Feeding ecology of green mirids: polyphagy and spatio-temporal dynamics across arid and agricultural environments

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Abstract

Green mirids, *Creontiades dilutus* (Hemiptera, Miridae), are polyphagous bugs and are endemic to Australia. These bugs feed on a variety of plant species that grow across massive expanses of subcoastal agricultural landscapes in eastern Australia, as well as in the arid continental interior. Molecular evidence, along with field surveys, have demonstrated that *C. dilutus* bugs move long distances between native vegetation in the arid interior and crops in the eastern states of Queensland and New South Wales. These bugs arrive in the subcoastal agricultural landscapes every summer, and are important pests of cotton (*Gossypium hirsutum* L.) (Malvaceae). They feed on the soft tissues of cotton plants, including the developing flowers, which results in a substantial loss of fruit (cotton bolls) and the feeding damage delays harvest through the crops taking time to compensate for these losses.

The seasonal invasions of bugs into cotton are influenced by the high mobility of these insects and their ability to use a wide variety of host plant species across arid and agricultural landscapes. Some plant species are relatively good hosts in supporting the production of high numbers of nymphs, whereas others produce few nymphs but may be used incidentally as shelter, and this may aid in the dispersal of the adults across long distances. The timing of invasions of *C. dilutus* bugs into cotton, and the pathways followed by them are poorly understood. Also, the general mechanisms by which these insects localize appropriate host plants have not been subject to much investigation.

Host plant availability in the arid continental interior is dependent on highly variable localized rainfall, and such areas are separated by large expanses of extremely dry regions containing few green plants. In agricultural systems, non-crop plants along roadsides and the margins of farms occasionally host low densities of bugs, but previous field surveys have not found high densities of *C. dilutus* that may act as a source of pests that invade cotton. Within farms several legume crops, such as lucerne (*Medicago sativa*) and pigeon pea (*Cajanus cajan*) (Malvaceae), routinely support high bug densities, whereas nearby cotton crops host substantially fewer bugs. Gut analyses conducted previously suggest that individual mirids do

move from lucerne into cotton, but they also move in the other direction, despite the differential numbers of bugs across these crops. Similar movement patterns across pigeon pea and cotton “boundaries” were evaluated in this thesis.

The ambiguity in the dispersal and host use patterns of *C. dilutus* bugs makes it difficult for pest managers to predict invasions of these insects into cotton, with accuracy. Consequently, researchers are not able to design effective management strategies. With a better understanding of dispersal and host use patterns of *C. dilutus* bugs, it may be possible to reduce the number of insecticide sprays in cotton if bugs could be attracted away from cotton by planting alternative hosts (trap crops), but these alternative hosts may inadvertently become local reservoirs of pests that move into cotton. A particular aim of this study is, therefore, to investigate aspects of the dispersal of these *C. dilutus* bugs across crop host species and the associated host localization behaviour of these insects. The ultimate goal is to use this information to form a conceptual model for the host localization process of *C. dilutus* bugs and provide a realistic framework to develop effective pest management decisions in cotton systems.

Specifically this thesis presents the results of: 1) surveys that asked pest managers about their perceptions of invasion patterns, 2) field surveys across vast arid and agricultural landscapes to identify which host species are used most consistently by bugs, 3) molecular evaluations to confirm feeding and movement of individuals across different hosts, and 4) behavioural experiments to identify host-associated cues used by bugs to localize specific plants.

Findings from this study indicate that most pest managers reported that the earliest seasonal infestations into cotton are associated with the proximity of cotton to legume crops and also with storms that move in from the arid regions to the west. Infestation patterns are consistent with multiple invasion events in each season and into each crop, and a gradual increase in bug numbers as nymphs develop into adults within squaring (flowering) cotton. Field surveys

in the arid zone found that the highest densities of bugs were found on *Cullen australasicum* (Fabaceae) and *Goodenia cycloptera* (Goodeniaceae), at a time prior to when cotton was planted, and on lucerne and pigeon pea in agricultural systems during the flowering period of cotton.

Similarly, too, bug densities were consistently much higher on pigeon pea than on cotton. *Creontiades dilutus* bugs were found in the field to feed on both pigeon pea and cotton, and frequently they move back-and-forth between these crops, as found across lucerne-cotton boundaries. Behavioural tests in the laboratory revealed that these bugs are arrested in the vicinity of pigeon pea and cotton by olfactory cues, but there was no evidence that olfactory cues alone attracted bugs to either host beyond a range of 2cm. Also, this is the first behavioural study that observed an increase of insect locomotion at night, suggesting that these bugs are essentially nocturnal.

Collectively, the results of this thesis indicate that *C. dilutus* bugs are produced in relatively greater numbers on specific plant species than on nearby alternative species (with cotton being a relatively poor host ecologically (although not economically)). Olfactory cues that arrest bug movement to a locality appear to have a stronger influence on settling patterns across host plant species than do olfactory cues that attract bugs towards plants. A general host localization model is proposed in which high densities of bugs develop on host plant species that maintain soft tissues (after receiving rain or growing on irrigated farmlands), then move across the landscape and land on plants until recognizing cues that arrest their movement. Other host species, such as cotton, are used if their primary hosts are not available. The implications of how these bugs persist in highly variable environments are discussed, and the implications for pest management are specified.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

Chapter 2:

Cappadonna, J.K., M.M. Miles, J.P. Hereward, G.H. Walter (2018). Invasions of green mirid (*Creontiades dilutus*) (Stål) (Hemiptera: Miridae) into cotton – perceptions of Australian crop consultants. Agricultural Systems 166: 70-78.

Chapter 3:

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Chapter 5:

Cappadonna J.K, Hereward J.P, Walter G.H. (2020). Diel activity patterns and arrestment behaviour in host associations of green mirids (*Creontiades dilutus*). Bulletin of Entomological Research 1–9.

Submitted manuscripts included in this thesis.

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Other publications during candidature

No other publications.

Contributions by others to the thesis

Gimme Walter and James Hereward made important contributions to the conception and design of the project. In regards to chapter 2, the initial data survey (in 1993) of crop consultants about their perspectives on pest infestations was provided by Melina Miles. In 2014, the Cotton Research and Development Crop Consultants Australia and Corporation and Crop Consultants Australia included questions designed by myself into their seasonal survey of consultants. All analyses were done by myself.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research Involving Human or Animal Subjects

No animal or human subjects were involved in this research.

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Keywords

arrestment, cotton, *Creontiades dilutus*, green mirid, insect dispersal, multiple host-use, refuge crop

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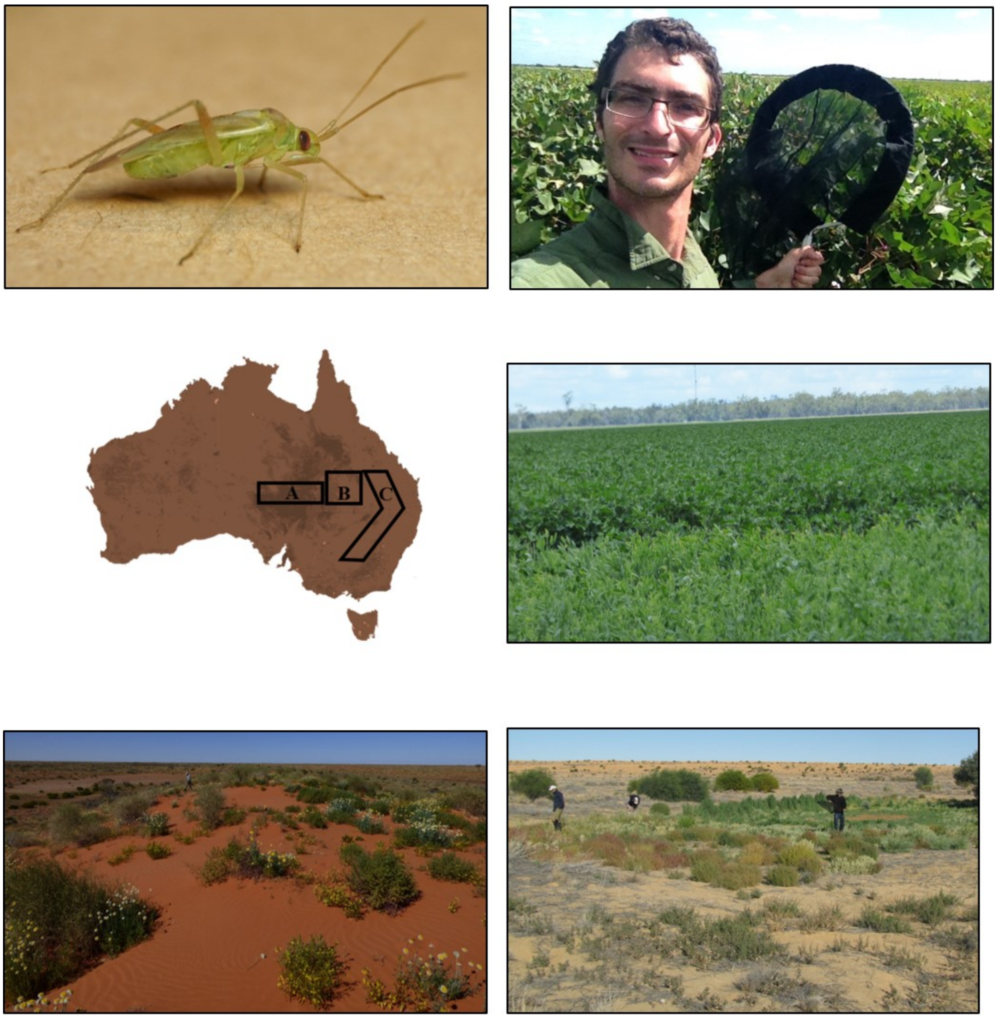
Frontispiece



Frontispiece. Clockwise from top left: adult green mirid (*Creontiades dilutus*) in the laboratory, sampling for green mirids in a cotton field, cotton growing next to pigeon pea crops, field surveys of a desert drainage, desert sand dunes with green mirid host plants, and the field survey locations (A – Simpson Desert, B – Channel Country floodplains, and C – the eastern cotton-growing region).

