobacco thrips, *F. fusca* as the main thrips species in the whole plant and leaf top samples in onion at both the locations. In Georgia, dark adult thrips in onion foliage were almost exclusively *F. fusca*. In comparison Riley and Batal (1998) reported 45% *F. fusca*, 23% *F. occidentalis* and 32% immatures on onion tops. Thrips were sampled during late January, mid February, early, mid and late March, April and early May. The mean number of thrips sampled over all dates indicated that the thrips populations were significantly higher during late April and early May (Fig. 3.8). The total number of thrips sampled at both the locations revealed *T. tabaci* as the dominant thrips species. *Frankliniella fusca*, a prevalent thrips species in onions also showed a stronger distribution over both the sampling locations (Fig. 3.9). Overall, the number of thrips sampled over all the dates was dominantly *T. tabaci* which is the only thrips species reported vectoring *Iris yellow spot*
virus in GA (Mullis et al. 2004). The VORC site is located in the main onion producing region in GA and the presence of *T. tabaci* as a main thrips species suggests it as an important vector of IYSV in the area. *Frankliniella fusca* was also found in significant numbers on onion plants during the survey. However, *F. fusca* has mainly been associated with *Tomato spotted wilt virus* (TSWV) in multiple cropping systems of the state (Salguero et al. 1991, Todd et al. 1996, Riley and Pappu 2000), not IYSV. In this survey other thrips species (e.g., *F. occidentalis* and *F. tritici*) were also identified, but were present in very low numbers. This survey suggests that *T. tabaci* is the main vector species of IYSV in onion crops in Georgia. *Frankliniella fusca* was also found as a prevalent pest of onions in Georgia. Since different thrips species were present in onions in Georgia, the role of *T. tabaci* in vectoring IYSV needs to be further investigated in the presence of competing thrips species.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the support of both the IPM CRSP pilot study program and that of the University of Georgia, Department of Entomology who’s funding made this project possible. We acknowledge the support of Maharashtra Hybrid Seed Company, Dawalwadi, Jalna, India which facilitated some of the thrips collections in Maharashtra. We would like to acknowledge Professor, taxonomist Dr. Rama Subba Rao, Research Associates, Ms. Diana Grace and Mr.Venkateswarlu, Acharya N. G. Ranga Agriculture University, Hyderabad, India for their support and help with thrips collections in Andhra Pradesh.
References Cited


and *Iris yellow spot virus* (family *Bunyaviridae*, genus *Tospovirus*) in Georgia. Plant Dis. 88: 1285.


Table 3.1 Sample locations in the two year thrips survey and sampling by State, District and Crop in India.

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>District</th>
<th>No. of villages</th>
<th>Crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006-2007*</td>
<td>Andhra Pradesh</td>
<td>Krishna</td>
<td>2</td>
<td>Onion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guntur</td>
<td>2</td>
<td>Chili pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rangareddy</td>
<td>3</td>
<td>Chili pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chittoor</td>
<td>1</td>
<td>Tomato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prakasam</td>
<td>2</td>
<td>Tomato</td>
</tr>
<tr>
<td></td>
<td>Maharashtra</td>
<td>Ahmednagar</td>
<td>4</td>
<td>Chili pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nasik</td>
<td>6</td>
<td>Onion, Chili pepper, Tomato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pune</td>
<td>6</td>
<td>Chili pepper, Tomato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Akola</td>
<td>2</td>
<td>Chili pepper, Tomato</td>
</tr>
<tr>
<td></td>
<td>Karnataka</td>
<td>Kolar</td>
<td>3</td>
<td>Tomato</td>
</tr>
<tr>
<td>2008**</td>
<td>Andhra Pradesh</td>
<td>Krishna</td>
<td>3</td>
<td>Onion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guntur</td>
<td>2</td>
<td>Chili pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Khammam</td>
<td>3</td>
<td>Chili pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rangareddy</td>
<td>3</td>
<td>Tomato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chittoor</td>
<td>4</td>
<td>Tomato</td>
</tr>
<tr>
<td></td>
<td>Maharashtra</td>
<td>Ahmednagar</td>
<td>4</td>
<td>Onions</td>
</tr>
</tbody>
</table>

**2006-2007***- Thrips were surveyed in 3 states, 10 districts and 31 villages in 3 crops (Onion, Tomato and Chili pepper).

**2008***- Thrips were surveyed in 2 states, 6 districts and 19 villages in 3 crops (Onion, Tomato and Chili pepper).
Table 3.2 Thrips vector species found in vegetable crops in India.

<table>
<thead>
<tr>
<th>States</th>
<th>Crops (associated viruses*)</th>
<th>Confirmed thrips species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andhra Pradesh</td>
<td>Chili pepper (CaCV)</td>
<td><em>Frankliniella schultzei, Thrips palmi</em></td>
</tr>
<tr>
<td></td>
<td>Tomato (PBNV/GBNV, CaCV)</td>
<td><em>Scirtothrips dorsalis</em></td>
</tr>
<tr>
<td></td>
<td>Onion (IYSV)</td>
<td><em>Frankliniella schultzei, Scirtothrips dorsalis</em></td>
</tr>
<tr>
<td>Maharashtra</td>
<td>Onions (IYSV)</td>
<td><em>Thrips tabaci</em></td>
</tr>
<tr>
<td></td>
<td>Chili pepper (CaCV)</td>
<td><em>Thrips palmi</em></td>
</tr>
<tr>
<td></td>
<td>Tomato (PBNV/GBNV, CaCV)</td>
<td><em>Frankliniella schultzei, Thrips hawaiiensis, Thrips palmi, Scirtothrips dorsalis</em></td>
</tr>
<tr>
<td>Karnataka</td>
<td>Tomato (PBNV/GBNV, CaCV)</td>
<td><em>Frankliniella schultzei, Thrips hawaiiensis</em></td>
</tr>
</tbody>
</table>

Figure 3.1 Map of India showing thrips samples collected in three states.

Sampled in 4 districts

Sampled in 1 district

Sampled in 5 districts
Figure 3.2a Ventral view of *Thrips tabaci*.
Figure 3.2b Ventral view of Scirtothrips dorsalis.
Figure 3.2c Ventral view of *Frankliniella schultzei*.
Figure 3.2d Ventral view of *Thrips palmi*. 
Figure 3.2e Ventral view of *Thrips hawaiiensis*.
Figure 3.3 Total number of thrips sampled by month for the survey during 2006-2008 in India.
Figure 3.4 Mean number of thrips by crop and genus for 2006-2008 in India.
Figure 3.5 Mean number of thrips in onions by state and genus for 2006-2008 in India.
Figure 3.6 Mean number of thrips in tomato by state and genus for 2006-2008 in India.
Figure 3.7 Mean number of thrips in chili peppers by state and genus for 2006-2008 in India.
Figure 3.8 Mean number of thrips sampled by month for 2008 and 2009 in Georgia, USA.
Figure 3.9 Total number of thrips sampled by location and thrips species for 2008-2009 in Georgia, USA.
CHAPTER 4
THRIPS (THYSANOPTERA: THRIPIDAE) COMPETITION IN ONIONS

1Chitturi, A and Riley D. G. 2010. To be submitted to Crop Protection
Abstract

Thrips competition studies were conducted under laboratory conditions to determine if *Thrips tabaci* would have a reproductive disadvantage when competing with another dominant thrips species, *Frankliniella fusca*, on onions. The *T. tabaci* used in this test was a biotype introduced to Georgia from Casma onion production region in Peru where this species is a serious problem. Three treatments were evaluated: *F. fusca* alone on onions, *T. tabaci* alone on onions, and then a combination of equal numbers of both species on onion plants. The first set of experiments used nymphs for initial infestation and the second used adult female thrips. Results from both competition studies indicated that *T. tabaci* exhibited a strong competitive behavior in the presence of *F. fusca*. Both the competition studies indicated that reproduction of the Peruvian biotype of *T. tabaci* used in these tests was not affected by the presence *F. fusca* on onions, but that *F. fusca* reproduction was decreased in the presence of *T. tabaci*. Field studies on the distribution of the thrips species *T. tabaci* and *F. fusca* indicated that thrips species tended to segregate from each other along the length of the onion foliage.

**Key words:** *Thrips tabaci, Frankliniella fusca*, competition, competitive exclusion, reproduction, distribution, onion foliage, thrips segregation.
Introduction

Vidalia sweet onion (*Allium cepa* L.) is one of the most important specialty vegetable crops in Georgia which also has worldwide recognition. Vidalia onions are short day onions, grown in ~10,000 acres with farm gate value worth $139 million in 2008 (Boatright and McKissick 2009). Vidalia onions are produced in a geographically defined within a specific area in Southeast Georgia, primarily on sandy loam soils. Thrips (Thysanoptera: Thripidae) are the primary arthropod pests in onion in Georgia (Riley and Batal 1998). The primary thrips species reported to occur in Georgia onions are tobacco thrips, *Frankliniella fusca* (Hinds), western flower thrips, *Frankliniella occidentalis* (Pergande) and onion thrips, *Thrips tabaci* (Lindeman) (Sparks and Riley 2007). Thrips cause damage to onions by feeding on the leaf surface by rasping plant cells resulting in silvering and curling of the leaves and subsequently reduces the bulb size (Childers 1997). *Thrips tabaci* is the only known vector species that transmits *Iris yellow spot virus* (IYSV) (genus tospovirus, family Bunyavidea) in onions (Nagata et al. 1999). The plant virus, IYSV, has been reported as a potential devastating pathogen of onion in the Western United States (Gent et al. 2006). In Georgia, IYSV was detected on onions in 2003 (Mullis et al. 2004).

As *T. tabaci* efficiently transmits IYSV and *F. fusca* is not reported to be a vector, it could be important to know if there is the possibility of competitive exclusion between these two thrips species. Recent, studies conducted in quantifying the competition between two thrips species indicated that thrips competition can result in competitive exclusion in the flowers of pepper (Paini et al. 2008). Since multiple thrips species can occur on onions (Riley and Batal, 1998, Sparks and Riley, 2007), a key question is to determine whether the presence of multiple thrips species can result in exclusion of the principal IYSV vector, *T. tabaci* on onion plants.
Thrips competition studies were conducted to determine if *T. tabaci* would have a reproductive disadvantage when competing with another common thrips species on onions. The *T. tabaci* used in this test was a recent biotype of this thrips species introduced to Georgia from the onion production region in Peru where IYSV is a serious problem (Nischwitz et al. 2007). This study was conducted to determine whether or not this new biotype had the potential to establish in an onion production region in Georgia that has traditionally been dominated by the thrips species *F. fusca* with occasional *F. occidentalis* (Riley and Batal, 1998, Sparks and Riley, 2007). In a field study, the occurrence of *T. tabaci* and *F. fusca* were studied to determine the preferential coexistence patterns of both the thrips species relative to leaf positions on the onion plant. We know there is diel periodicity of onion thrips in relation to dispersion and occurrence on onion plants (Sites et al. 1991), but we wanted to test for spatial segregation by thrips species. As *T. tabaci* and *F. fusca* are currently the common thrips species in onions, these studies are important in understanding the behavior of the thrips species in the Vidalia onion region. The main objectives of this study were i) to test for competitive exclusion of *T. tabaci* by equal numbers of *F. fusca* on Vidalia type onion plants and ii) to determine the distribution of two thrips species, *T. tabaci* and *F. fusca* relative to time and leaf position and look for segregation on onion plants in the field.

**Materials and Methods**

Competition experiments were conducted during the fall 2004 and spring 2008 at the Coastal Plain Experiment Station, Tifton, GA. For both experiments three different treatments were used to test for possible exclusion of *T. tabaci* by *F. fusca* by testing for reproduction of each individual species alone on onion versus an equal combination of both species. The *T. tabaci* colony (Peruvian strain originally from the Casma region) was collected from the onion
cull piles in the Vidalia onion region, Reidsville, GA, USA and maintained on whole onion plants for use in these experiments. *Frankliniella fusca* colony was maintained on greenbeans. For the first set of experiments, randomly picked 2\textsuperscript{nd} - 3\textsuperscript{rd} instar nymphs from each colony were used for the experiments while for the second set of experiments, randomly picked female *T. tabaci* adults of undetermined ages were used for the experiments.

In the first test, we placed one onion transplant (*Allium cepa* var. Savannah Sweet) a ~0.5 cm diameter thickness at the 3-5 leaf stage in a 946 ml (32 oz) styrofoam cup (Bart Container Corp., Madison, MI) and sprayed to run-off with 1% Safer Soap solution (Woodstream Corp. Lititz, PA). Plants were allowed to dry, and then 3\textsuperscript{rd} instar nymphs were placed as per different treatments above on the plant and covered with a fine nylon screened plastic exclusion cage and the edge sealed with parafilm (Fig. 4.1). Onions were held for one week and then inspected to verify no thrips emerged from the plants, i.e., no contamination. The treatments applied to a single onion plant-isolation cage (replicated 6 times) were: 1) 10 immature *T. tabaci*, 2) 10 immature *F. fusca*, and 3) 10 immature *F. fusca* + 10 immature *T. tabaci*. Two replications were placed into each of three Percival Growth Chambers having a temperature of 27.5°C and photoperiod of 12:12. Cages were undisturbed for 2 weeks and then the top cage was removed and inspected for adults. Adults were removed and placed in a 7 ml vial containing 70% EtOH being labeled by treatment and date. Cages were then closed above to remove adult thrips with parafilm leaving immature thrips for another week and then repeated eight times using the procedure outlined. This was continued after another week when all thrips were collected from the cages. All adult thrips were identified to species and number recorded for each inspection date.
In the second set of experiments, onion seeds, *Allium cepa* var. Pegasus hybrid onion (Seminis, Oxnard, CA) were placed in germination trays and allowed to germinate and grown until two leaf stage in growth chambers at 25°C. The seedlings were then transferred to the greenhouse and transplanted into 4-inch pots using ‘LT 5 Mix’ (Sun Gro Horticulture Dist. Bellevue, WA). Plants were visually checked to maintain them as insect free as possible and watered every alternate day. Tops and roots of the onion seedlings at 3 leaf stage were trimmed off and the pruned plant was placed into a 13 × 100 mm glass test tube (Borex ® Disposable culture tubes). The tube was wrapped with parafilm and a wet cotton ball at the base of the tube provided enough moisture for the seedling (Fig. 4.2). The treatments applied to a single onion plant-isolation test tube (replicated 8 times) were: 1) 4 female adult *T. tabaci*, 2) 4 female adult *F. fusca*, and 3) 2 female adult *F. fusca* + 2 female adult *T. tabaci*. Female adult thrips four per plant were placed in the test tubes using a zero number brush, and the test tubes were sealed with a fine copper mesh screen using parafilm. Thrips were allowed to oviposit for 5 days and after every 5 days the remaining adults were collected and transferred to a new onion seedling. This was continued until 20 days to allow maximum oviposition by adult thrips. The total number of adults that emerged from each test tube were counted and collected in vials with 70% EtOH.

To determine the distribution of the adult thrips species in the field, 10 onion plants were randomly selected in field plots at VORC, Reidsville, GA. To measure the distribution of thrips adults on onion plants, each plant was evenly divided into top, middle and basal sections. Observations were recorded late season in the field relative to the plant positions during late April and late May and adults were counted on all the green leaves of the onion plant. Leaf sheaths that were dry and unwrapped around the base of the plant were not considered. The locations of adults on the plant were recorded by thrips species for every 15 minutes starting at
8.30 a.m in the morning for 2 hours. Frankliniella fusca adults were separated from T. tabaci adults based a 10 x magnification hand lens, so there was limited taxonomic accuracy; however, it was deemed sufficient to test for possible segregation of species on the plant.

Data from these randomized complete block design experiments were analyzed using PROC ANOVA (SAS Institute 1990). Fisher’s least significant difference method was used for determining the difference in thrips treatments using PROC ANOVA with \( \alpha = 0.05 \).

**Results and Discussion**

In the first set of experiments, starting with immatures, the mean total number of adults and immatures produced by treatments, over the four weeks varied significantly (Table 4.1). In the first week, T. tabaci alone and F. fusca alone tended to produce higher number of immatures than when the species were combined (Fig 4.3a). When the species were combined, there was no significant effect observed on the total number of immatures produced (\( F = 2.19, df = 2, 14, P = 0.1492 \)). However, there was a significant effect on the total number of adults produced by T. tabaci (\( F = 3.81, df = 2, 14, P = 0.0478 \)) and F. fusca (\( F = 11.22, df = 2, 14, P = 0.0012 \)). In the second week, a similar trend was observed where T. tabaci alone and F. fusca alone produced higher number of immatures (Fig. 4.3b). When the species were combined, a significant effect was observed on the number of immatures produced (\( F = 12.29, df = 2, 14, P = 0.0008 \)). There was a significant effect observed on the adults of both species, T. tabaci (\( F = 7.60, df = 2, 14, P = 0.0058 \)), but no significant effect on adults of F. fusca (\( F = 2.86, df = 2, 14, P = 0.0909 \)). In the third week, T. tabaci alone and F. fusca alone produced higher number of immatures than when the species were combined (Fig. 4.3c). When the species were combined, a significant effect was observed on the number of immatures produced (\( F = 4.16, df = 2, 14, P = 0.0381 \)). There was a significant effect on adults of both species, T. tabaci (\( F = 3.94, df = 2, 14, P = 0.0440 \)) and F. fusca (\( F = 7.94, \))
$df = 2, 14, P= 0.0050$) with a similar trend as the first week. In the first three weeks when the species were combined, the number of immatures reduced significantly compared to the uncombined treatments, but it was not clear if the nymphs were being killed by one of the thrips species or if fecundity was lower (Fig. 4.3a, b and c). In the fourth week, $F. fusca$ alone tended to produce higher number of immatures than $T. tabaci$ alone (Fig 4.3d), but the effect was not significant ($F= 0.43, df = 2, 14, P= 0.6592$). As in the second week, a similar trend was observed with a significant effect on adults of $F. fusca$ ($F=10.04, df = 2, 14, P= 0.0020$) but not on adults of $T. tabaci$ ($F= 2.42, df = 2, 14, P= 0.1249$). Overall results combined over all four weeks indicated that, $T. tabaci$ alone and $F. fusca$ alone tended to produce significantly higher number of immatures (Table 4.1). There was a significant reduction in the number of immatures produced when the species were combined ($F= 6.73, df = 2, 14, P= 0.0090$). As expected, there was a significant effect on adults of both species, $F. fusca$ ($F=14.28, df = 2, 14, P= 0.0004$) and $T. tabaci$ ($F= 8.36, df = 2, 14, P= 0.0041$). In the combined treatment, $T. tabaci$ produced eight times more number of adults than $F. fusca$.

In the second experiment which used female adults, the mean total number of adults and immatures produced by the treatments, over 20 days varied significantly (Table 4.2). In the first period, $T. tabaci$ alone tended to produce more number of immatures than $F. fusca$ alone (Fig 4.4a). There was a significant effect on the number of immatures produced when the species were combined ($F= 5.52, df = 2, 14, P= 0.0171$). A significant effect on adults of both species was observed, $F. fusca$ ($F=6.78, df = 2, 14, P= 0.0087$) and $T. tabaci$ ($F= 6.55, df = 2, 14, P= 0.0098$). In the combined treatment, $T. tabaci$ had no significant reduction of adults while $F. fusca$ did. In the second 5-day period, a similar trend was observed where $T. tabaci$ alone tended to produce higher number of immatures than $F. fusca$ alone (Fig. 4.4b). There was a significant
effect on the number of immatures when the species were combined \((F= 11.27, df = 2, 14, P= 0.0021)\). As expected, there was a significant effect on adults of both species, \(T. tabaci\) \((F= 7.92, df = 2, 14, P= 0.0050)\) and \(F. fusca\) \((F=92.38, df = 2, 14, P=< 0.0001)\). In the third 5-day, a similar trend was observed (Fig. 4.4c). There was a significant effect on the number of immatures when the species were combined \((F= 22.67, df = 2, 14, P= <0.0001)\). There was also a significant effect on adults of both species, \(T. tabaci\) \((F= 6.35, df = 2, 14, P= 0.0109)\) and \(F. fusca\) \((F=29.97, df = 2, 14, P= <0.0001)\) again with the same trend. By end of 15 days, the number of immatures reduced significantly in the combination treatments than alone. In the last period (16-20 days), \(T. tabaci\) alone tended to produce higher number of immatures than \(F. fusca\) alone (Fig. 4.4d), but this was not significant \((F= 2.01, df = 2, 14, P= 0.1712)\). There was a significant effect on adults of \(F. fusca\) \((F=6.0, df = 2, 14, P= 0.0131)\), but not \(T. tabaci\) \((F= 2.31, df = 2, 14, P= 0.1363)\).

Results combined over 20 days indicated that, \(T. tabaci\) alone and in combination produced significantly higher number of immatures than \(F. fusca\) alone \((F= 17.61, df = 2, 14, P= 0.0002, Table 4.2)\). There was a significant effect on adults of both species, \(T. tabaci\) \((F= 24.90, df = 2, 14, P= <0.0001)\) and \(F. fusca\) \((F=33.56, df = 2, 14, P= <0.0001)\). In the combined treatment, \(T. tabaci\) had no significant reduction of adults while \(F. fusca\) had a significant reduction in the adults. In the combined treatment, \(T. tabaci\) produced four times more number of adults than \(F. fusca\).

Results summarized from the two competition studies indicated that \(T. tabaci\) exhibited a strong competitive behavior in the presence of \(F. fusca\). Both the competition studies indicated that the reproduction by Peruvian type of \(T. tabaci\) used in these tests was not affected by the presence of \(F. fusca\) whether placed together in the immature stages or as adults. In fact, this
biotype of onion thrips appeared to out-compete *F. fusca*, at least under laboratory conditions. This was surprising given that *F. fusca* has been the native thrips species infesting onion in the Vidalia onion growing area. Both thrips species, when alone, tended to reproduce similarly. However, *T. tabaci* produced 8-fold more adults (Table 4.1) and 4-fold more adults (Table 4.2) than *F. fusca* in the combined state when starting with immatures and adults, respectively. In the presence of *T. tabaci*, *F. fusca* reproduced less adult offspring per female than when developing in isolation.

An intriguing result is what happened to the nymphs in the first experiment. Overall thrips reproduction for *T. tabaci* and *F. fusca* was 8.2 and 7.2 offspring per immature in the isolated state versus 4.2 offspring per immature in the competitive state. This suggested possible mortality of immature thrips or reduced fecundity when thrips species were combined on the same onion plant. Given the greater number *T. tabaci* adults after one generation, the likely scenario is that *T. tabaci* is interfering with the development of immature stages of *F. fusca*. We think that direct nymph competition might be the mechanism for competitive displacement of thrips species in these experiments based on the differences between the first and second experiments. Regardless, these studies indicated the *T. tabaci* should dominate in mixed thrips populations, which means that the transmission of IYSV is likely to increase in onions in the Vidalia onion production region of Georgia if this efficient vector increases where *F. fusca* has historically dominated. An important question remaining is whether or not combination of two different thrips species affects the IYSV transmission behavior of *T. tabaci*.

Field study results on the distribution of thrips adults within the onion foliage recorded in the field during late April and late May of fall 2009 indicated that, when species were combined, *T. tabaci* appeared to occur more at the top portions of the leaf in onion plant in the presence of
F. fusca (Fig. 4.5). Adult thrips species analyzed during late April also indicated that T. tabaci appeared to prefer the top portions of the leaf followed by middle and basal portions of the leaf on the onion plant while F. fusca was observed in the middle followed by basal and top portions (Fig. 4.6). A significant leaf position and plant interaction was observed for T. tabaci ($F = 51.71, df = 2, 288, P = <.0001$) and F. fusca ($F = 180.42, df = 2, 288, P = <.0001$). During late May T. tabaci was seen more on the middle potions of the plant followed by top and basal portions while F. fusca tended to be more towards the middle followed by bottom and top portions of the leaf (Fig 4.7). A significant treatment interaction was observed by leaf position and plant for T. tabaci ($F = 99.22, df = 2, 288, P = <.0001$) and F. fusca ($F = 150.97, df = 2, 288, P = <.0001$).

Overall, the adult thrips species analyzed over both the dates and leaf positions suggested that T. tabaci spatially segregated from F. fusca along leaf length. The distribution of T. tabaci and F. fusca by time indicated that there was no temporal displacement of thrips species. Both the species were seen occurring on the onion more during the morning hours between 8:45 a.m to 9:30 a.m than in the later part of the day (Table 4.3). Based on these limited late season observations, it appears that T. tabaci and F. fusca have the tendency to segregate along leaf length in the field.