Clockwise from top left: adult green mirid (*Creontiades dilutus*) in the laboratory, sampling for green mirids in a cotton field, cotton growing next to pigeon pea crops, field surveys of a desert drainage, desert sand dunes with green mirid host plants, and the field survey locations (A – Simpson Desert, B – Channel Country floodplains, and C – the eastern cotton-growing region).

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List of abbreviations used in the thesis

Bt	*Bacillus thuringiensis*
DNA	Deoxyribonucleic Acid
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
PCR	Polymerase Chain Reaction

Chapter 1. General Introduction

Green mirids (*Creontiades dilutus*, Stål) (Hemiptera: Miridae) are small herbivorous bugs endemic to Australia, and found across vast expanses of the arid continental interior and in subcoastal agricultural environments (Malipatil and Cassis 1997). These bugs are considered to be highly polyphagous, because individuals have been captured from over 90 plant species across 15 families (Hereward and Walter 2012). They are captured on legumes (Fabaceae) in greater numbers than on plants in other families (Hereward and Walter 2012). However, they are best known as important pests of cotton (*Gossypium hirsutum*) (Malvaceae) (McColl et al. 2011, Wilson et al. 2013).

Cotton crops are damaged when mirid bugs lacerate plant tissues with their stylets while secreting saliva that destroys the cell walls of hosts (Hori and Miles 1993, Taylor 1995). Bugs then feed on the liquified plant contents of the soft terminals, flower buds, and young fruits of cotton (Bishop 1980, Foley and Pyke 1985, Chinajariyawong 1988). Severe infestations of cotton typically result in a substantial loss of bolls (the fruit), which requires cotton to remain in the ground longer so that plants can compensate for these losses (Khan 1999, McColl et al. 2011). This delayed harvest increases management costs, and risks lint being stained by mould if it should rain (as harvest is close to the start of the wet season) (Khan 1999, McColl et al. 2011).

The ability of these bugs to use multiple hosts across diverse environments underpins their spatio-temporal patterns of invasions into crops (Stinner et al. 1983, Kennedy and Storer 2000). That is, the high mobility of *C. dilutus* adults, along with their extreme polyphagy and ability to produce offspring on many different host species contributes to their pest status in cotton systems (Hereward and Walter 2012, Hereward et al. 2013a). Individual bugs move across “boundaries” between different crops, weeds, and native vegetation (Miles 1995, Khan 1999, Hereward et al. 2013a). Eggs laid on non-cotton hosts likely produce nymphs that are in areas that do not receive insecticide sprays intended to control cotton pests, and these local hosts may become sources of bugs immigrating into cotton.

The high mobility and broad diets of these bugs also likely allows them to persist in the remote arid continental interior where host plant availability is driven by highly variable rain patterns, with local rainfall often separated by hundreds of kilometres of dry country that supports little plant growth (Miles 1995, Morton et al. 2011, Hereward and Walter 2012, Nano and Pavey 2013). The overall densities of host plants are low across arid environments, but these regions also include isolated patches of *Cullen australasicum* and *C. cinereum* (Fabaceae), which grow in clay drainages and host relatively high numbers of *C. dilutus* bugs, and these species have been suggested to be among their primary host plants (Hereward and Walter 2012). Bug densities on *Cullen* hosts in arid environments are often greater (per sampling unit) than densities on cotton in agricultural regions (Bodnaruk 1992, Miles 1995, Mensah and Khan 1997, Khan 1999, Hereward and Walter 2012, Hereward et al. 2013a).

Seasonally, *C. dilutus* bug numbers across arid and agricultural landscapes are inverse, with higher numbers in arid environments during winter prior to when cotton is planted, and with more bugs in cotton systems in summer when the arid interior is too hot to support the growth or persistence of the herbs and forbs that sustain these bugs (Miles 1995, Hereward and Walter 2012). Gene flow studies indicate that these bugs disperse from the arid interior into the more eastern agricultural systems (Hereward et al. 2013b). The specific immigration pathways from arid environments onto cotton farms remains ambiguous, but invasions may be aided by the presence of alternative host plants in the cotton-growing regions, the arid continental interior, and perhaps even in the vast area between them.

The development of effective control strategies requires a detailed ecological understanding of why these bugs move across host species (Walter 2003, McColl et al. 2011, Wilson et al. 2013), but the ecological drivers responsible for the immigration of *C. dilutus* bugs onto cotton has remained elusive. The aim of this study is to evaluate the spatio-temporal dynamics of *C. dilutus* bugs across agricultural and natural landscapes to determine the behavioural mechanisms that underpin their movement into cotton. Several authors have identified various research directions that would be particularly useful for developing control strategies for *C. dilutus* bugs, and which are less dependent on insecticide use, as well as for understanding their ecology more generally. These include identifying: the environmental factors that cause adults to initiate flights across long distances (Hereward et al. 2013a), the hosts plant species that retain bugs away from cotton (Mensah and Khan 1997), and the plants that consistently host relatively high numbers of bugs (in contrast to those that are used only occasionally, as incidental hosts) (Hereward and Walter 2012). Section 1.1 (below) reviews the pest status and management of these bugs, and Section 1.2 outlines the structure of this thesis in respect to the specific research questions addressed.

1.1. Pest status and management of *Creontiades dilutus* in cotton

Cotton monocultures are planted across large areas of eastern Australia in the states of Queensland and New South Wales (Fitt 1994). For much of the early years of the cotton industry growers planted non-transgenic "conventional" cotton (Constable et al. 2011). During this period frequent insecticide sprays targeted lepidopteran pests primarily, and this routine spraying incidentally kept *C. dilutus* numbers low (Fitt 1994, Constable et al. 2011). In the 1980s and 1990s many cotton-growers switched from highly persistent broad-spectrum insecticides to less persistent compounds (Bishop 1980), and they subsequently adopted transgenic *Bt* cotton that is toxic to lepidopteran pests and resulted in fewer sprays (Wilson et al. 2013). As of 2019 *Bt* cotton, however, is ineffective against *C. dilutus* because the toxin does not affect bugs, and the overall reduction of toxic sprays simultaneously released these insects from incidental control (Fitt 1994). The relative increase in *C. dilutus* numbers resulted in cotton-growers spraying for these bugs in their own right for the first time (Constable et al. 2011, Wilson et al. 2013).

Management of *C. dilutus* pests relies on chemical control (McColl et al. 2011), and excessive sprays may lead to the development of resistance in these bugs or other pest species in the field (Wilson et al. 2013). Establishing trap crops that attract particular pest insects away from valuable crops, or intercepting the insects while they are immigrating into those crops, may minimize total insecticide use by concentrating pests to small fields of less valuable plants (Hokkanen 1991). Lucerne (= alfalfa, *Medicago sativa*) (Fabaceae) has been proposed as a trap crop for *C. dilutus* pests (Mensah and Khan 1997, Fitt 2000, Wilson et al. 2018), but few growers have opted to replace a portion of the cotton they plant with relatively low-value lucerne (Wilson et al. 2013).