Acknowledgements

We thank the Coastal Plain Experiment Station for providing facilities and technical support for this research. Funding was provided in part by the IPM CRSP and by the Department of Entomology.
References


Table 4.1 Total adult and immature thrips emerged over all weeks on onion transplants infested with 10 immatures per plant during 2004.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thrips tabaci total</th>
<th>Frankliniella fusca Total</th>
<th>Immatures total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. tabaci</td>
<td>26.8a</td>
<td>0.0b</td>
<td>54.7a</td>
</tr>
<tr>
<td>F. fusca</td>
<td>0.0b</td>
<td>21.0a</td>
<td>50.8a</td>
</tr>
<tr>
<td>Both species</td>
<td>25.0a</td>
<td>2.9b</td>
<td>13.6b</td>
</tr>
</tbody>
</table>

1Means (n=8) within columns are not significant if followed by the same letter, LSD Test, P<0.05.
Table 4.2 Total adult and immature thrips collected at 5 day intervals on onion seedlings infested with 4 adult females per plant during 2008.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thrips tabaci total</th>
<th>Frankliniella fusca total</th>
<th>Immatures total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. tabaci</td>
<td>59.7a</td>
<td>0.0b</td>
<td>37.7a</td>
</tr>
<tr>
<td>F. fusca</td>
<td>0.0 b</td>
<td>47.9a</td>
<td>8.7b</td>
</tr>
<tr>
<td>Both species</td>
<td>52.6a</td>
<td>13.0b</td>
<td>31.0a</td>
</tr>
</tbody>
</table>

1Means (n=8) within columns are not significant if followed by the same letter, LSD Test, P<0.05.
Table 4.3 Distribution of mean number of *Thrips tabaci* and *Frankliniella fusca* adults on onion plants relative to time.

<table>
<thead>
<tr>
<th>Time (am)</th>
<th>Mean number of <em>Thrips tabaci</em></th>
<th>Mean number of <em>Frankliniella fusca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>8: 30 a. m.</td>
<td>1.78a</td>
<td>4.66abc</td>
</tr>
<tr>
<td>8: 45 a. m.</td>
<td>1.91a</td>
<td>5.88a</td>
</tr>
<tr>
<td>9: 00 a. m.</td>
<td>2.08a</td>
<td>5.20ab</td>
</tr>
<tr>
<td>9: 15 a. m.</td>
<td>1.45ab</td>
<td>4.90abc</td>
</tr>
<tr>
<td>9: 30 a. m.</td>
<td>1.48ab</td>
<td>4.38bcd</td>
</tr>
<tr>
<td>9: 45 a. m.</td>
<td>1.03b</td>
<td>3.76cde</td>
</tr>
<tr>
<td>10: 00 a. m.</td>
<td>1.03b</td>
<td>3.05ed</td>
</tr>
<tr>
<td>10:15 a. m.</td>
<td>1.08b</td>
<td>2.96ed</td>
</tr>
<tr>
<td>10:30 a. m.</td>
<td>0.88b</td>
<td>3.03ed</td>
</tr>
<tr>
<td>10: 45 a. m.</td>
<td>0.95b</td>
<td>2.76e</td>
</tr>
</tbody>
</table>

1Means (n=10) within the columns are not significant if followed by the same letter (LSD, P<0.05).
Figure 4.1 Onion transplants placed in 1 quart styrofoam cups to test for possible exclusion of *Thrips tabaci* by *Frankliniella fusca* during 2004.
Figure 4.2 Onion seedlings placed into 13 × 100 mm glass test tubes to test for possible exclusion of *Thrips tabaci* by *Frankliniella fusca* during 2008.
Figure 4.3a Thrips numbers by species for week 1 during 2004. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.3b Thrips numbers by species for week 2 during 2004. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.3c Thrips numbers by species for week 3 during 2004. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.3d Thrips numbers by species for week 4 during 2004. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.4a Thrips numbers by species for 0-5 days during 2008. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.4b Thrips numbers by species for 6-10 days during 2008. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.4c Thrips numbers by species for 11-15 days during 2008. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.4d Thrips numbers by species for 16-20 days during 2008. Columns topped by different letters indicate significant differences between totals, LSD (P<0.05).
Figure 4.5 Distribution of *Thrips tabaci* and *Frankliniella fusca* on onion plants in the field relative to leaf position over all. Means by columns within species are significantly different if topped by different letters, LSD Test, $P<0.05$. 
Figure 4.6 Distribution of *Thrips tabaci* and *Frankliniella fusca* on onion plants in the field relative to leaf position during late April 2009. Means by columns within species are significantly different if topped by different letters, LSD Test, $P<0.05$. 

![Graph showing the distribution of Thrips tabaci and Frankliniella fusca on onion plants with top, middle, and base divisions indicated. The bars for each division are labeled with different letters: a, b, and c.](image-url)
Figure 4.7 Distribution of *Thrips tabaci* and *Frankliniella fusca* on onion plants in the field relative to leaf position during late May 2009. Means by columns within species are significantly different if topped by different letters, LSD Test, $P<0.05$. 
CHAPTER 5

DISTRIBUTION, SETTLING AND OVIPOSITION OF THRIPS TABACI

(THYSANOPTERA: THRIPIDAE) ON ONION FOLIAGE

_______________________

1Chitturi, A and Riley, D. G. 2010. To be submitted to Environmental Entomology
Abstract

The behavioral patterns of *Thrips tabaci*, distribution, settling and oviposition were investigated on onion foliage relative to leaf position and leaf length at pre-bulbing stages of the crop. Settling and oviposition behavior of *T. tabaci* were investigated at the 3-leaf stage and 6-8 leaf stage of onion plants relative to different leaf positions and every 2 cm segment of the leaf length. The thrips behavior parameters were quantified on the above ground portion of onion plants from the distal end of the bulb or leaf sheath “neck” through the tips of the foliage. Results from the 3-leaf stage onion plants indicated that distribution of thrips adults, immatures and eggs were skewed towards the base of the plant. For the older 6-8 leaf stage onion plants, results indicated that both settling and oviposition behavior of *T. tabaci* also tended to be more towards the base of the plant. The distribution of thrips nymphs was similar to the egg distribution in the onion plant studies. Thus, while onion leaves remain straight during early season *T. tabaci* showed a clear preference for settling and ovipositing towards the base of the plant near the wrapped leaf sheath.

**Keywords:** Onion thrips, *Thrips tabaci*, distribution, settling, oviposition, 3-leaf stage, 6-8 leaf stage, leaf position, 2 cm leaf segments, leaf length, plant bioassay.
Introduction

Onion (*Allium cepa* L.) is a high-value specialty vegetable crop in Georgia popular worldwide as Vidalia Sweet Onions. Onion is an important vegetable grown as a winter crop in 12,000 acres with a total value of $108,560 million in 2008 and $82,908 million in 2009 (NASS, 2009). *Thrips tabaci* (Onion thrips) (*Thysanoptera: Thripidae*) is an important pest in onions in Georgia (Sparks and Riley 2007) and worldwide in all onion growing areas (Jenser and Szènàsi 2004). *Thrips tabaci* is the only vector species known to transmit *Iris Yellow Spot virus* (IYSV) (Nagata et al. 1999, Kitzman 2001). In Georgia, IYSV was first reported on Vidalia onions in 2003 (Mullis et al. 2004) and ever since its first report the virus has been found every year in onion growing regions in the state. Thrips are well adapted to seek narrow spaces in which to live and feed (Kirk 1997). In onions the narrow spaces at the base of the neck are tightly appressed by the inner leaves resulting in an attractive niche for thrips populations (Rossiter 1980). Where thrips settle and feed on the onion plant is where transmission of IYSV is likely to occur. Since the distribution of IYSV on the onion plant has been difficult to characterize (Smith et al. 2006), we began to unravel this problem by first looking at the distribution of two critical *T. tabaci* behaviors on onion foliage, settling and oviposition. Studies have reported on the field distribution of *T. tabaci* populations in onion (Edelson et al. 1986) and within-plant distribution on cabbage (Mustafa 1986, Stoner and Shelton 1988), leek (Theunissen and Legutowska 1991) and cucumber (Razdoburdin and Sinelniov 2000). Only recent studies conducted in Australia on onion thrips in onion fields provided better resolution of the within-leaf distribution of eggs on the onion plants (Mo et al. 2008), but further studies were needed to relate the distribution of settling of adults and nymphs relative to eggs. The current study was conducted to determine the distribution of onion thrips adults, nymphs and eggs on the onion plant relative to leaf length.
The main objective of this study was to characterize the behavior of *T. tabaci* on the above ground portion of onion plant from the distal end of the immature bulb or leaf sheath “neck” to the apical tips of the foliage. Settling and oviposition behavior of *T. tabaci* were investigated on onion foliage relative to leaf position and leaf length under laboratory conditions where adults and nymphs could be more closely monitored.

**Materials and Methods**

Settling and oviposition behavior of *T. tabaci* were investigated at 3-leaf stage and 6-8 leaf stage of onion plants relative to leaf position L1 (inner most), L2 and L3 (outer most) for every 2 cm of the leaf length (Fig. 5.1) using 12 replicates. The two behavior parameters were observed by setting up a whole plant bioassay using an insect cage measuring 47.5x47.5x 47.5 cm with fine mesh screen (MegaView Science Co., Taichung, Taiwan) (Fig. 5.2). Onion seeds cv. Pegasus Hybrid Onion (Seminis, Oxnard CA) were placed in germination trays and allowed to germinate and grow until the two leaf stage in growth chambers at 25°C. The seedlings were then transferred to the greenhouse and transplanted into 10 cm pots using ‘LT 5 Mix’ (Sun Gro Horticulture Dist. Bellevue, WA). Plants were visually checked to maintain them as insect free as possible and watered every alternate day. Before introducing the thrips, onto the 3-leaf and 6-8 leaf onion plants, the surface of the each pot was sealed with parafilm to prevent escape of thrips into the soil. Four adults of *T. tabaci* were released on 3-leaf onion plants and 6 adults were placed on 6-8 leaf stage onion plants and placed in the insect cage. Adults were allowed to settle the day before quantifying behavior. To quantify the settling behavior, thrips adults and emerging nymph positions on the leaf were recorded every 30 minutes for 4 hours each day for 5 days. For oviposition, studies thrips were allowed to oviposit for 6 days. Oviposition sites indicated by purple rings were counted by following the lacto-phenol acid fuschin staining
technique detailed by Nuessly et al. (1995) and Parella and Rob (1982). To determine the oviposition sites, the intact leaf tissues from the plant were cut from the base plant and decolorized by boiling 3-5 minutes in the lacto phenol acid fuschin solution while working under a fume hood. Stained leaves were cooled for 3-5 hrs and excess stain was removed with warm water. Leaves were examined under a stereo microscope for oviposition sites indicated by eggs and purple rings (hatched eggs) and counted for each 2 cm section of the leaf.

Data was analyzed using PROC GLM (SAS Institute 1990) with plants as replicates. Fisher’s least significant difference method was used for determining mean separation by leaf segment using PROC GLM with $\alpha = 0.05$.

Results

_Thrips tabaci settling on 3-leaf onion plant by leaf position and leaf length:_ _Thrips tabaci_ on 3-leaf onion plants showed a clear preference for the base of the foliage across all leaves (Table 5.1). In the 3-leaf onions we consider “0’ cm as the length of the contiguous leaf sheath from the distal end of the immature bulb to where the leaves separated. Settling of _T. tabaci_ adults and immatures on the inner most leaf (L1) was also skewed towards the base of the plant (Fig. 5.3a). A significant treatment effect was observed with the adult thrips settling and leaf segment by cm ($F=17.04$, $df=15$, 1893, $P= <0.001$). As much as 81% of adults concentrated on leaf segment 2-8 cm with less at the base and towards the tip. Similarly, 61% of immature stages of thrips concentrated on leaf segment 2-8 cm with less at the base and towards the tip. A significant treatment effect was observed with the adult thrips settling and leaf segment by cm ($F=2.46$, $df=15$, 1893, $P= 0.0014$). Settling of _T. tabaci_ adults and immatures on L2 tended towards the middle of the plant (Fig. 5.3b) with only 47% of adults concentrated on leaf segment 2-8 cm and none towards the tip. However, there was no significant treatment effect observed
with the adult thrips settling by leaf segment \((F=1.45, df=15, 1893, P=0.1139)\). Similarly, 70% of immatures concentrated on leaf segment 6-14 cm. A significant treatment effect was observed with the immature thrips settling by leaf segment \((F=1.81, df=15, 1893, P=0.0281)\). On the outermost leaf (L3), both adults and immatures were concentrated at base of the onion plant (Fig. 5.3c). As much as 60% of the adults concentrated on the basal leaf segment. A significant treatment effect was observed with the adult thrips settling and leaf segment by cm \((F=205.0, df=15, 1893, P<0.001)\). Similarly, 78% of immatures were concentrated on the basal leaf segment, but counts were highly variable \((F=1.0, df=15, 1893, P=0.4564)\). Settling of *T. tabaci* adults and immatures over all leaves was skewed towards the base of the plant. Adults and immatures concentrated on the base of the leaf segment followed by the middle and almost none towards the tip of the leaves (Table 5.1).

**Oviposition in 3-leaf onion plant:** Oviposition of *T. tabaci* by leaf length on 3-leaf onion plants showed a clear preference towards the base. On the inner most leaf (L1), 52% of the eggs were concentrated on the leaf segment 4-8 cm with less in the middle and towards the tip (Fig. 5.4a). A significant treatment effect was observed with oviposition by leaf segment \((F=6.64, df=15, 383, P<0.001)\). A similar trend was seen with *T. tabaci* oviposition on L2 (Fig. 5.4b), with 76% of the eggs concentrated on the leaf segment 2-10 cm \((F=8.98, df=15, 383, P<0.001)\). Oviposition of *T. tabaci* on outer most leaf (L3) exhibited a similar pattern (Fig. 5.4c) \((F=5.67, df=15, 383, P<0.001)\). Overall, oviposition of *T. tabaci* was similarly distributed as the adults and immature, with the difference that eggs occurred all the way to the leaf tip (Table 5.1).

**Thrips tabaci settling on 6-8 leaf onion plant:** For 6-8 leaf stage of onion plant, the center three upright leaves were considered for observing settling and oviposition. Settling of *T.
tabaci adults and immatures on a 6-8 leaf onion plant was similar to that of the 3-leaf onion plant, except that immature were more clumped at the base (Table 5.1). On the inner most leaf (L1) 89% of the observed adults concentrated on leaf segment 2-10cm (Fig. 5.5a) \((F=15.86, df=15, 1343, P<0.001)\). Also, 92% of immatures concentrated on leaf segment 2-8 cm \((F=1.85, df=15, 1343, P=0.0246)\). A similar trend was observed with the settling of T. tabaci adults on L2 (Fig. 5.5b) with 93% of adults concentrated on leaf segment 2-12 cm \((F=4.80, df=15, 1343, P<0.0001)\). No immatures were seen on the very base or tip of the leaf \((F=0.96, df=15, 1343, P=0.4965)\). On the leaf (L3), T. tabaci adults were concentrated on the leaf segment “0”cm and 95% of immatures were concentrated on leaf segment 2-8 cm \((F=118.70, df=15, 1343, P<0.001)\). Settling of T. tabaci adults and immatures on the 6-8 leaf stage relative to leaf segment was skewed towards the base of the plant at leaf segment 0 cm, followed by adults settling on the leaf segments below 6 cm from the base (Table 5.1).

**Oviposition in 6-8 leaf onion plant by leaf position and leaf length:** Oviposition of T. tabaci by leaf length on 6-8 leaf onion plant also showed the same preference towards the base of the plant (Table 5.1). On the inner most leaf (L1) 73% of the eggs were concentrated on the leaf segment 2-6 cm with less in the middle and none towards the tip \((F=13.83, df=15, 165, P<0.001)\). A similar trend was observed with T. tabaci oviposition on L2 (Fig. 5.6b) with 79% of eggs concentrated on the leaf segment 2-8 cm \((F=15.08, df=15, 165, P<0.001)\). On L3 81% of eggs were concentrated on the leaf segment 0-4 cm \((F=7.34, df=15, 165, P<0.001)\). Overall, oviposition of T. tabaci was skewed towards the base of the plant below 6 cm across all the three leaves (Table 5.1).
**Discussion**

The distribution of thrips oviposition by leaf length was skewed towards the base of the plant, very similar to that reported by Mo et al. (2008). However, the distribution of adults and immatures was even more clumped towards the base of the plant than eggs. Differences between the mobile and immobile stages of thrips could simply relate to eggs being a cumulative measure of activity. Thrips are generally thigmokinetic/thigmotactic, preferring to spend time in or near tight protected spaces on the plant surface, such as on the side of folded leaf surfaces or in tightly wrapped leaf sheaths (Kirk 1997). The slower moving, soft bodied immature thrips particularly benefit from this location from predators such as *Orius* spp. In the laboratory studies, both the adults and immatures of *T. tabaci* showed a strong preference for settling toward the base of the plant near the wrapped leaf sheath in both the young 3 leaf stage onions (Fig. 5.4a, b, c) and the older 6-8 leaf stage (Fig. 5.5a, b, c). In the early stages of the plant growth, the onion leaves are straight. As the plants mature, leaves begin to have extensive leaf folding which provides additional protected spaces for thrips higher up on the leaf tube. In the 6-8 leaf older onion plants, the distribution of eggs still occurred significantly towards the base of the plant (Fig. 5.9a, b, c) and the distribution of adult settling was even lower (Fig. 5.5a, b, c). This was because the leaf separation was based on the two inner most leaves which at the 6-8 leaf stage was higher than where the oldest leaves split off from the main sheath. The thrips egg distribution data is consistent with that reported by Mo et al. (2008) and the adult settling data showed that, although clumped at the base, thrips must be spending some time out on the leaf laying eggs. The data indicates that the distribution of thrips nymphs was more similar to the egg distribution in the onion plant studies. Thus, immatures must be taking more time to migrate to the protected sheaths than adults.
This distribution of thrips along leaf length is of interest because in onion IYSV has also been reported in greater frequency close to the bulb near the base of the leaves (Kritzman 2001). Smith et al. 2006 reported a different distribution of IYSV in leek, but the plant structure is different in leek and thrips distribution will also likely be affected by this structure. Further studies are needed to specifically relate the distribution of thrips to the distribution of IYSV within the onion plant structure.

**Acknowledgements**

We thank the Coastal Plain Experiment Station for providing facilities and technical support for this research. Funding was provided by the Department of Entomology, University of Georgia.
References Cited


staining *Liriomyza trifolii* (Diptera: Agromyzidae) eggs and stipples within cos lettuce leaves. Florida Entomologist. 78: 258-264.


Table 5.1 Mean settling of *Thrips tabaci* adults, immatures and oviposition by leaf length.

<table>
<thead>
<tr>
<th>Leaf length (cm)</th>
<th>3-leaf stage onion plant</th>
<th>6-8 leaf onion plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adults</td>
<td>Immatures</td>
</tr>
<tr>
<td>0</td>
<td>0.41a</td>
<td>0.65a</td>
</tr>
<tr>
<td>2</td>
<td>0.06b</td>
<td>0.17b</td>
</tr>
<tr>
<td>4</td>
<td>0.05bc</td>
<td>0.09bc</td>
</tr>
<tr>
<td>6</td>
<td>0.02cd</td>
<td>0.15bc</td>
</tr>
<tr>
<td>8</td>
<td>0.02d</td>
<td>0.17b</td>
</tr>
<tr>
<td>10</td>
<td>0.01de</td>
<td>0.12bc</td>
</tr>
<tr>
<td>12</td>
<td>0.01de</td>
<td>0.12bc</td>
</tr>
<tr>
<td>14</td>
<td>0.01de</td>
<td>0.18b</td>
</tr>
<tr>
<td>16</td>
<td>0.01de</td>
<td>0.12bc</td>
</tr>
<tr>
<td>18</td>
<td>0.01de</td>
<td>0.05bc</td>
</tr>
<tr>
<td>20</td>
<td>0.01de</td>
<td>0c</td>
</tr>
<tr>
<td>22</td>
<td>0de</td>
<td>0c</td>
</tr>
<tr>
<td>24</td>
<td>0e</td>
<td>0c</td>
</tr>
<tr>
<td>26</td>
<td>0e</td>
<td>0c</td>
</tr>
<tr>
<td>28</td>
<td>0e</td>
<td>0c</td>
</tr>
<tr>
<td>30</td>
<td>0e</td>
<td>0c</td>
</tr>
</tbody>
</table>

Means (n=12) within the columns are not significant if followed by the same letter (LSD, P<0.05).
Figure 5.1 Leaf positions for the onion seedling to determine settling by *Thrips tabaci* adults and immatures.
Figure 5.2 Potted 3-leaf onion plants placed in an insect cage measuring 47.5×47.5×47.5 cm
Figure 5.3a Settling of *Thrips tabaci* adults and immatures on L1 (innermost leaf) at 3-leaf stage onion plant.
Figure 5.3b Settling of *Thrips tabaci* adults and immatures on L 2 (inside leaf) at 3-leaf stage onion plant.
Figure 5.3c Settling of *Thrips tabaci* adults and immatures on L3 (outermost leaf) at 3-leaf stage onion plant.
Figure 5.4a Oviposition of *Thrips tabaci* on L1 (innermost leaf) at 3-leaf stage onion plant.
Figure 5.4b Oviposition of *Thrips tabaci* on L 2 (inside leaf) at 3-leaf stage onion plant.
Figure 5.4c Oviposition of *Thrips tabaci* on L3 (outermost leaf) at 3-leaf stage onion plant.
Figure 5.5a Settling of *Thrips tabaci* adults and immatures on L1 (innermost leaf) at 6-8 leaf stage onion plant.
Figure 5.5b Settling of *Thrips tabaci* adults and immatures on L 2 (inside leaf) at 6-8 leaf stage onion plant.
Figure 5.5c Settling of *Thrips tabaci* adults and immatures on L3 (outer leaf) at 6-8 leaf stage onion plant.
Figure 5.6a Oviposition of *Thrips tabaci* on L1 (innermost leaf) at 6-8 leaf stage onion plant.
Figure 5.6b Oviposition of *T. tabaci* on L 2 (inside leaf) at 6-8 leaf stage onion plant.
Figure 5.6c Oviposition of *Thrips tabaci* on L 3 (outer leaf) at 6-8 leaf stage onion plant.
CHAPTER 6

WITHIN LEAF DISTRIBUTION OF THRIPS EGGS AND *IRIS YELLOW SPOT VIRUS*

ON ONION FOLIAGE IN THE FIELD\(^1\)

---

\(^1\)Chitturi, A., Riley, D. G., Claudia, N., Gitaitus, R., and Mullis, S. 2010. To be submitted to *J. Entomological Science*. 
Abstract

Studies were conducted to determine the within-leaf distribution of thrips eggs and IYSV in the field. The distribution of eggs was observed relative to crop age, leaf position and 2 cm leaf segments relative to leaf length from the leaf sheath at the distal end of the bulb to the leaf tip. The within leaf distribution of the eggs was generally skewed towards the base of the plant. The within leaf distribution of IYSV in onion plants was determined in the field and greenhouse by testing leaf segments for the presence of virus by DAS ELISA. In 2007, the distribution of IYSV was not evenly distributed within the onion leaves and also skewed towards the base of the onion plant. In 2009 the number of onion leaf segments that tested IYSV positive in the field samples as well in the greenhouse samples was significantly higher on the leaf segments within 2-10 cm from the sheath at the base of the onion plant with less number of positive IYSV leaf segments in the middle and towards the tip of the leaves. Results from these studies suggest that the distribution of thrips eggs and IYSV positive segments can display similar patterns.

Key words: Within leaf distribution, Onion, *Thrips tabaci*, Egg distribution, IYSV distribution.
Introduction

*Iris yellow spot virus* (IYSV) genus *Tospovirus*, family Bunyaviridae has been detected as a potential devastating pathogen of onion (*Allium cepa*) infecting all major onion growing regions worldwide (Smith et al. 2006). IYSV was first reported from Netherlands (Cortês et al. 1998) and is considered to be transmitted exclusively by onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) (Nagata et.al 1999, Kitzman et al. 2001). Economic damage due to IYSV in onion is variable, but it has been reported to cause up to 100% loss in 1999 (Pozzer et al. 1999) and up to 60% losses in onion bulb production (Kitzman et al. 2001). In recent years, the estimated economic losses due to outbreaks of IYSV in bulb and seed onion crops in the USA are reported to be $480,000 (Pappu et al. 2009). Considering the distribution and spread of IYSV, studies have been conducted to determine the incidence and distribution of virus in onions (Kitzman et al. 2001) and leek (Smith et al. 2006). Studies conducted in both crops indicated the distribution of virus was localized and not uniform within the leaves on the plant. In onion, IYSV has been reported to occur in greater frequency close to the bulb near the base of the leaves (Kitzman et al. 2001). Recent studies conducted in Australia on the within plant distribution of onion thrips indicated that eggs were concentrated above the basal sections of the onion plant (Mo et al. 2008). Based on these studies, it was hypothesized that the oviposition sites of thrips on the onion leaf were related to the within leaf distribution of IYSV on the plant.

It is proposed that oviposition is a relatively good indicator of thrips settling and feeding which in turn should be related to the distribution of IYSV on onion foliage if infection is mainly localized around the point of inoculation. The main objective of this study was to quantify the within leaf distribution of *T. tabaci* eggs and IYSV on onion plants in the field.
Materials and Methods

Thrips egg distribution in onion plants: For thrips egg distribution measurements, 10 randomly selected onions plants were collected biweekly during March, April and May of 2009 in the field plots at the Vidalia Onion and Vegetable Research Center (VORC), Reidsville, GA. Onion plants were cut close to the ground, sealed in a zip lock bag and brought back to the Coastal Plain Experiment Station, Tifton, GA for quantifying the egg laying sites in the leaf tissue. All the leaves were stained and counted for eggs. But the eggs were seen only in the younger leaves and hence thrips eggs were quantified only in the center three youngest leaves for all the samples. The leaf positions for all the stages of onions sampled in the onion plant were designated L1 (youngest leaf) followed by L2 and L3 (third from center). To determine the oviposition sites, the entire intact leaf was cut from the plant and by following the lacto- phenol acid fuschin staining technique detailed by Nuessly et al. (1995) and Parella and Rob (1982) onion leaves were decolorized by boiling 3-5 minutes while working under a fume hood. Stained leaves were cooled for 3-5 hrs and excess stain was removed with warm water and then examined under a stereo microscope for thrips eggs and oviposition sites indicated by purple rings. Thrips eggs and egg laying sites were counted for every 2cm length from the split at the base of the onion plant.