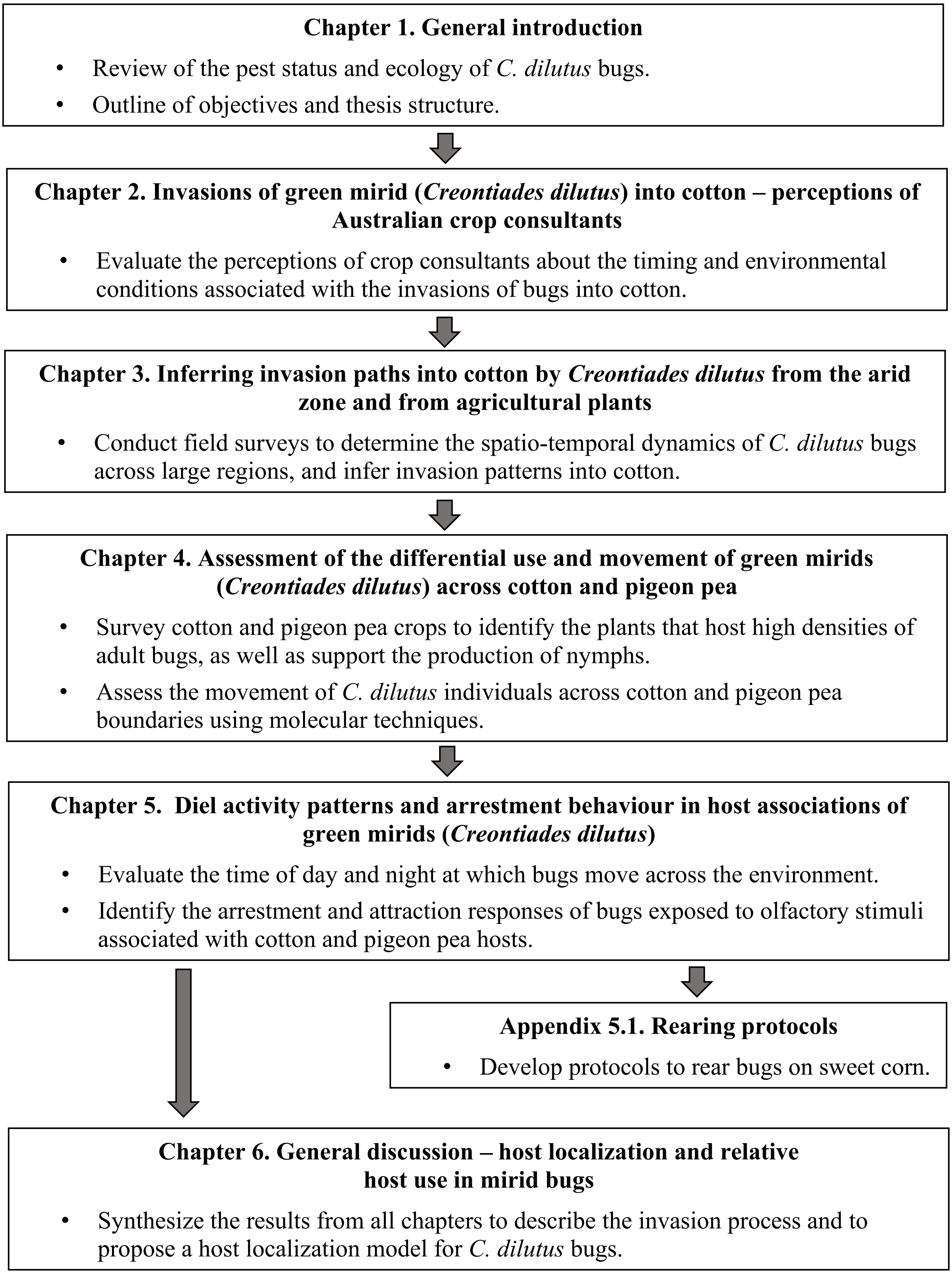
There are crops, other than lucerne, that are planted along with cotton that may act as a trap crop by retaining bugs away from cotton. For example, growers are required to establish refuges for lepidopteran bollworm pests as part of an insecticide resistance strategy (Downes et al. 2010, CRDC 2019b), and the most commonly planted refuge crop is pigeon pea (*Cajan Cajanus*) (Fabaceae) (Doyle and Coleman 2007, Wilson et al. 2013). Pigeon pea routinely hosts more *C. dilutus* bugs than cotton in adjacent fields, and has been suggested to be a potential source of bugs that move into cotton (Lawrence et al. 2007, CRDC 2014).

Planting lucerne is expected to reduce bug numbers in nearby cotton (Mensah and Khan 1997). By contrast, planting pigeon pea near to cotton crops has been suggested to increase numbers in cotton (Lawrence et al. 2007). This ambiguity stems from the fact that the host associations of *C. dilutus* bugs are poorly understood in relation to their movement across different host species. Only a few studies have assessed the movement of individual *C. dilutus* bugs directly, using intercept traps (Lowor et al. 2009b, Hill 2013) and molecular techniques (Hereward and Walter 2012, Hereward et al. 2013a). Also the movements of these insects have been inferred by modelling wind patterns (Hill 2017). In general, the behavioural aspects of the host localization process of these bugs have not been well studied. The omission is significant because an understanding of when and why these insects remain on particular host species, leave them, and accumulate in them would help interpret their movement patterns and host-use patterns.

1.2. Objectives and thesis structure

The objective of this study is to investigate the ecological factors and behavioural processes that lead to the movement of *C. dilutus* bugs across host plant species, with a focus on their seasonal invasions into cotton. Specific research questions and their investigation are structured into four chapters (Fig. 1.1). An appendix outlining rearing protocols that were developed to facilitate behavioural experiments is also included. The first two of these chapters (2 and 3) evaluate the spatio-temporal patterns of bugs across agricultural and arid landscapes. Chapter 2 presents the results of multiple questionnaire surveys that asked crop consultants about how they perceive the invasion patterns of *C. dilutus* adults into cotton, and the evaluation of seasonal reports published in industry magazines. These consultant surveys and industry reports helped direct the research questions in the subsequent chapters, by identifying the environmental factors that may influence invasions, as perceived by professionals that monitor the same farms for bugs every year. Chapter 3 presents field survey results that evaluated host-use by *C. dilutus* adults and nymphs across a massive geographical expanse that included a section of the arid interior of Australia and agricultural environments which are in the subcoastal eastern part of the continent.

The other chapters (4 and 5) evaluate the behavioural mechanisms associated with the movement of bugs in respect to two hosts, cotton and pigeon pea (*Cajanus cajan*) (Fabaceae). Chapter 4 presents the findings of field surveys and gut content analyses using molecular methods to identify the direction of movement of these bugs across the boundaries that separate these crop species. Chapter 5 is an assessment of the behaviour of *C. dilutus* bugs associated with the arrestment and attraction responses to olfactory stimuli that derive from cotton and pigeon pea hosts. Chapter 5 also identifies the time of day or night (the diel period) in which these insects are most active. The thesis concludes with a general discussion (Chapter 6) that synthesizes the findings of these chapters, proposes a conceptual host localization model for *C. dilutus* bugs, and identifies ecological factors that likely promote the invasion of these endemic Australian pests into cotton farms.



Chapter 1. General introduction

- Review of the pest status and ecology of *C. dilutus* bugs.
- Outline of objectives and thesis structure.

Chapter 2. Invasions of green mirid (*Creontiades dilutus*) into cotton – perceptions of Australian crop consultants

- Evaluate the perceptions of crop consultants about the timing and environmental conditions associated with the invasions of bugs into cotton.

Chapter 3. Inferring invasion paths into cotton by *Creontiades dilutus* from the arid zone and from agricultural plants

- Conduct field surveys to determine the spatio-temporal dynamics of *C. dilutus* bugs across large regions, and infer invasion patterns into cotton.

Chapter 4. Assessment of the differential use and movement of green mirids (*Creontiades dilutus*) across cotton and pigeon pea

- Survey cotton and pigeon pea crops to identify the plants that host high densities of adult bugs, as well as support the production of nymphs.
- Assess the movement of *C. dilutus* individuals across cotton and pigeon pea boundaries using molecular techniques.

Chapter 5. Diel activity patterns and arrestment behaviour in host associations of green mirids (*Creontiades dilutus*)

- Evaluate the time of day and night at which bugs move across the environment.
- Identify the arrestment and attraction responses of bugs exposed to olfactory stimuli associated with cotton and pigeon pea hosts.

Appendix 5.1. Rearing protocols

- Develop protocols to rear bugs on sweet corn.

Chapter 6. General discussion – host localization and relative host use in mirid bugs

- Synthesize the results from all chapters to describe the invasion process and to propose a host localization model for *C. dilutus* bugs.

Fig. 1.1. Thesis structure and objectives in the investigation of *Creontiades dilutus* bugs, with respect to each chapter.

The contents of the following section (Chapter 2) have previously been published. I (Justin Cappadonna) am responsible for the concept and design of the study and have conducted all statistical evaluations. Gimme Walter and James Hereward contributed editorial revisions to the manuscript. Melina Miles contributed data in regards of the consultant surveys from field seasons in the 1992/93 field season (see Section 2.2.1).

Chapter 2. Invasions of green mirid (*Creontiades dilutus*) (Stål) (Hemiptera: Miridae) into cotton – perceptions of Australian crop consultants

Abstract

Green mirids, *Creontiades dilutus* (Stål) (Hemiptera, Miridae), are primary pests of cotton (*Gossypium hirsutum*) (Malvaceae) in eastern Australia. Severe infestations delay crop development and reduce yield through boll drop (fruit loss). Most cotton farmers employ crop consultants to monitor mirids and recommend pest management options. Effective pest management needs to account for both increased pest numbers within cotton (i.e. the production of mirid offspring), and the movement of mirids into crops (invasions).

The objective of this study is to evaluate what is known about environmental processes that influence mirid invasions by assessing records on mirid infestations spanning the 1976/77 through 2015/16 cotton seasons. The primary records of mirid invasion are qualitative surveys conducted in 1993 and 2014 that asked consultants about their monitoring efforts. These qualitative surveys are supplemented by seasonal reports of pest pressures across eastern Australia, and sporadic field surveys that quantified mirid densities.

Although each consultant monitored only a small portion of the overall agricultural landscape, there was agreement that the earliest seasonal mirid invasions were associated with squaring (flower bud forming) cotton and were influenced by nearby vegetation. There was, however, substantial variation in the responses regarding local factors that may influence mirid invasions. The importance of distinguishing between mirid invasions and the production of offspring within cotton is discussed. Methods are proposed that would improve the spatial and temporal resolution of mirid monitoring efforts across the agricultural landscape.

2.1. Introduction

Green mirids, *Creontiades dilutus* (Stål) (Hemiptera, Miridae), are polyphagous plant bugs endemic to Australia. They invade cotton (*Gossypium hirsutum*) (Malvaceae) annually (Chinajariyawong, 1988; Khan, 1999; Miles, 1995), where their feeding substantially reduces yield through the loss of fruits (i.e. boll drop) and delays crop maturity (Cotton Research and Development Corporation (Australia), 2016). Delays in maturation extend the duration that crops are in the field, which in turn increases the number of fertilizer and pest management applications, as well as increasing the risk of exposing lint to rain (associated with increased fungal growth).

During winter, green mirids are more abundant in the natural landscapes of the arid continental interior than they are in sub-coastal agricultural landscapes, but in summer the relationship is reversed (Hereward et al., 2013b). Molecular evidence indicates population connectivity and gene flow across arid and agricultural landscapes (Hereward et al., 2013b). This, combined with the almost simultaneous increase of green mirid numbers in crops across vast regions (Miles, 1995), suggests mirids are invading cotton from remote regions. The exact timing and the ecological influences of green mirid invasions into cotton, however, have proved difficult to determine.

To minimize crop loss, most cotton growers in Australia rely on the advice of crop consultants with respect to the management of green mirids (Whitehouse, 2011). Consultants regularly monitor crops for the build-up of mirid numbers, then recommend when insecticide should be sprayed. Spray thresholds have been established at 2 mirids/m of row detected during beat sheet pest surveys in cool regions, or 4 mirids/m in warmer regions (Cotton Research and Development Corporation (Australia), 2016). Spraying at mirid densities below these thresholds should be avoided to prevent flare-ups of non-mirid pests (Constable et al., 2011; Fitt et al., 1994a), but allowing mirid densities to increase beyond these thresholds risks excessive crop damage. Whitehouse (2011), demonstrated that although growers and consultants mostly spray at threshold, some still make “insurance sprays” below threshold.

Effective control of mirids depends on consultants accurately identifying where and when mirids have invaded cotton prior to mirid populations reaching threshold densities. During periods of optimal cotton growth, when temperatures are 22-30ºC (Cotton Research and Development Corporation (Australia), 2017; Reddy et al., 1992), mirids develop from egg to adult in about 15 days (Khan et al., 2009; Pyke et al., 1985). This means it is critical to reduce mirid numbers immediately after thresholds are reached to prevent rapid population growth. Early notice of where populations have established may indicate the location and timing of when populations will reach thresholds, which would allow cotton growers to prepare for a quick response.

Three major challenges are associated with assessing green mirid invasions into cotton crops. First, mirids can be difficult to detect in cotton because adults are cryptic, extremely flighty, highly mobile, and are patchily distributed across agricultural landscapes (Bodnaruk 1992). Second, consultants tend to focus their monitoring efforts in cotton fields. As a result, observations of any potential sources of mirid invasions from alternative hosts are scarce. Third, no systematic green mirid monitoring program exists for the entire cotton-growing region. Each consultant monitors only a few farms (Whitehouse, 2011), and from their local observations they develop impressions about the patterns and causes of mirid infestations. While consultants may share their perceptions of mirid infestations with colleagues, there is no centralized depository of the quantified mirid counts from their monitoring efforts. As a consequence, particular regions may have relative assessments of the intensity of mirid presence, but no quantified baseline is available to compare mirid invasions across regions or seasons.

In the absence of quantified mirid surveys that cover the entire Australian cotton-growing region, qualitative mirid observations by consultants who are familiar with mirid build-ups may identify factors associated with invasions. These potential factors include: seasonal changes in temperature or host availability in the continental interior, shifts in wind patterns that can bring individuals into agricultural landscapes, the presence of attractive cotton developmental stages, and the spillover of mirids from alternative host species. These are not mutually exclusive of one another.

The impressions of consultants about mirid invasions may well identify invasion processes relevant to mirids across the agricultural landscape, or it may only represent idiosyncratic patterns for specific localities. In either case, the perceptions of consultants are important for two reasons. First, regardless of the degree of uniformity of mirid invasions of cotton across the agricultural landscape, the perceived patterns of pest build-ups by consultants strongly influence how green mirids are managed in Australia. Second, a consensus about the patterns of mirid arrivals in cotton would indicate that mirid invasions might be driven by similar ecological factors across a vast geographical area, whereas regional differences among consultants would indicate that invasions might be due to local context-specific factors. With the assistance of consultant input, researchers can prioritize which environmental factors may be worthwhile for future investigations.

Consultants routinely share their opinions about management strategies and their observations of green mirid population growth in cotton, but these records are scattered across the scientific literature, unpublished theses, industry reports, and anecdotal observations. These records rarely address invasions explicitly. When consultants have been asked about their views on mirids it is typically in the context of the establishment of spray thresholds (Miles, 1995; Whitehouse, 2011), seasonal reports of local pest pressure for *The Australian Cottongrower* (an industry magazine), or in annual surveys of their pest status commissioned by the *Cotton Research and Development Corporation* (Australia).

The objective of this study was to identify the patterns of invasions into cotton by green mirids throughout eastern Australia. This was done by assessing observations of mirid infestations by consultants that monitor cotton pests, as well as reviewing published pest reports that have accumulated between the 1976/77 through the 2015/16 cotton seasons. Any consistencies in the consultant perceptions or infestation reports about mirids were evaluated in respect to the timing and location of the earliest mirid detections for each season.

2.2. Methods

Four sets of records were evaluated to assess the observations and perceptions of green mirid invasions by crop consultants. The initial two data sets were from independent qualitative surveys enquiring about the perceptions of consultants about the earliest mirid detections in a specific season, general invasion patterns, and the environmental factors associated with mirid arrivals (Section 2.2.1). The final two data sets are from published quantitative mirid counts conducted sporadically between 1976 and 2003 by different researchers (Section 2.2.2), as well as from pest detections reported in seasonal summaries for all years between 2007 and 2016, inclusive, (Section 2.2.3). The methods associated with each dataset and analysis are described in detail below.

2.2.1 Qualitative consultant surveys

The first qualitative survey was conducted at the end of the 1992/93 season (hereafter called the "1993 survey"). This 1993 survey consisted of a questionnaire that was mailed to members of the Queensland Crop Consultant's Association, or it was given directly to participants in a meeting of the NSW Cotton Consultant's Association. The second survey was conducted 21 years later at the end of the 2013/14 season (hereafter called the "2014 survey"). This 2014 survey was part of a larger annual online assessment of various management issues administered by the *Cotton Research and Development Corporation* (Australia) and *Crop Consultants Australia*.

The 1993 survey enquired about the districts in which consultants worked, as well as the developmental stage of cotton and the month in which green mirids were first detected (Table 2.1). Crop stages were categorized as seedling (pre-reproductive), squaring (developing flower buds), or flowering cotton. In respect to the arrival dates of mirids, the responses from some months were pooled with those from consecutive months. September and October records were pooled because only a few farms plant cotton in central Queensland during the final two weeks of September. December, January, and February records were also pooled because initial mirid detections were rare during the final 2 months of the season.

Table 2.1. Qualitative survey questions about *Creontiades dilutus* invasions into cotton crops. Crop consultants participated in two separate surveys, in 1993 and 2014. Survey year is followed by the survey questions, with answer choices in parentheses (see text for details).