IYSV distribution in onion plants: Distribution of IYSV within the onion foliage was determined from field collected onions at VORC, Reidsville, GA and in greenhouse at the Coastal Plain Experiment station, Tifton, GA. IYSV in mature onion plants was measured by selecting the onion plants at random during the fall of 2007 and 2009. Onion plants were cut close to the ground, sealed in a zip lock bag and brought back to the lab to determine the presence of IYSV by leaf segment. In the first year, 90 onion plants were sampled and all the
leaves with symptomatic lesions or signs of heavy thrips feeding were selected for IYSV testing. Each leaf was cut into 2.5 cm sections and tested for the presence of IYSV by double-antibody sandwich enzyme linked immunosorbent assay (DAS ELISA). In the second year, 10 randomly selected onion plants were collected during mid March and mid April and three leaves from each plant were cut into 2 cm sections and tested for the presence of IYSV by DAS ELISA.

Experiments to determine distribution of IYSV in the greenhouse plants was done at the Coastal Plain Experiment Station, Tifton, GA. Onion bulbs with viruliferous *T. tabaci* were collected from cull piles in Reidsville, GA and bulbs were potted in 10 cm pots using ‘LT 5 Mix’ (Sun Gro Horticulture Dist. Bellevue, WA). Onion bulbs with viruliferous thrips were maintained in the greenhouse and allowed to grow. Plants raised from the onion bulbs were grown in the greenhouse for natural infection of IYSV by the viruliferous *T. tabaci* populations. Five onion plants at 6-8 leaf stage were selected at random and three leaves from each plant were cut into 2 cm sections and tested for the presence of IYSV by DAS ELISA. The average absorbance values of the negative checks at 405nm plus four standard deviations were considered as a positive threshold for IYSV in the experiments (Bandla et al. 1994). For determining the within-leaf distribution of IYSV in onion plants, a total of 96 leaves from both field and greenhouse were tested for every 2 cm leaf segments relative to leaf length.

Data on the egg and IYSV distribution were analyzed using PROC GLM (SAS Institute 1990). Fisher’s least significant difference method was used for determining treatment differences using PROC GLM with $\alpha = 0.05$.

**Results**

**Thrips distribution of eggs in onion plants:** The summed distribution of thrips eggs for the onion plants collected in the field analyzed by 2 cm leaf segments clearly showed a
distribution skewed towards the base of the onion plant (Fig. 6.1). Egg distribution analyzed by date starting from 2-3 leaf stage in late February indicated that the eggs were spread out across the leaf length with 43% of eggs concentrated on the leaf segment 2-10 cm (Fig. 6.2). Eggs were spread out across the leaf length in the middle and towards the tip of the leaves. A significant treatment effect was observed on thrips egg distribution and leaf length in cm ($F = 1.63, df = 24, 417, P = 0.0318$). The mean number of eggs sampled during early March tended to be towards the base of the onion leaf with 82% of the eggs on 2-16 cm of the leaf segments (Fig. 6.3). There was no significant treatment effect observed on thrips egg distribution and leaf length in cm ($F = 1.39, df = 20, 364, P = 0.1218$). The mean number of eggs during mid March followed a similar pattern of distribution in the eggs (Fig. 6.4) with no significant treatment effect ($F = 1.30, df = 23, 600, P = 0.1595$). The distribution of eggs during late March indicated a more spread out pattern of the eggs across the leaf length with 58% of eggs concentrated on the leaf segments 14-34 cm (Fig. 6.5). Eggs were spread out across the leaf length towards the base and tip of the leaves. There was a significant treatment effect on thrips egg distribution and leaf length in cm ($F = 1.56, df = 30, 522, P = 0.0313$). The distribution of eggs in the field on the onion plants during mid April were skewed towards the base of the plant (Fig. 6.6). 78% of the eggs were concentrated on the leaf segments 2-24 cm followed by eggs distribution towards the tip of the leaves. There was no significant treatment effect on thrips egg distribution and leaf length in cm ($F = 1.03, df = 25, 687, P = 0.4183$). The egg distribution in late April was bimodal with 56% of eggs concentrated on 0-16 cm leaf segments followed by egg distribution towards the middle and tip of the leaves (Fig. 6.7). There was a significant treatment effect on thrips egg distribution and leaf length in cm ($F = 2.58, df = 21, 416, P = 0.0002$). This is when many of the leaves in the field are “flagging,” bending in the middle of the leaf creating habitat for thrips in the leaf fold. By the
end of season in early May the distribution of eggs followed the earlier pattern with egg
distribution skewed more towards the base of the plant (Fig. 6.8). 70% of the egg distribution
was concentrated 2-16 cm leaf segments with egg distribution in the middle and none towards
the tip of the leaves. There was no significant treatment effect on thrips egg distribution and leaf
length in cm ($F= 1.00, df= 23, 456, P= 0.4668$).

The mean number of eggs distributed in the field relative to leaf position L1 (youngest
leaf in the plant) were skewed towards the base of the plant (Fig. 6.9). 69% of the eggs were
concentrated on 2-14cm leaf segments. There was a significant treatment effect on the egg
distribution relative to leaf length in cm and leaf position ($F= 3.36, df= 17, 903, P= <0.0001$).
Egg distribution within the leaf position L2, followed the earlier pattern with 63% of eggs were
concentrated on 2-22cm leaf segments (Fig. 6.10) with a significant treatment effect on the egg
distribution relative to leaf length in cm and leaf position ($F= 1.63, df= 30, 1514, P= 0.0174$).
For the leaf position L3, the distribution of eggs was skewed towards the base of the plant (Fig.
6.11). 91% of the eggs were concentrated on 10-18 cm leaf segments. There was a significant
treatment effect on the egg distribution relative to leaf length in cm and leaf position ($F= 0.71,
df= 23, 1161, P= 0.8391$). Over all, the sum total of eggs analyzed by date, leaf position and
every 2 cm relative to leaf length from the split at the base of the onion plant indicated that the
egg distribution was skewed more towards the base of the onion plant (Fig. 6.1).

**IYSV distribution in onion plants:** Results on the distribution of IYSV within the onion
foliage collected from the field samples during spring 2007 indicated that IYSV is extremely
clumped (Fig. 6.17) towards the base of onion leaf tissue relative to leaf length ($F=4.30, df= 19,
492, P=<0.0001$). The results from mid March 2007, indicate that IYSV distribution within the
leaf was highly variable and the distribution of the virus was skewed towards the base of onion
plant from the split at the base of the onion plant (Fig. 6.12). Over all, the total number of IYSV positive onion leaf segments analyzed over all the leaves in mid March indicated that the number of IYSV positive segments were 63% higher on 5.1-15 cm leaf segments with a less distribution of the virus in the middle and towards the tip of the onion leaves. Only 27.5% of individual 2.54 cm segments (n=527) tested positive and the range ran from a low of only 5% of segments testing positive to a high of 91% of the leaf segments testing positive. There was no leaf in which 100% of the segments tested positive.

Results on the distribution of IYSV within the onion foliage collected from the field samples during mid March and mid April of 2009 indicated that the distribution of the IYSV was random, but still biased towards the base of the onion plant (Fig. 6.13). The number of onion leaf segments that tested positive per leaf relative to leaf length in mid March were 71% more on 2-18 cm leaf segments followed by 25% on 20-24 cm leaf segments. There was no significant effect on the number of IYSV positive leaf segments and leaf length (F= 0.98, df= 17, 167, P= 0.4858). In mid April, the number of onion leaf segments that tested positive were 71% more on 2-26 cm leaf segments with less number of positive leaf segments towards tip of the leaves (Fig. 6.14), but again the effect was only marginally significant (F= 1.34, df= 25, 189, P= 0.1389). Over all, the total number of IYSV positive onion leaf segments analyzed over all the leaves and both dates (Fig. 6.15) indicated that the distribution of IYSV was 80% more on 2-24cm leaf segments. There was no significant treatment effect observed on the number of IYSV positive leaf segments and leaf length (F= 1.15, df= 25, 378, P= 0.2835).

Results on the distribution of IYSV within the onion foliage collected from the greenhouse during fall 2009 indicated a bimodal distribution of the IYSV towards the base and middle of the onion plant (Fig. 6.16) The number of onion leaf segments that tested positive per
leaf relative to leaf length was 66% more on 2-18 cm leaf segments with less number of positive leaf segments towards the tip of the leaves. However, no significant treatment effect was observed on the number of IYSV positive leaf segments and leaf length ($F= 1.14$, $df=19$, 332, $P= 0.3088$). The distribution on sprouted bulbs was likely more random due to IYSV over a longer period of time both during bulb formation and later in the greenhouse. Comparing sums from Fig. 6.1 and Fig 6.12, the within–leaf distribution of eggs on the onion plants significantly correlated with the number of IYSV positive segments for the field samples tested in fall 2007 ($R= 0.92$, $P<0.001$). Similarly a significant correlation was observed for both field and greenhouse samples collected during fall 2009 ($R= 0.80$, $P<0.001$). Thus there were some similarities between egg and IYSV distributions within the plant relative to leaf length from the split at the base of the onion plant.

**Discussion**

Within-leaf distribution of thrips eggs in the field was skewed more towards the base of the onion plant. Results on the distribution of IYSV within the onion foliage collected from the field samples during 2007, 2009 and the greenhouse indicated that IYSV is extremely clumped in the onion leaves. However, the number of onion leaf segments that tested IYSV positive per leaf from the base of the plant in the field samples, as well in the greenhouse samples tended towards the base of the onion plant with less number of positive IYSV leaf segments in the middle and towards the tip of the leaves. Thus, initially it appears that where there are more thrips oviposition sites there is higher incidence of IYSV in onion leaf tissue. There was no leaf in which 100% of the segments tested positive. These results show that distribution of detectable virus in onion leaves is highly variable and localized in distribution. We propose that the
distribution of IYSV in onion leaf tissue is highly associated with thrips presence on the leaf, i.e.
IYSV has limited systemic distribution in the onion plants and is concentrated near the points of
inoculation by thrips.

Acknowledgements

We thank the Plant pathology department at Coastal Plain Experiment Station, Tifton for
providing facilities to run the ELISA experiments. We thank the Vegetable Entomology staff
Jackie Davis and Donnie Cook for their help in collecting the cull pile onions at Reidsville, GA
and student workers Jessica Kalina, Sarah Williford and Ronnie Smith for their help in setting up
the experiments and maintaining the thrips colonies. Funding was provided by the Department of
Entomology, University of Georgia.
References Cited


Figure 6.1 Total number of thrips eggs sampled in the field relative to leaf length in 2009.
Figure 6.2 Mean number of thrips eggs sampled in the field during late February 2009.
Figure 6.3 Mean number of thrips eggs sampled in the field during early March 2009.
Figure 6.4 Mean number of thrips eggs sampled in the field during mid March 2009.
Figure 6.5 Mean number of thrips eggs sampled in the field during late March 2009.
Figure 6.6 Mean number of thrips eggs sampled in the field during mid April 2009.
Figure 6.7 Mean number of thrips eggs sampled in the field during late April 2009.
Figure 6.8 Mean number of thrips eggs sampled in the field during early May 2009.
Figure 6.9 Mean number of thrips eggs sampled in the field relative to leaf position L1.
Figure 6.10 Mean number of thrips eggs sampled in the field relative to leaf position L2.
Figure 6.11 Mean number of thrips eggs sampled in the field relative to leaf position L3.
Figure 6.12 Total IYSV positive leaf segments per length of onion leaf sampled in the field during mid March 2007.
Figure 6.13 Total IYSV positive leaf segments per length of onion leaf sampled in field during mid March 2009.
Figure 6.14 Total IYSV positive leaf segments per length of onion leaf sampled in the field during mid April 2009.
Figure 6.15 Total IYSV positive leaf segments per length of onion leaf sampled over both dates in the field during 2009.
Figure 6.16 Total IYSV positive leaf segments per length of onion leaf sampled in the greenhouse 2009.
Figure 6.17 IYSV incidence on onion leaf in the field relative to leaf length from base of the onion plant collected in 2007 (Each column represents an individual onion leaf in cm).
CHAPTER 7

THRIPS TRANSMISSION OF *IRIS YELLOW SPOT VIRUS* TO ONIONS ¹

¹Chitturi, A., Riley, D. G., Pappu, H. R., Bag, S., Mullis, S. 2010. To be submitted to *Plant Disease*
Abstract

Bioassays for transmission of Iris yellow spot virus (IYSV) with *Thrips tabaci* adults using onion seedlings were conducted at the Coastal Plain Experiment Station, Tifton, GA, using 3-leaf onion seedling placed in a glass test tube. Potentially viruliferous *T. tabaci* adults (collected from IYSV symptomatic positive plants) were placed on healthy 3-leaf onion seedlings and exposed at different lengths of time and using different numbers of viruliferous *T. tabaci* adults per plant. The onion seedlings used in the transmission studies were tested with DAS ELISA for percentage of IYSV transmission at different exposure times relative to number of thrips. The thrips used for the transmission studies were tested individually with NSs ELISA to determine the vector status of each *T. tabaci* adult. The proportion of confirmed vectors to successful transmission events based on plant ELISA tests was then compared. Results from the transmission studies indicated that 64% IYSV transmission can occur very rapidly, within 15 minutes. After 6 hours over 80% of the thrips transmissions were successful, confirming that *T. tabaci* is a highly efficient vector of IYSV in onions. DAS ELISA positive transmissions correlated with NSs positive vectors (R=0.66).