	Consultant demographics
1993	• Which district do you work in? • Are green mirids currently included in your cotton monitoring program?
2014	• In which region/s are the cotton clients based? • For how many seasons have you worked consulting in cotton?
	Earliest Seasonal Mirid Detections
1993	• When do green mirids first appear in cotton in your district? i. Approximately the same calendar time each year (please specify) ii. At a particular stage in crop growth ("seedling," "squaring," "flowering," "boll setting") iii. Timing dependent on some other factor (please specify)
2014	• Thinking about 2012/13 and 2013/14 cotton seasons, when was the earliest detection of mirids in cotton across ALL farms and fields. Select ONE 'time' for each season. (Answer options included 2-week calendar categories starting in the 2nd half of September and ending in the 2nd half of March, "no mirids," and "don't remember") • Thinking about 2013/14 cotton crops over which you/the business consulted, across how many hectares were mirids first detected in fields when crops were at the following stages of development? ("After emergence and before first square," "between first square and before peak flower," "after peak flower," "no mirids")
	Mirid invasion patterns
1993	• What is the usual pattern of mirid infestation? ("Gradual build up in numbers," "influx of adults over a short period," "frequent influxes of adults over a longer period," and "other")
2014	• Thinking about the arrival of green mirids in cotton in a given season, which statement do you agree with the most? ("Green mirids build up at different times and places across fields/farms," "green mirids initially appear at one time across all or most fields," "green mirids initially appear at one time in the same locations each season") • From your own experiences, describe the situations where you are most likely to find the earliest mirids of the season: e.g. proximity to other farms, crops, vegetation, wind direction?

Consultants were also asked to judge the best description of the overall mirid invasion pattern from the three choices provided (Table 2.1). The aim of this question was to determine if consultants perceived that mirid invasions occurred at different places and dates throughout the season, at once over a short period across all fields, or was best characterized as a gradual build-up of mirid numbers through the season.

In the 2014 survey, the cotton stages “after emergence” and up to the detection of the “first square” corresponded to the pre-reproductive seedlings of the 1993 survey. Cotton stages from the first square through peak flowering corresponded to the “squaring” and “flowering” stages of the 1993 survey. Both surveys included a developmental period following peak flowering characterized by the development of bolls prior to harvest.

Further, the 2014 survey asked consultants to estimate the total area of farmland in which they first detected mirids in emerging, squaring, or flowering cotton. The phrasing of this question allowed for multiple “first” mirid detections for each consultant. Multiple responses occurred if a consultant initially detected mirids at a particular cotton stage in one field but had an initial mirid detection at a different cotton stage in another field. Since this analysis was concerned with the first mirid detection for each consultant across all monitored fields, each consultant was assigned a single response for the earliest crop stage in which green mirids were detected. By reclassifying responses into exclusive categories, double counting multiple observations by consultants was avoided.

The 2014 survey addressed the same issues as the 1993 survey, but the specific questions and answer choices were phrased differently (Table 2.1). The differences between surveys aimed to improve the clarity of the questions by aligning more closely to the current language and management activities of consultants in the 2013/14 season. The different phrasing between surveys, however, likely influenced the interpretation of these questions. Therefore, the responses from each survey were independently evaluated, but there were no statistical evaluations formally comparing the 1993 and 2014 surveys.

Statistical assessments of consultant responses to the fill-in-the-blank style questions of the surveys were conducted with either “binomial proportion tests” (comparing 2 alternative responses), or with “pairwise comparisons of proportions” with Holm-Bonferroni corrections for multiple comparisons (R Core Team 2017). Choices that did not receive responses were omitted to avoid artificially increasing the statistical significance of the remaining responses.

2.2.2 Field studies

Each participant in the consultant surveys (see above) will likely interpret the questions differently from their peers. This is due, in part, to the differences in the methodology of designing the surveys, but also the subjective nature of assessing the perceptions of complex ecological processes in the context of crop management by individual consultants. To help link the views of consultants about mirid invasions with relevant ecological processes, findings from the qualitative consultant surveys were compared with quantitative field surveys that documented mirid invasions into cotton.

To be included in this analysis, quantitative field surveys must have been published in peer reviewed scientific journals or have been presented in unpublished PhD theses using standard sampling techniques. Furthermore, sampled cotton fields must have been unsprayed, and sampling at regular intervals must have started prior to the earliest known observation of mirids for that season.

2.2.3 District reports

Nine seasons of district reports (2007/08 through 2015/16, inclusive) were assessed for green mirid observations to supplement the findings of the consultant surveys (above). The districts were located across the states of Queensland and New South Wales (Fig. 2.1), and the naming convention follows that of *The Australian Cottongrower.* District reports frequently addressed the green mirid concerns in terms of explicit descriptions of population growth patterns across cotton growing regions, or more generally as records of where insecticide sprays were required.

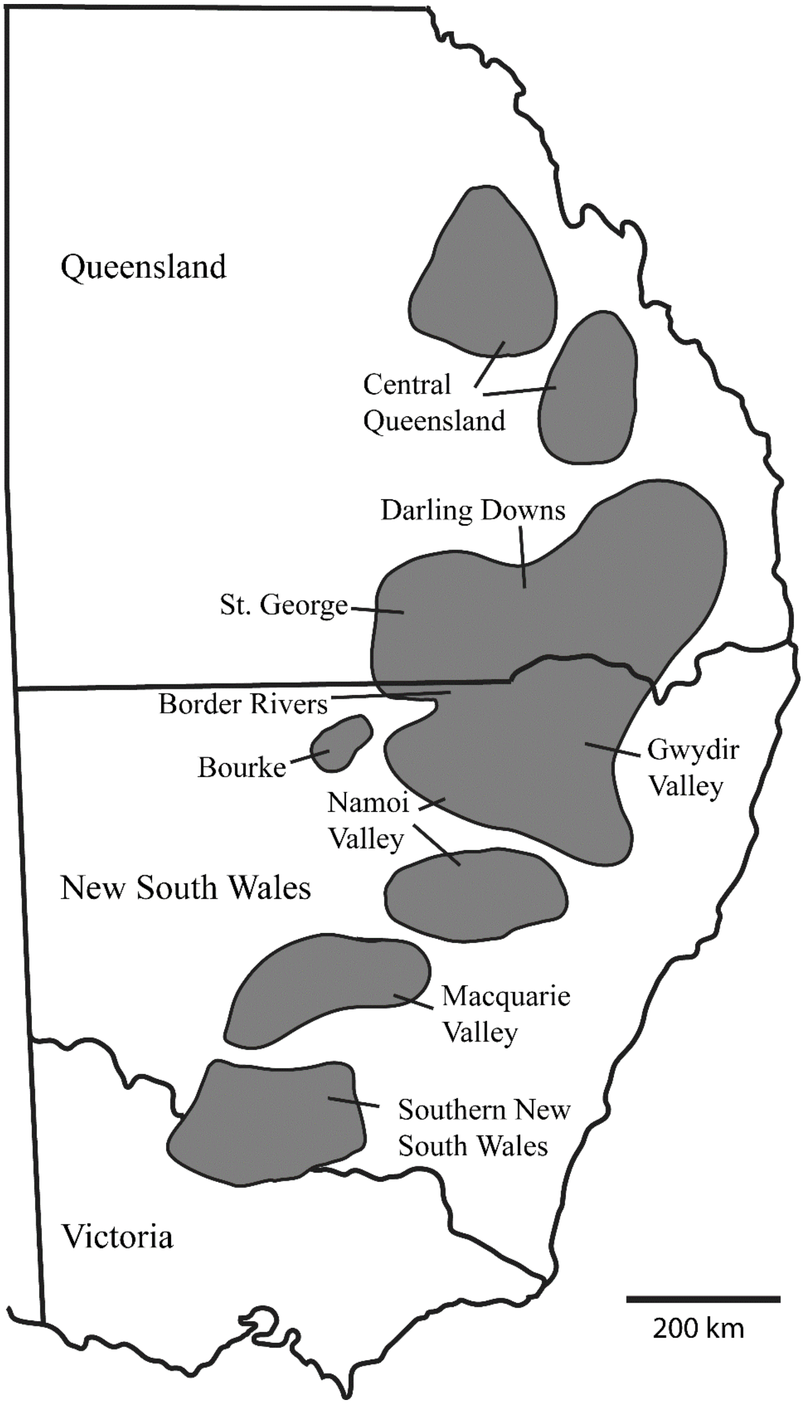


Fig. 2.1. Cotton-growing districts of eastern Australia (grey areas), including the district names used by crop consultants (adapted from *The Australian Cottongrower*, Greenmount Press).

These records were summarized by classifying the intensity of mirid "pest pressure" (i.e. the vulnerability of crops to increasing numbers of mirids) into three categories. "Severe" infestations required at least three insecticide sprays targeting mirids, or they were scored as such when consultants reported unusually high mirid densities. Moderate (= "Mod.") infestations required only one or two sprays, or consultants reported mirid densities as being "typical". "Low" infestation classifications characterized fields without mirids or, if present, they were at low densities and did not require insecticide applications. There were no formal statistical evaluations on these records, because of an insufficient sample size across seasons (n = 9). This summary of pest pressure across vast landscapes is discussed in the context of localized mirid increases and proposed monitoring programs.

2.3. Results

2.3.1 Qualitative consultant surveys

Fifty-one consultants responded to the 1993 survey (13 from Queensland and 38 from New South Wales). An additional 51 consultants responded to the 2014 survey (18 from Queensland, 29 from New South Wales, and 4 worked in both states) (see Fig. 2.1 for district locations). The sample size for each question varies, because some consultants did not respond to all questions. Twenty-two percent of the participants of the 2014 survey were consulting for cotton growers during 1993, but they did not necessarily participate in both surveys.

All consultants that responded in both the 1993 and 2014 surveys detected green mirids. Several consultants who responded in 1993 also noted that mirids were abundant every year beginning from the 1987-88 season, except for low numbers in the 1991-92 season.

Initial invasions of mirids were observed after the emergence of cotton seedlings, and before the completion of flowering (Fig. 2.2). Observations of initial mirid invasions were less frequent as crops developed. All initial mirid detections were prior to the "boll setting" and "peak flowering" stages. Pairwise comparisons of proportions (with Holm-Bonferroni corrections for multiple comparisons) are reported for analyses of all cotton stage categories. These analyses

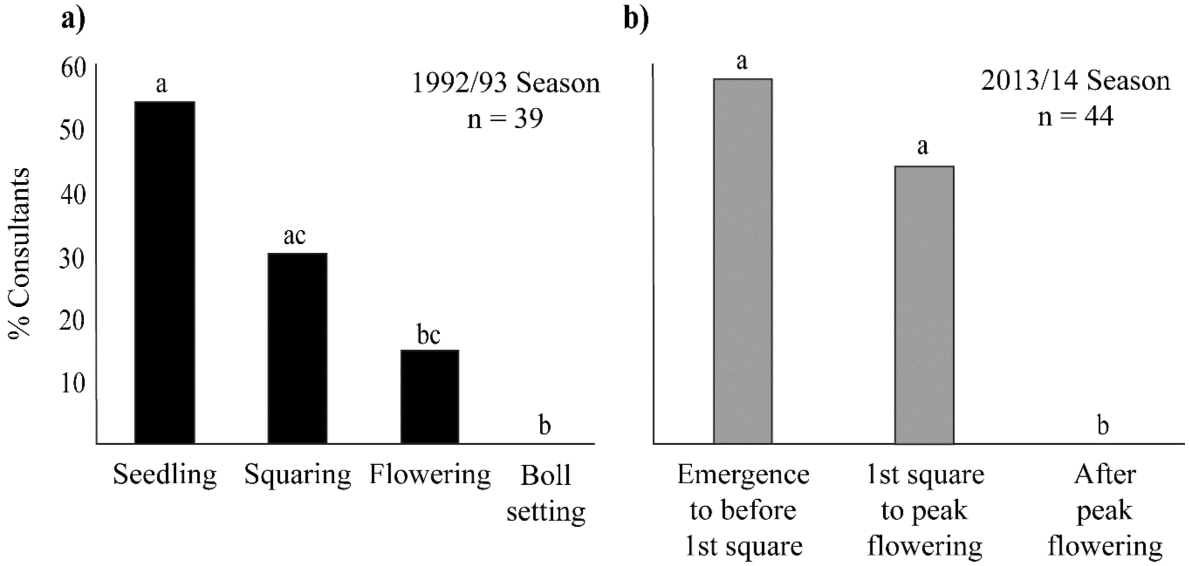


Fig. 2.2. Earliest *Creontiades dilutus* detection relative to cotton stage (pairwise comparisons of proportions with Holm-Bonferroni corrections for multiple comparisons); a) 1993 survey; b) 2014 survey. Statistical comparisons were calculated separately for each survey. Different letters, within seasons, reflect significantly different results at p < .05.

were repeated when the “boll forming” and “after peak flowering” categories were excluded (both lacked mirid detections). Excluding these categories did not change the statistical significance relationships for their respective analyses (not presented).

September and January were associated with few new mirid invasions (Fig. 2.3). All consultants had detected mirids prior to February and March, so these months were excluded from statistical analyses. In 1993, most consultants reported observing initial mirid invasions in September, October and November. In 2014, most consultants observed invasions later in the season, November and December. There was only 1 initial mirid invasion in September, and 3 detections in January during the 2013/14 season.

The duration that cotton was exposed to potential mirid invasions varied by date. Very little cotton was grown during September, in each season, so infestations effectively began in October. The period of September and October following the emergence of cotton seedlings was 45 days, 30 days for November, and 62 days for December and January.

In 1993, no significant differences were detected in the proportion of consultants who perceived mirid invasions as taking place with a gradual build up in numbers, a single influx, or as multiple influxes over a longer period (Fig. 2.4). In 2014, a significantly higher proportion of consultants characterized green mirid invasions as occurring over multiple locations on different dates throughout the season, as compared to one influx across all fields, or as an influx at a particular region of a farm which builds from there (Fig. 2.4b).

Consultants were asked about the environmental conditions associated with the earliest detection of green mirids. Several consultants identified multiple situations in which they found mirids (Table 2.2). Consultants responding to the 1993 survey attributed mirid arrival to incoming storms and wind (58%), as well as to local mirid sources from nearby crops (21%) and weeds (21%). Consultants did not specify any invasion associations with native plants, but they may not have distinguished between “weeds” and “native species”. When combined into the logical functional groups “weather” and “non-cotton hosts”, however, there was no significant difference between the proportion of responses.

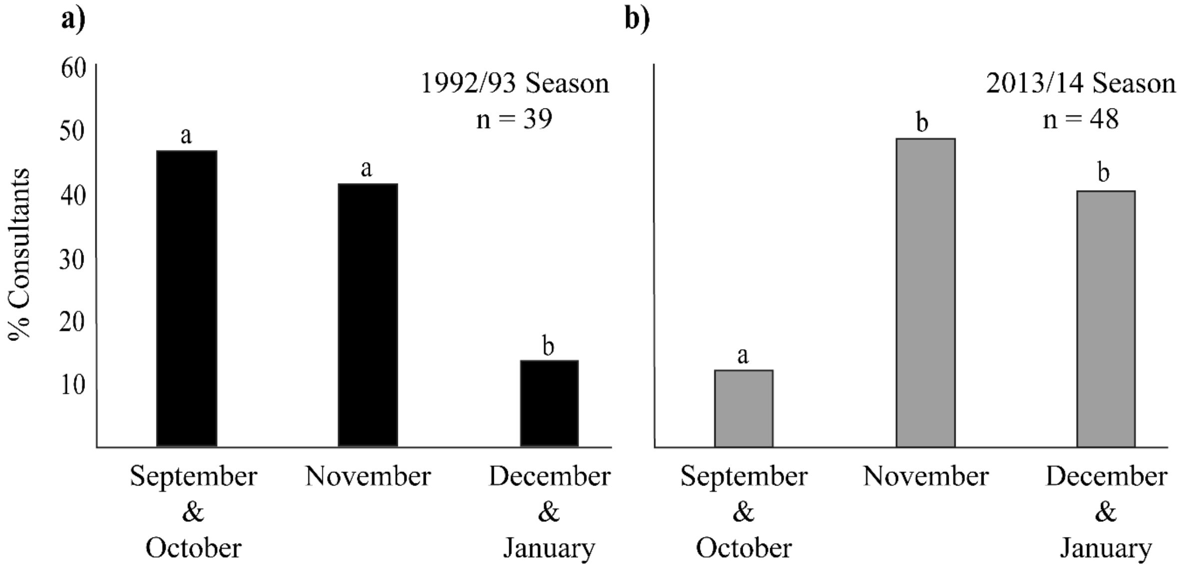


Fig. 2.3. Earliest *Creontiades dilutus* detection relative to month of the cotton-growing season (pairwise comparisons of proportions with Holm-Bonferroni corrections for multiple comparisons); a) 1993 survey; b) 2014 survey. Statistical comparisons were calculated separately for each survey. Different letters, within seasons, reflect significantly different results at $p < .05$.