**Keywords:** Iris yellow spot virus, *Thrips tabaci*, Onion thrips, transmission efficiency, DAS ELISA, NSs ELISA.
Introduction

*Iris yellow spot virus* (IYSV) a distinct member of the genus *Tospovirus*, family *Bunyaviridae*, is a plant virus vectored by thrips (*Thysanoptera*: Thripidae). Currently, only one vector species in the genera of Thrips, *Thrips tabaci* (onion thrips) Lindeman is confirmed to be the vector of IYSV (Nagata et al. 1999, Kitzman et al. 2001). IYSV was first reported in 1998 from Netherlands infecting Iris and leek (Cortês et al. 1998), subsequently virus has been reported infecting onion (*Allium cepa*) in many onion growing regions worldwide (Smith et al. 2006). Currently, IYSV infects at least 47 plant species (Gent et al. 2006), including onions (Gera et al. 1998, Pozzer et al. 1999), garlic (Robène-Soustrade, 2005), ornamental crops such as lisianthus (Kirtzman et al. 2001) and alstroemeria (Okuda et al. 2001), and certain weed species (Pappu et al. 2006, Sampangi et al. 2007, Nischwitz et al. 2007). IYSV is reported to be in 6 continents in 24 countries (CABI/EPPO, 2009). In Georgia, Vidalia sweet onions are a high value specialty crop cultivated as a short day winter crop generating an estimated farm gate value of $139 million annually (Boatright and Mc Kissick, 2009). IYSV was first observed in Vidalia onion growing region of the state in 2003 (Mullis et al. 2004). Since the first report of IYSV in 2003, no quantitative economic losses have been reported, however virus has been found every year in all the onion growing regions in the state (Nischwitz et al. 2007). In Georgia onions, primarily three thrips species, *Frankliniella fusca* (tobacco thrips), *F. occidentalis* (western flower thrips) and *T. tabaci* (onion thrips) have been reported to occur (Riley and Sparks 2004, Sparks et al. 2005). *Frankliniella fusca* and *F. occidentalis* are the major vectors species of *Tomato spotted wilt virus* (TSWV) in multiple cropping systems of the state (Riley and Pappu 2000, 2004) while *T. tabaci* is the only vector of IYSV reported in onions (Mullis et al. 2004) and the weed species, Spiny Sow thistle (*Sonchus asper*) (Nischwitz et al. 2007). Like other tospoviruses (Ullman et al. 2002, Whitfield et al. 2005), IYSV is acquired and transmitted by
second larval instars and adults after circulation and replication in the vector (Ullman et al. 1992, Wijkamp et al. 1993). Studies were conducted on the transmission of IYSV by *T. tabaci* in onion seed crop (Bulajić et al. 2009), onion seedlings (Kitzman et al. 2001) and Lisianthus (Kitzman et al. 2000). Even though the transmission of IYSV by *T. tabaci* is known (Kritzman et al. 2001), the efficiency of transmission has not been well worked out. To the best of our knowledge there are no published data on the length of exposure time, relative to numbers of viruliferous thrips required to transmit IYSV to onions.

Tomato spotted wilt virus (TSWV) in viruliferous thrips using antibodies to TSWV structural proteins (Allen et al. 1991, Cho et al. 1989, Cho et al. 1991). These proteins are present in the digestive tract of the insect that has fed on virus infected plant (Ullman et al. 1992). Detection of virus specific non structural protein (NS) could be used to distinguish thrips that have ingested the virus but cannot transmit the virus from those thrips where virus is replicated resulting in transmissability of the virus (Bandla et al. 1994). We tested the individual thrips used in these studies with NSs ELISA to confirm their vector status. In order to determine the transmission of IYSV by *T. tabaci* adults, the proportion of NSs ELISA confirmed vectors to successful transmission events based on plant ELISA tests and symptomology was compared.

The main objectives of this study were i) to determine the length of time required for IYSV transmission, ii) to determine the number of thrips required for successful transmission of IYSV to occur on the host plant, and iii) to compare IYSV in individual thrips using non structural enzyme-linked immunosorbant assay (NSs ELISA) to plant transmission data confirmed with DAS ELISA.
Materials and Methods

Bioassays for transmission of *Iris yellow spot virus* using onion seedlings were conducted in spring 2009 at the Coastal Plain Experiment Station, Tifton, GA. A simple bioassay transmission protocol was developed where 3-leaf onion seedlings were placed into a 20 × 150 mm glass test tube (Fisher brand disposable culture tubes) with roots wrapped in a wet cotton ball to provide enough moisture for the seedling. Potentially viruliferous adult *T. tabaci* collected from infected IYSV onion plants were placed in the test tube using a fine (# 0) brush, and the test tube was sealed with a super fine (200 mesh) copper screen (TWP Inc., Berkeley CA) wrapped around the edges with parafilm.

*Thrips tabaci colonies:* For source of virus, onion bulbs with viruliferous *T. tabaci* were collected from cull piles in Reidsville, GA and bulbs were potted in 4-inch pots using ‘LT 5 Mix’ (Sun Gro Horticulture Dist. Bellevue, WA). Onion bulbs with viruliferous thrips were maintained in the greenhouse and allowed to grow until symptoms appeared on the plants. Onion plants were tested with double antibody sand witch enzyme linked immunosorbant assay (DAS ELISA) for IYSV (Agdia, Inc Elkhart, IN) and *T. tabaci* adults collected from IYSV positive plants were used for transmission bioassay.

Virus free *T. tabaci* colonies were maintained on onion plants (Var Pegasus) potted in 10 cm pots and maintained in the laboratory under standard conditions. The plants were checked with DAS ELISA and *T. tabaci* adults from the virus free plants were used as the source for control individuals to check transmission tests.

Transmission assays: Healthy onion sets were procured from Arizona (Sunbelt Transplants Inc. Buckeye, AZ) and the seedlings were tested with DAS ELISA before using them for actual transmission studies. These clean seedlings were placed into 20 × 150 mm glass
test tube (Fisher brand disposable culture tubes) and the trimmed root ball was wrapped with a wet cotton ball to provide enough moisture for the seedlings. In the first bioassay, 4 potentially viruliferous *T. tabaci* adults (collected from IYSV symptomatic positive plants) were placed on healthy 3-leaf stage onion seedlings and exposed for different lengths of time and in the second bioassay, different numbers of *T. tabaci* adults were used to determine if thrips numbers affected transmission of IYSV. In both tests, thrips were collected after plant exposure into 1X PBST buffer for non structural protein enzyme linked immunosorbant assay (NSs ELISA) testing (Bag et al. 2010). The lengths of exposure time tested were 0 (check), 0.25, 0.5, 1, 6, 12 and 24 hours with an average 16 replications per treatment. The number of potentially viruliferous thrips tested were 1, 4, 8 and 12 adults with an average 54 replicates per treatment. Thrips were all allowed to feed for 5 days before removal from the plant. Due to shortage of 3-leaf onion seedlings, the second bioassay was carried out during mid March (03/17) and mid April (05/11). For transmission studies with single thrips adults were allowed to feed on the plant for 5 days and then thrips were collected off the plants in 1X PBST buffer for NSs ELISA testing and these results were compared to the DAS ELISA values of the onions from which they were collected. Seedlings were grown in the test tubes to allow time for virus development in the plant and the tested with DAS ELISA for the presence of IYSV.

**NSs ELISA:** Non structural enzyme-linked immunosorbant assay was used to detect IYSV in individual thrips. NSs antiserum was provided by Dr. Hanu Pappu, Department of Plant Pathology, Washington state University. Thrips collected in 1XPBST (sodium chloride 8gm, sodium phosphate dibasic 1.15g, potassium phosphate monobasic 0.2g, potassium chloride 0.2g, Tween 20 0.5g) were removed from the vials with a zero number brush and a single thrips was placed in each well on a 12 well Coors Tek spot plate (Coors Tek Inc). Individual thrips were
ground with a 1.5 ml microcentrifuge plastic pestle (USA Scientific Plastics) in 50 µl of ELISA extraction buffer (0.01M sodium phosphate potassium buffer Ph 7.4, containing 0.02% sodium azide (w/v), 0.8% sodium chloride (w/v), 0.05% Tween 20 (v/v) and 2% polyvinyl pyrrolidone mol wt 40,000 (w/v) (MP Biomedicals LLC, Solon, OH) added per well. The buffer extract is transferred to 96 well flat bottom immune plate (Nalge Nunc International, Rochester, NY) and four to six negative controls (healthy *T. tabaci* adults), two positive controls (*T. tabaci* from IYSV positive plants) and one well of extraction buffer was added to the plate and incubated at 37ºC for 2 hours. Plate is washed three times with 1XPBST allowing 3 minutes incubation for each wash at room temperature. The plate is then blocked with Bovine Serum Albumin (BP 1600 Fisher Bio reagents, 1%BSA) in 1XPBS (0.8% sodium chloride (w/v), 0.2%, potassium dihydrogen phosphate (w/v), 0.14% sodium phosphate dibasic (w/v) for 2 hours and incubated at 37ºC. The plate was washed three times as stated earlier then 75 µl of primary antibody (NSs) was diluted in dilution buffer, pH 7.4 (1X PBST with 0.2% BSA (w/v), 2% PVP (w/v) and 0.02% sodium azide (w/v), stored at 4 ºC) and incubated at 37ºC for 2 hours. The plate was washed for three times as stated above. Secondary antibody (IgG-alkaline phosphatase) (goat-anti rabbit Sigma A7539) (Dilution -1:5000) was added and incubated at 37ºC for 2 hours. The plate was washed for three times as stated above. Then 75 µl of substrate solution (1mg/ml pNPP in 1M diethanolamine buffer containing 0.5Mm MgCl2 and 0.02% sodium azide) was added and incubated at 37ºC for 1 hour. The ELISA readings were measured spectrophotometrically at 405nm using an ELx 800 plate reader (Bio-Tek Instruments, Winooski, VA). The average absorbance values of the negative checks at 405nm plus four standard deviations were considered as a positive threshold for IYSV in the experiments (Bandla et al 1994). Data on the IYSV transmission studies was analyzed using PROC GLM (SAS Institute 1990). Fisher’s least
significant difference method was used for determining treatment differences using PROC GLM with $\alpha = 0.05$. A single analysis of variance was conducted for all test runs using individual plants as replicates.

**Results**

The bioassays conducted on IYSV transmission studies documented an unusually high level of virus infection in a *T. tabaci* colony (70 to 80%) in the greenhouse at the Coastal Plain Experiment Station at Tifton, GA. In a preliminary bioassay 88% of transmission was achieved using a minimum of 4 thrips per plant from this highly viruliferous colony. Results from the time transmission studies indicate that IYSV transmission can occur very rapidly, with 15 minutes providing 64% successful transmission (Fig. 7.1). The percentage of successful transmission for the other lengths tested were 70% for 30 min, 70% for 1 hour, 80% for 12 hours and 88% for 24 hours. There was a significant treatment effect observed with the percentage of IYSV transmission by thrips and different time lengths ($F= 21.73$, $df= 8$, 133, $P=<0.0001$). Also, the plant ELISA absorbance values were significantly affected by the exposure time. The absorbance values at 405 nm ranged from 0.2, 0.56, 0.78, 0.76, 0.83 to 0.99 at 0.25, 0.5, 1.0, 6.0, 12.0 and 24 hours, respectively. There was a significant effect on the plant ELISA absorbance values and exposure time ($F= 6.70$, $df= 8$, 133, $P= <0.0001$). The percentage of IYSV transmission increased with increasing exposure time, but it was clear that after 0.5 hours, there was little improvement of transmission efficiency. The DAS ELISA absorbance value at 405 nm clearly indicated a higher absorbance value with increase in time. However, we are not sure how this specifically relates to virus titer in the plant.

NSs ELISA for individual thrips in the 0.25, 0.5, 12 and 24 hour time treatments did vary with individual thrips from 0.25, 0.5 and 24 hours treatment testing 100% positive while 12 hour
treatment tested only 50% positive. Similarly there was a significant treatment effect observed for the percentage of NSs positive thrips and different time intervals \((F= 12, \ df= 4, 15, P=0.0001)\). However, with the total percentage of viruliferous thrips being high (93%) and using 4 thrips per time interval, there was no likely effect from low virus frequency.

The second bioassay with different thrips numbers indicated that IYSV transmission can occur with one viruliferous \textit{T. tabaci} adult with 90% transmission efficiency when thrips were allowed to feed for 5 days (Table 7.1). The different thrips numbers tested for IYSV transmission had no significant effect on the transmission \((F= 147.23, \ df= 7, 395, P=0.0001)\), again due to the high percent of viruliferous thrips. With one adult we did observe the lowest absorbance value of any the treatments (0.41 at 405nm). For 4 thrips and greater there was a corresponding increase in the absorbance values (Table 7.1). If anything, the results from the IYSV transmission studies with different numbers showed a tendency of decreasing % transmission with more thrips, so 1-4 thrips is sufficient when dealing with a highly viruliferous population of \textit{T. tabaci}. There was a significant treatment effect observed for the DAS ELISA values across different thrips numbers \((F= 30.26, \ df= 7, 395, P=0.0001)\).

Check assays for IYSV transmission were conducted with virus free \textit{T.tabaci} adults for different thrips number treatments. Results from the check tests indicated that all of the plants tested negative for IYSV following feeding bioassays with 1, 4, 8 and 12 virus free thrips (Table 7.1). For the check treatments we used 35 plants for 1 thrips, 40 plants for 4 thrips, 47 plants for 8 thrips and 38 plants for 12 thrips to test for IYSV transmission. The DAS ELISA absorbance value at 405nm for the different treatments tested were all low indicating the absence of IYSV in plant tissue.

The NSs ELISA test for individual thrips within the different treatments confirms that
overall there was a relatively high percentage of viruliferous adults used for the second bioassay (61%). The individual thrips from the transmission studies with different thrips numbers testing positive with NSs were 11 out of 20 thrips for 1-thrips, 34 out of 44 thrips for 4-thrips, 17 out of 28 thrips for 8-thrips and 8 out of 22 thrips for the 12-thrips treatment tested positive (Table 7.2). However, percentage of NSs positive thrips for different thrips treatment groups did vary significantly. There was no significant treatment interaction observed for the percentage of NSs positive thrips and different thrips numbers ($F= 3.86, df= 3, 110, P=<0.0115$).

To compare IYSV in individual thrips using plant transmission data confirmed with DAS ELISA to NSs ELISA, the thrips treatment were analyzed using correlation coefficient. The positive IYSV transmission as detected by DAS ELISA significantly correlated with NSs positive individuals ($n=55, R=0.66, P<0.0001$). We observed that 22% of the observations on the % viruliferous as determined by DAS ELISA plant testing vs. direct testing of thrips with NSs did not match, but of these 22%, 92% were positive NSs with a negative DAS. Thus this could just represent unsuccessful transmission events. Also, there was a lack of consistency in the percent viruliferous in the experiments. We determined that this variation was coming mainly from the two sample dates. The bulk of the thrips samples tested for NSs ELISA were collected from two different sample dates (Table 7.2, 7.3). The thrips populations collected during the second week of March had high percentage viruliferous $T. tabaci$ adults which was indicated by a high percentage of 100% NSs positive thrips (Table 7.2) while the thrips populations from second week of May were less viruliferous testing significantly low only 36% of individual thrips were NSs positive (Table 7.3). This confounded treatment effects with colony effects.
**Discussion**

Transmission studies for IYSV indicate that transmission of IYSV to onions can also occur in as little as 15 minutes feeding time with viruliferous *T. tabaci*. If IYSV infected onion thrips are present in significant numbers early in the growing season, there is a high likelihood of significant infection of onions. With such a rapid transmission period, management options for the thrips vectors are limited to prevention (e.g., host plant resistance to IYSV, inhibition of thrips feeding, prevention of thrips alighting on plants, reduction viruliferous thrips populations before the onion crop is planted (Riley et al 2000, 2004). One such example of a preventative cultural control in Georgia that is currently being practiced is the destruction of IYSV/thrips infested cull piles. This is critical if the onion seedling/transplant production fields are located close to these sources of infection. Early inoculation of the virus to onion plants can potential cause greater losses directly to the crop and provide inoculums for secondary spread of viruliferous thrips across the onion production region.

**Acknowledgements**

We thank the Plant pathology department and Entomology-Virology lab at Coastal Plain Experiment Station, Tifton for providing facilities to run the ELISA experiments. We thank the Vegetable Entomology staff Jackie Davis and Donnie Cook for their help in collecting the cull pile onions at Reidsville, GA and student workers Jessica Kalina, Sarah Williford and Ronnie Smith for their help in setting up the experiments and maintaining the thrips colonies. Funding was provided by the Department of Entomology, University of Georgia.
References Cited


Table 7.1 Transmission of *Iris yellow spot virus* (IYSV) to healthy onion plants with different number treatments and check treatments of adult *T. tabaci* as detected by DAS ELISA.

<table>
<thead>
<tr>
<th>Thrips treatment</th>
<th>N</th>
<th>% DAS positive</th>
<th>DAS ELISA reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>90a</td>
<td>0.41c</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>90a</td>
<td>0.77b</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>88a</td>
<td>0.79b</td>
</tr>
<tr>
<td>12</td>
<td>58</td>
<td>82a</td>
<td>1.0a</td>
</tr>
<tr>
<td><em>C1</em></td>
<td>35</td>
<td>0c</td>
<td>0.28cd</td>
</tr>
<tr>
<td><em>C4</em></td>
<td>40</td>
<td>0c</td>
<td>0.33cd</td>
</tr>
<tr>
<td><em>C8</em></td>
<td>47</td>
<td>0c</td>
<td>0.17de</td>
</tr>
<tr>
<td><em>C12</em></td>
<td>38</td>
<td>0c</td>
<td>0.05e</td>
</tr>
</tbody>
</table>

1. Means within the columns are not significant if followed by the same letter, LSD Test, $P<0.05$.

2. *C 1, 4, 8, 12 were check treatments with different non-viruliferous thrips numbers.
Table 7.2 Detection of *Iris yellow spot virus* (IYSV) in thrips using the non structural protein enzyme-linked immunosorbent assay (NSs ELISA) with different numbers of thrips transmitted to healthy onion plants conducted during mid March 2009.

<table>
<thead>
<tr>
<th>Thrips treatment</th>
<th>N</th>
<th>% NSs positive</th>
<th>NSs ELISA reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Infected</td>
<td>20</td>
<td>100a</td>
<td>0.92b</td>
</tr>
<tr>
<td>4-Infected</td>
<td>44</td>
<td>77a</td>
<td>2.03a</td>
</tr>
</tbody>
</table>

Means within the columns are not significant if followed by the same letter, LSD Test, $P<0.05$. 
Table 7.3 Detection of *Iris yellow spot virus* (IYSV) in thrips using the non structural protein enzyme-linked immunosorbent assay (NSs ELISA) with different numbers of thrips transmitted to healthy onion plants conducted during early May 2009.

<table>
<thead>
<tr>
<th>Thrips treatment</th>
<th>N</th>
<th>% NSs positive</th>
<th>NSs ELISA reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Infected</td>
<td>28</td>
<td>61a</td>
<td>2.09a</td>
</tr>
<tr>
<td>12-Infected</td>
<td>22</td>
<td>36a</td>
<td>0.43b</td>
</tr>
</tbody>
</table>

Means within the columns are not significant if followed by the same letter, LSD Test, $P<0.05$. 
Figure 7.1 Transmission of IYSV by adult *Thrips tabaci* to healthy onion plants at different time intervals.
CHAPTER 8

SUMMARY

This dissertation focused on five objectives relative to *Thrips tabaci* as a major thrips vector of IYSV in onions in India and Georgia, USA.

The first objective was to survey thrips in vegetable production systems where tospoviruses occurs. The first survey was conducted in India for two years 2006-2008, collecting the potential thrips vector species of tospoviruses in onions and other vegetables cropping systems. A second survey was conducted in Georgia, USA on onions for potential thrips vectors of IYSV. Different thrips vector species infesting the vegetable crops were collected by three sampling techniques, beat cup method for plant foliage, plant samples and whole plant inspection. Five potential tospovirus vector species of thrips were identified in the survey and the tospovirus occurrence was listed for each of the states by crop to suggest possible associations. The distribution of thrips genera across the crops surveyed showed a dominance of *Thrips* sp. in onion, *Scirtothrips* sp. in chili pepper and *Frankliniella* sp. in tomato. In Georgia, this survey identified onion thrips, *Thrips tabaci*, as the main vector species of IYSV present in onion. *Frankliniella fusca* was also found as a prevalent pest of onions in Georgia.

The second objective was to investigate the thrips competition between the two main thrips species in Georgia onions, *T. tabaci* (onion thrips) and *F. fusca* (tobacco thrips). Thrips competition studies were conducted under laboratory conditions to determine if the onion thrips *T. tabaci*, a biotype introduced to Georgia from Casma onion production region in Peru, would have a reproductive advantage or disadvantage when competing with another dominant thrips species on onions, *F. fusca*. Two sets of experiments were conducted over two years to test for
possible negative effects on thrips reproduction on onion plants. Also a field study was conducted to determine the occurrence of the common of two thrips species, *T. tabaci* and *F. fusca* on onion plants in the field. The Peruvian biotype of *T. tabaci* used in these tests was not affected reproductively by the presence *F. fusca* on onions. In fact, *T. tabaci* reproduced more vigorously than *F. fusca* when the two species were confined on an onion plant. Field studies on the occurrence of the thrips species *T. tabaci* and *F. fusca* indicated that both species tended to segregate from each other on the top and middle portions of the onion foliage. Thus, thrips competition is detectable both in the lab and under field conditions.

For the third objective, the behavioral patterns of *T. tabaci*, distribution, settling and oviposition were investigated on onion foliage relative to leaf position and leaf length at pre-bulbng stages of the crop. Settling and oviposition behavior of *T. tabaci* were investigated at the 3- leaf stage and the 6-8 leaf stage of onion plant relative to different leaf positions and every 2cm segment of the leaf length. Both the adults and immatures of *T. tabaci* showed a strong preference for settling toward the base of the plant near the wrapped leaf sheath in both the young 3 leaf stage onions and the older 6-8 leaf stage. Oviposition of *T. tabaci* by leaf length on 3- leaf stage onions and 6-8 leaf onion plant also showed a preference towards the base of the plant.

For the fourth objective, studies were conducted to quantify the within-leaf distribution of *T. tabaci* eggs and IYSV in the field. The distribution of eggs was observed relative to crop age, leaf position and 2 cm leaf segments relative to leaf length from the leaf sheath at the distal end of the bulb to the leaf tip. Distribution of IYSV within the onion foliage was determined in the field and in the greenhouse for 2007 and 2009. The within distribution of the eggs was skewed more towards the base of the plant in 2009. The distribution of IYSV was skewed towards the
base of the onion plant in 2007 and also appeared to be more random within onion leaves in 2009. Overall, there were similarities between the distribution of the virus and thrips eggs relative to onion leaf length. IYSV appeared to be highly clumped at any sample time, unlike what we would expect for a highly systemic virus.

For the fifth objective, two bioassays were conducted for transmission of IYSV with *T. tabaci* adults using 3-leaf onion seedlings. In the first bioassay, 4 potentially viruliferous *T. tabaci* adults (collected from IYSV symptomatic positive plants) were placed on healthy 3-leaf onion seedlings and exposed at different lengths of time and in the second bioassay, different numbers of 1, 4, 8 and 12 viruliferous *T. tabaci* adults were used to determine thrips transmission of IYSV. The thrips used for the transmission studies were tested individually with NSs ELISA to determine the IYSV vector status of *T. tabaci* adults. The proportion of confirmed vectors to successful transmission events based on plant ELISA tests in the experiments were highly correlated. IYSV transmission studies indicated that IYSV transmission can occur very rapidly within 15 minutes with 64% transmission efficiency and DAS ELISA confirmed transmissions correlated with NSs confirmed vectors.