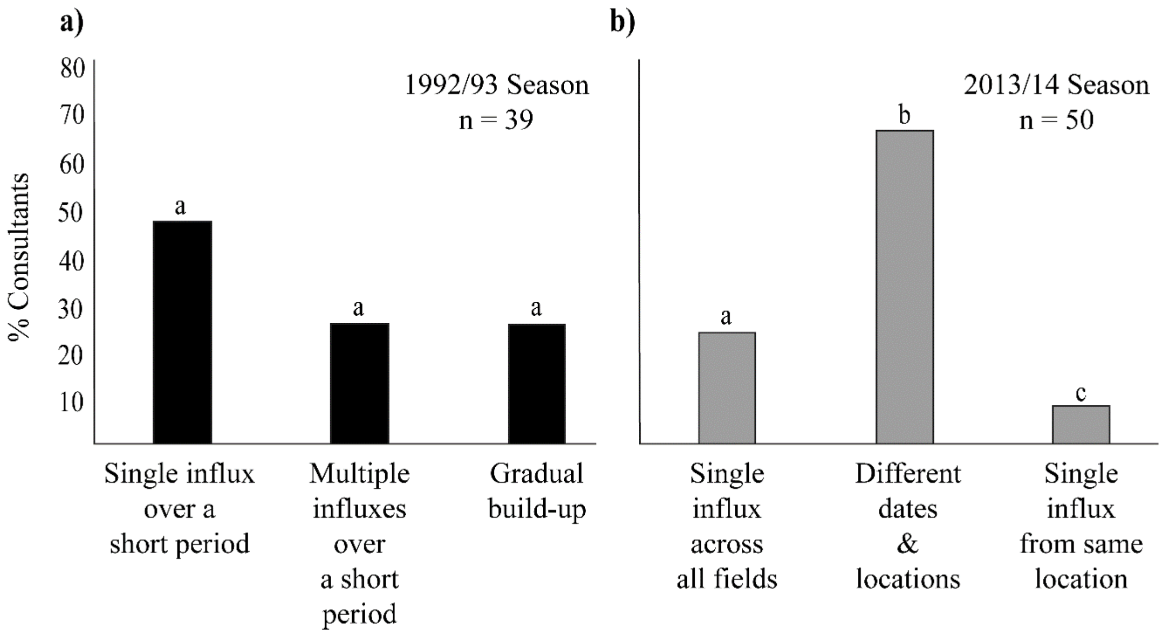


Fig. 2.4. Patterns of earliest *Creontiades dilutus* detection (pairwise comparisons of proportions with Holm-Bonferroni corrections for multiple comparisons); a) 1993 survey; b) 2014 survey. Statistical comparisons were calculated separately for each survey. Different letters are significantly different at $p < .05$.

Table 2.2. Consultant perceptions of environmental influences associated with the earliest detection of *Creontiades dilutus* bugs. Consultants were surveyed at the end of the 1992/93 (n=16) and 2013/14 (n=43) seasons. Binomial proportion tests of functional groups were conducted independently for each season (*=statistical significance at $p < .05$, NS=non-significance). Forty percent of consultants identified multiple environmental influences. Responses are reported as a percent of the total.

Environmental influences	**Seasons (% responses)**		**Descriptions of mirid associations**
	1992/93	**2013/14**	
Storms / weather fronts / rain	58	10	Mirid influx follows storm fronts or winds. One response associated mirids with wet/mild springs (phrasing of this response implied alternative hosts may also be involved).
Non-cotton crops	21	16	Mirids frequently associated with legumes, but also on wheat, sunflowers & winter pulse crops.
Weeds / pastures	21	8	Mirids associated with unsprayed roadsides, grasslands & pastures.
Native plants / rivers / lakes	–	21	Mirids associated with trees, and natural vegetation along rivers / lakes / roads.
Cotton development	–	8	Mirids found on earliest planted, largest, or most developed cotton (as the canopy closed over).
Western direction	–	7	Mirids found on western fields on a farm, or western winds.
Eastern direction	–	1	Mirids move in from crops grown in eastern regions, but in one instance mirids simultaneously moved in from the west.
Random / no idea	–	5	Mirid patterns are highly variable, no obvious environmental influences.
Total	100	100	

In the 2014 survey, consultants associated mirid invasions with a broader variety of environmental factors (Table 2.2), including the influence of native plant species, developmental stage of cotton crops, and the direction from which mirids invade. When all 8 environmental factors (including consultants that did not observe associations with any factor, Table 2.2) were statistically evaluated, there was substantial variation in their perceptions of these factors (binomial proportion test, $\chi^2 = 14.8$, $n = 43$, $p < 0.05$). To identify which factors were particularly important, pairwise proportion tests were conducted. An analysis using all 8 factors did not have sufficient statistical power for multiple comparisons, so the responses were pooled (post hoc) into 5 logical functional groups: “weather”, “non-cotton hosts”, “cotton developmental stage”, “geographical direction”, and “no factor identified”. The subsequent pairwise analysis indicated that the presence of non-cotton plants was the factor most often associated with initial mirid invasions (Table 2.3). This post hoc analysis did not identify which type of host plant was most influential, but about a quarter of the respondents identified crop hosts or native plants as being associated with initial mirid invasions (Table 2.2).

With regard to weather and geographical direction, it is likely that these factors interact with each other as well as with host availability more generally. Storms that move in from the west, for instance, may be associated with consultants observing mirid invasions after storms in their western fields. If these storms bring rain, then host abundance may increase. Questionnaire surveys may not be the most effective method to discriminate between the interacting factors, but these surveys do identify the factors noticed by the people who are most familiar with the farms on which they work.

2.3.2 Field studies

Records of six quantified mirid field surveys are available for the period between the 1976/77 and 2002/03 seasons (inclusive) included in this analysis (Table 2.4). The mean duration between the initial detection of mirids and peak mirid abundance was 25 days. Most mirids were initially detected when cotton was squaring (consistent with consultant surveys, see above). Only two surveys, from Central Queensland, initially detected mirids from cotton seedlings

Table 2.3. Pairwise comparisons for proportions of environmental functional groups reported by consultants to be associated with the earliest *Creontiades dilutus* detections in the 2013/14 season (n = 43) (see Table 2.2 for descriptions). P-values were corrected for multiple comparisons using the Holm adjustment. NS = not statistically significant.

	Non-cotton hosts	**Weather**	**Cotton hosts**	**Geographical direction**
Weather	$p < 0.001$	–	–	–
Cotton hosts	$p < 0.001$	NS	–	–
Geographical direction	$p < 0.001$	NS	NS	–
No factor identified	$p < 0.001$	NS	NS	NS

Table 2.4. Quantitative surveys of *Creontiades dilutus* invasions into cotton in eastern Australia. District names are those published in *The Australian Cottongrower* (Greenmount Press). Abbreviations for months are as follows: "Nov." = November, "Dec." = December, and "Jan." = January.

Season	District	Cotton Stage	Date of Initial Mirid Detection		References
			Adults	Nymphs	
1976/77	Darling Downs	Squaring	Mid Dec.	Late Jan.	(Bishop, 1980)
1977/78	Darling Downs	Squaring	Early Jan.	Early Feb.	
1985/86	Central Queensland	Seedlings & Squaring	19 Nov.	Not reported	(Chinajariyawong, 1988)
1986/87	Central Queensland	Seedling	21 Nov.	11 Dec.	
1992/93	Namoi Valley	Squaring	21 Dec.	Not reported	(Fitt et al., 1994a)
1992/93	Central Queensland	Squaring	10 Sep.	17 Sep.	(M. M. Miles, 1995)
1993/94	Central Queensland	Squaring	28 Oct.	5 Nov.	
1992/93	Namoi Valley	Not reported	2 Nov.	1 Dec.	(Mensah & Khan, 1997)
1993/94	Namoi Valley	Not reported	22 Nov.	6 Dec.	
1993/94	Gwydir Valley	Not reported	30 Nov.	10 Dec.	

(Chinajariyawong 1988). Initial mirid detection varied by date, ranging from late September to early January. Detection of nymphs always followed the initial detection of adults, which is likely the result of successful oviposition once adults have arrived at cotton. It is expected that if adults were previously present in cotton, then both adults and nymphs would have been detected in the earliest surveys.

2.3.3 District reports

In general, the pest pressure of green mirids reported in district reports varied across seasons and regions (Table 2.5). Severe infestations were reported exclusively for all districts in Queensland and the Border Rivers in northern New South Wales. These infestations were always in November and December. No severe infestations were reported for the southernmost districts. Every instance of severe mirid pressure reported for St. George matched severe infestations in either the Darling Downs or Border rivers (the two closest districts) (Fig. 2.1, Table 2.5). There was no indication that reports of severe infestations in Central Queensland were correlated with such infestations in other districts.

2.4 Discussion

2.4.1 Arrival patterns of green mirids

Pest numbers increase in crops due to the arrival of new individuals, and the production of offspring (Rajapakse and Walter, 2007). The interaction of these processes can make pest management more challenging. For example, mirids that arrive and lay eggs at a specific site, without moving to new locations, may have a negligible impact on crop production. If, however, mirids lay eggs at different sites while moving across farms, then the subsequent nymph emergence may result in a near simultaneous increase in pest pressure across many crop paddocks. Managing low densities of newly arrived mirids and higher densities of established mirids may require different approaches, such as luring incoming individuals away before they arrive at cotton, or applying insecticides targeted at resident populations. Distinguishing between invasions and within crop pest production, however, can be challenging. When cotton growers

Table 2.5. Assessments of *Creontiades dilutus* pest pressure derived from district reports published in *The Australian Cottongrower* (Greenmount Press). Infestations were considered "severe", moderate (= "mod."), or "low" (see text for details). Blank cells in the table indicate "low" classifications. Districts included from left to right, reflect their relative geographic localities from north to south. Districts above the same horizontal line are within 200 km from the neighbouring districts included in this analysis. The Gwydir Valley and Bourke districts were not included because so few records were available from them.