*Thrips tabaci* appears to be the main vector of IYSV in both Georgia and India. Based on these studies, the IYSV distribution is highly clumped and appears to be closely associated with the presence of thrips (eggs, adults or immatures) on the plant relative to leaf length. If IYSV does in fact stay close to the point of inoculation in cool season onion plants, then controlling thrips could have a greater impact on the spread of the virus through the onion foliage than previously thought. Unfortunately, the transmission time for IYSV with *T. tabaci* is very short, in as little as 15 minutes. Thus preventative controls are still going to be the most desirable option for trying to manage the thrips vectors of IYSV on onions.
REFERENCES


Overwintering hosts and wingform of thrips, Franklioniella spp., in Georgia (Thysanoptera: Thripidae): implications for management of spotted wilt disease.

Environ. Entomol. 21: 121-128.


Kumar, N. K. K., and Rawal, R. D. 1999. Onion thrips, Thrips tabaci, a vector of onion tospovirus. Insect Environ. 5: 52


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-08/SB41-08.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-09/SB41-09.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-10/SB41-10.html


Nuessly, G. S., Nagata, R. T., Skiles, E. S., Christenson, J. R. 1995. Techniques for differential staining Liromyza trifolii (Dipetra: Agromyzidae) eggs and stipules within cos lettuce
leaves. Florida Entomologist. 78: 258-264.


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-01/SB41-01.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-02/SB41-02.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-03/SB41-03.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-04/SB41-04.html


website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-05/SB41-05.html

website: http://pubs.caes.uga.edu/caespubs/pubcd/SB41-06/SB41-06.html


APPENDIX 1

FIELD DATA ON THRIPS SURVEY CONDUCTED DURING 2006-2008 IN INDIA.
Appendix 1: Field data on thrips survey conducted during 2006-2008 in India.

<table>
<thead>
<tr>
<th>Yr</th>
<th>Mon</th>
<th>Date</th>
<th>Vial</th>
<th>State</th>
<th>District</th>
<th>Province</th>
<th>Field</th>
<th>Crop</th>
<th>Thr</th>
<th>Frank</th>
<th>Scir</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2206</td>
<td>GNTCH1</td>
<td>AndhraPradesh</td>
<td>Guntur</td>
<td>Prathipadu</td>
<td>Vpadu</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>12</td>
<td>2706</td>
<td>GNTCH2</td>
<td>AndhraPradesh</td>
<td>Guntur</td>
<td>Lamfarm</td>
<td>Guntur</td>
<td>Chillies</td>
<td>0</td>
<td>2</td>
<td>107</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON1</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Vpalem</td>
<td>Onion</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON2</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Vpalem</td>
<td>Onion</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON3</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Vpalem</td>
<td>Onion</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON4</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Kpalem</td>
<td>Onion</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON5</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Kpalem</td>
<td>Onion</td>
<td>44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2306</td>
<td>UNDON6</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Undavalli</td>
<td>Kpalem</td>
<td>Onion</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Dec</td>
<td>12</td>
<td>2206</td>
<td>GDON1</td>
<td>AndhraPradesh</td>
<td>Prakasam</td>
<td>Mundlapadu</td>
<td>Burujupalli</td>
<td>Onion</td>
<td>82</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2006</td>
<td>May</td>
<td>05</td>
<td>2506</td>
<td>MDPT1</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Mdnpalliwest</td>
<td>Tomato</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>May</td>
<td>05</td>
<td>2506</td>
<td>MDPT2</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Mdnpalliwest</td>
<td>Tomato</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>May</td>
<td>05</td>
<td>2506</td>
<td>MDPT3</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Mdnpalliwest</td>
<td>Tomato</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>May</td>
<td>05</td>
<td>2506</td>
<td>MDPT4</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Mdnpalliwest</td>
<td>Tomato</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>May</td>
<td>05</td>
<td>2506</td>
<td>MDPT5</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Mdnpalliwest</td>
<td>Tomato</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2406</td>
<td>HYDT1</td>
<td>AndhraPradesh</td>
<td>RangaReddy</td>
<td>Chevella</td>
<td>Khanapur</td>
<td>Tomato</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2406</td>
<td>HYDT2</td>
<td>AndhraPradesh</td>
<td>RangaReddy</td>
<td>Chevella</td>
<td>Ghanapur</td>
<td>Tomato</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2406</td>
<td>HYDT4</td>
<td>AndhraPradesh</td>
<td>RangaReddy</td>
<td>Chevella</td>
<td>Ghanapur</td>
<td>Tomato</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2506</td>
<td>HYDT3</td>
<td>AndhraPradesh</td>
<td>RangaReddy</td>
<td>Chevella</td>
<td>Allewada</td>
<td>Tomato</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>Nov</td>
<td>11</td>
<td>2506</td>
<td>HYDON1</td>
<td>AndhraPradesh</td>
<td>RangaReddy</td>
<td>Chevella</td>
<td>Allewada</td>
<td>Onion</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Date</td>
<td>Code</td>
<td>Location</td>
<td>Suburb</td>
<td>State</td>
<td>Area</td>
<td>Crop</td>
<td>Batches</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>------------------</td>
<td>--------</td>
<td>--------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
<td>------</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>2006 May</td>
<td>KART1</td>
<td>Karnataka</td>
<td>Kolar</td>
<td>Kolar</td>
<td>Kolarbypass</td>
<td>Tomato</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KART2</td>
<td>Karnataka</td>
<td>Kolar</td>
<td>Kolar</td>
<td>Kolarslum</td>
<td>Tomato</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KART3</td>
<td>Karnataka</td>
<td>Kolar</td>
<td>Kolar</td>
<td>Gangapuram</td>
<td>Tomato</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KART4</td>
<td>Karnataka</td>
<td>Kolar</td>
<td>Kolar</td>
<td>Kolarbypass</td>
<td>Tomato</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006 Jun</td>
<td>MAHT1</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Narayangao</td>
<td>Aliephata</td>
<td>Tomato</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT2</td>
<td>Maharashtra</td>
<td>Akola</td>
<td>Akola</td>
<td>Kalas</td>
<td>Tomato</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT3</td>
<td>Maharashtra</td>
<td>Akola</td>
<td>Akola</td>
<td>KalasBK</td>
<td>Tomato</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT4</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Nasik</td>
<td>Chandori</td>
<td>Tomato</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT5</td>
<td>Maharashtra</td>
<td>Akola</td>
<td>Akola</td>
<td>Kalas</td>
<td>Tomato</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT6</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Othur</td>
<td>Zunnar</td>
<td>Tomato</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT7</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Othur</td>
<td>Zunnar</td>
<td>Tomato</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHT8</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Narayangao</td>
<td>Narayangao</td>
<td>Tomato</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC1</td>
<td>Maharashtra</td>
<td>Akola</td>
<td>Akola</td>
<td>Kalas</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC2</td>
<td>Maharashtra</td>
<td>Akola</td>
<td>Akola</td>
<td>Kalas</td>
<td>Chillies</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC3</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Ahmadnagar</td>
<td>SangamnerRd</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC4</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Nasik</td>
<td>Niphad</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC5</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Sangamnar</td>
<td>Wuvrgaopaska</td>
<td>Chillies</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC6</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Zunnur</td>
<td>Golegao</td>
<td>Chillies</td>
<td>5</td>
<td>1</td>
<td>17</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC7</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Zunnur</td>
<td>Thikarwardi</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC8</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Parner</td>
<td>Supavill</td>
<td>Chillies</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC9</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Rajgurunagar</td>
<td>Rashe</td>
<td>Chillies</td>
<td>0</td>
<td>2</td>
<td>15</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC10</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Zunnur</td>
<td>Thikarwardi</td>
<td>Chillies</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAHC11</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Zunnur</td>
<td>Thikarwardi</td>
<td>Chillies</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Code</td>
<td>State</td>
<td>District</td>
<td>Sub-District</td>
<td>Commodity</td>
<td>Type</td>
<td>Quantity</td>
<td>Grade</td>
<td>Remarks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>----------------</td>
<td>-----------</td>
<td>--------------</td>
<td>------------</td>
<td>------</td>
<td>----------</td>
<td>-------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006 Jun</td>
<td>060306</td>
<td>Maharashtra</td>
<td>Pune</td>
<td>Zunnur</td>
<td>Thikarwardi</td>
<td>Chillies</td>
<td>2</td>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006 Jun</td>
<td>060106</td>
<td>Maharashtra</td>
<td>Nasik</td>
<td>Yeola</td>
<td>Andursool</td>
<td>Onion</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nevasa</td>
<td>Nevasarural</td>
<td>Onion</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nandur</td>
<td>Nandurural</td>
<td>Onion</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Onion</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nevasa</td>
<td>Nevasarural</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nevasa</td>
<td>Nevasarural</td>
<td>Chillies</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan</td>
<td>011608</td>
<td>Maharashtra</td>
<td>Ahmadnagar</td>
<td>Nimgauvjali</td>
<td>Nimgauvjali</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Mar</td>
<td>032008</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Vempalli</td>
<td>Tomato</td>
<td>1</td>
<td>12</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Mar</td>
<td>032008</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Chippili</td>
<td>Tomato</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Mar</td>
<td>032008</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Yidigipalli</td>
<td>Tomato</td>
<td>0</td>
<td>27</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Mar</td>
<td>032008</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Yidigipalli</td>
<td>Tomato</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Code</td>
<td>District</td>
<td>Village</td>
<td>Description</td>
<td>Quantity</td>
<td>Price</td>
<td>Weight</td>
<td>Nature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>----------</td>
<td>-------</td>
<td>--------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Mar</td>
<td>MDPT5</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Yidigipalli</td>
<td>Tomato</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDPT6</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Yidigipalli</td>
<td>Tomato</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDPT7</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Obukkapalli</td>
<td>Tomato</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDPT8</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Obukkapalli</td>
<td>Tomato</td>
<td>14</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDPT9</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Obukkapalli</td>
<td>Tomato</td>
<td>0</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDPT10</td>
<td>AndhraPradesh</td>
<td>Chittoor</td>
<td>Madanapalli</td>
<td>Obukkapalli</td>
<td>Tomato</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Apr</td>
<td>KHMC1</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Thirnaypalem</td>
<td>Chillies</td>
<td>0</td>
<td>3</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC2</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Thirnaypalem</td>
<td>Chillies</td>
<td>3</td>
<td>0</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC3</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Chintapalli</td>
<td>Chillies</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC4</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Chintapalli</td>
<td>Chillies</td>
<td>2</td>
<td>1</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC5</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Chintapalli</td>
<td>Chillies</td>
<td>2</td>
<td>1</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC6</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Venkatapuram</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHMC7</td>
<td>AndhraPradesh</td>
<td>Khammamrurl</td>
<td>Venkatapuram</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007 Dec</td>
<td>UNDON1</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Penumaka</td>
<td>Onion</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON2</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Penumaka</td>
<td>Onion</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON3</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Penumaka</td>
<td>Onion</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON4</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Yerrabalem</td>
<td>Onion</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON5</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Yerrabalem</td>
<td>Onion</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON6</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Yerrabalem</td>
<td>Onion</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON7</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Kpalem</td>
<td>Onion</td>
<td>167</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON8</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Kpalem</td>
<td>Onion</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON9</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Kpalem</td>
<td>Onion</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UNDON10</td>
<td>AndhraPradesh</td>
<td>Krishna</td>
<td>Kpalem</td>
<td>Onion</td>
<td>153</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Code</td>
<td>District</td>
<td>Village</td>
<td>Crop</td>
<td>Source</td>
<td>Quantity</td>
<td>Weight</td>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>----------------</td>
<td>-----------</td>
<td>------------</td>
<td>--------</td>
<td>----------</td>
<td>--------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>GNTCH1</td>
<td>Andhra Pradesh</td>
<td>Guntur</td>
<td>Gunturural</td>
<td>Yetukuru</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>GNTCH2</td>
<td>Andhra Pradesh</td>
<td>Guntur</td>
<td>Gunturural</td>
<td>Kurnuthala</td>
<td>Chillies</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>GNTCH3</td>
<td>Andhra Pradesh</td>
<td>Guntur</td>
<td>Gunturural</td>
<td>Kurnuthala</td>
<td>Chillies</td>
<td>0</td>
<td>1</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT1</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Asmathpur</td>
<td>Tomato</td>
<td>0</td>
<td>12</td>
<td>25</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT2</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Asmathpur</td>
<td>Tomato</td>
<td>0</td>
<td>23</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT3</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Japala</td>
<td>Tomato</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT4</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Japala</td>
<td>Tomato</td>
<td>0</td>
<td>64</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT5</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Manchal</td>
<td>Tomato</td>
<td>0</td>
<td>50</td>
<td>20</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008 Jan 01</td>
<td>HYDT6</td>
<td>Andhra Pradesh</td>
<td>RangaReddy</td>
<td>Ibrahimptnm</td>
<td>Manchal</td>
<td>Tomato</td>
<td>0</td>
<td>25</td>
<td>23</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>