Season	Central Queensland	Darling Downs	St. George	Border Rivers	Namoi Valley	Macquarie Valley	Southern New South Wales	References
2007/08		Mod.	Severe	Severe	Mod.			(Dowling (ed), 2007, 2008a)
2008/09		Mod.	Mod.	Mod.				(Dowling (ed), 2008b, 2009a)
2009/10		Mod.						(Dowling (ed), 2009b, 2010b)
2010/11	Mod.	Mod.				Mod.	Mod.	(Dowling (ed), 2010a, 2011a)
2011/12			Mod.		Mod.	Mod.		(Dowling (ed), 2011b, 2012a)
2012/13	Severe			Mod.		Mod.		(Dowling (ed), 2012b, 2013a)
2013/14		Severe	Mod.	Severe	Mod.	Mod.	Mod.	(Dowling (ed), 2013b, 2014b)
2014/15	Mod.	Mod.		Mod.	Mod.	Mod.	Mod.	(Dowling (ed), 2014a, 2015b)
2015/16	Mod.	Severe	Severe	Mod.		Mod.	Mod.	(Dowling (ed), 2015a, 2016b)

report “bad” seasons, with regard to mirid infestations, they may be responding to large influxes of insects, but also to the duration that mirids have been present in their fields.

Green mirids found in cotton must have moved in from other host species, because cotton is completely harvested (removed from the environment) each season and replanted in the following September and November. Field surveys of farms and the surrounding landscapes indicate that during winter mirids are most abundant in the arid landscapes west of cotton farms (Miles 1995, Hereward et al. 2013a).

Evidence from quantified field studies suggests that mirid numbers build-up gradually as a result of within-crop offspring production (Table 2.4). Surveys across different districts over a 17-year span, each initially found low densities of adults, followed by detections of nymphs about 23 days later. Nymphs complete development within 15 days at typical cotton-growing temperatures (Khan et al., 2009; Pyke and Foley, 1985), so this observed population growth is consistent with the expected pattern of nymph emergence from eggs laid at previously unoccupied crops by a few newly arrived adults.

The perceptions of consultants about the overall patterns of mirid invasions varied, but most consultants did not view mirid invasions as a single influx. In 1993, just under 50% of consultants viewed mirid invasions as a single pulse over a short period, whereas in 2014, only 35% of consultants viewed mirids as a single influx. Of the consultants that characterized mirid invasions as a single influx in 2014, observations were split between mirids arriving across all fields or originating from a single location.

Furthermore, consultants differed about the time of year and crop stage in which they perceived that most mirid invasions occurred. In 1993, initial mirid invasions were reported to be more common from September through November. In contrast, in 2014 most invasions were reported to be later, from November through December. Consultants in both 1993 and 2014 associated mirid invasions with seedling and squaring cotton, but despite these shared perceptions, in 2014 several consultants linked mirid invasions with the canopy closure of larger crops. These surveys did not explicitly address why some consultants associated mirid invasions with early-season non-reproductive cotton, while others associated invasions with mid-season mature crops. It is possible that there is a decreased detection rate of mirids once canopies have closed.

The variation in consultant responses may be due either to the differences in phenology (i.e. crop stage), or the associated management, between cotton varieties grown in 1993 and 2014. In 1993, all cotton crops were conventional non-transgenic varieties, but since 1996 the most commonly planted cotton are transgenic varieties toxic to important lepidopteran pests (Wilson et al., 2018; 2013). It is unlikely that mirids changed their invasion patterns due to variation in cotton phenology because, in general, non-transgenic and transgenic cotton have similar developmental rates. In eastern Australia, the development of the first flower buds (squaring) for Bollgard II cotton is only 7-8 days longer than the conventional varieties used in 1993 (personal communication, Dr. Paul Grundy, Queensland Department of Agriculture and Fisheries). This difference is not large enough to explain the difference between 1993 and 2014 in crop stage during which mirid invasions were reported to occur.

Instead, it is more likely that consultants were more vigilant for pests earlier in the 1992-93 season than in the 2013-14 season due to differences in insecticide management practices. For early-season cotton prior to 1994, the recommended insecticide application threshold was “exceedingly low” (0.2 mirids/m of plant row) (Fitt et al., 1994a) when mirids were expected to be difficult to detect due to their low densities. Furthermore, to minimize the development of insecticide resistance by pests, permits for the newest insecticides in 1993 established a relatively short period in which they could be applied (personal communication, Dr. Paul Grundy, Department of Agriculture and Fisheries). If growers waited until after the cut-off dates recommended for newer products, then they may have had to use older, less effective chemistries. Collectively, the perceived vulnerability of young crops and short permitting dates, likely encouraged consultants to make management decisions based on their impressions of pest numbers within the first 1-2 months after planting.

In contrast, by the start of the 2013-14 season, the approach to managing mirids had changed. Insecticide spray thresholds were revised in 1994-95 to allow for higher mirid densities (reviewed by Miles (1995)). Spray guidelines for the 2013-14 season cautioned against excessive early-season insecticide-use to avoid the development of insecticide resistance in non-mirid pests and included higher threshold adjustments for warmer regions where plants were better able to compensate for mirid damage (Cotton CRC Extension Team, 2013). As a consequence, consultants in 2014 had a longer opportunity to monitor their farms before selecting insecticides. This longer monitoring period, may have allowed consultants to observe slower increases in numbers or field-specific arrivals of mirids.

2.4.2 Use of cotton and alternative hosts by mirids

Consultants agreed that most mirids invaded farms when cotton crops were either in the seedling or squaring stages (Fig. 2.2). In 2014, a few consultants also reported that mirids invaded once cotton was beginning to "close over" (Table 2.2), which coincides with the squaring stages (CRDC 2019a). These qualitative findings of consultant perspectives were similar to the quantitative field studies that were conducted between 1976 and 1994 (Table 2.4).

Consultant opinions differed regarding the dates during which initial mirid invasions were perceived to occur (Fig. 2.3). Most consultants observed invasions in November, but those surveyed in 1993 also reported invasions in September and October, whereas those surveyed in 2014 reported many invasions in December. Two possible explanations could account for this discrepancy, and they are not mutually exclusive. First, seedlings may have been more intensely monitored in the 1992/93 season relative to the 2013/14 season. Sampling delicate seedlings for mirids relies on visual inspection, because the crops would be damaged if alternative methods were used (CRDC 2019b). In the 1992/93 season between 69-82% of consultants visually inspected crops for mirids (Miles, 1995), but only 11% of consultants visually inspected crops in the 2013/14 season (CRDC 2019b). Alternatively, the environmental conditions that potentially promoted early season invasions in the 1992/93 season may not have been present until much later in the 2013/14 season.

It is difficult to evaluate independently the influence of crop stage and date on early season mirid invasions. Typically, cotton is planted in late September or October as soil temperatures warm, so cotton squaring usually occurs in November and early December (CRDC 2019a). Various environmental factors, however, may shift the normal planting and squaring periods. For example, unusually cold conditions or severe storms may delay crop maturation (e.g. Dowling, 2016b, p. 58), and dry conditions may encourage farmers to shorten their season to minimize irrigation costs (e.g. Dowling, 2016b, p. 65).

Despite the correlation between crop stage and month, the evidence suggests that mirid invasions occur in response to the presence of squaring cotton rather than being related to time of year. Squaring cotton is known to be vulnerable to feeding mirids (Bishop, 1980; Chinajariyawong, 1988), and mirids prefer young squares over larger, more developed squares (Chinajariyawong, 1988). Field surveys over 17 years show that initial mirid invasions vary across 5 months, but almost always occur when cotton is squaring (Table 2.4). In addition, the field surveys conducted by Miles (1995) as well as by Khan and Mensah (1997) detected high numbers of mirids in nearby lucerne (*Medicago sativa*), but invasions into cotton did not occur until after squaring began.

While the ultimate source of mirid populations may be from remote regions (Hereward et al., 2013b; Miles, 1995), their presence in agricultural settings prior to detection in cotton suggests that movement by mirids into cotton is also promoted by local environmental factors. If the influx of mirids is reliant on local factors, then it is expected that invasion patterns would be site specific with mirids responding to their immediate surroundings. This may help explain why there is no consensus as to whether mirids arrive as a single pulse, in multiple waves, or gradually over time (Fig. 2.4). This is also consistent with the reports of many consultants in 2014 that mirids arrive at different times and locations across all fields.

Consultants identified a variety of context-specific environmental conditions that may influence mirid invasions. Most of these environmental influences relate to the presence of alternative hosts (Table 2.2). The build-up of mirid numbers on weeds and non-cotton crops has been observed for many years, but the view that natural vegetation growing in uncultivated lands are sources of invading mirids has become more widely accepted since 1993. Additional field surveys are needed to identify which non-cotton hosts support early season mirids, but previous feeding studies have shown that these insects restrict feeding to soft plant tissues associated with seedlings (Bishop 1980, Chinajariyawong 1988) or developing flowers (Miles 1995). Green mirids have occasionally been observed to feed on dead or dying insects, but they do not prey upon insects in substantial numbers (Chinajariyawong 1988), so mirid invasions are unlikely to be a response to increased insect prey.

This association with alternative host plants is notable because 73% of growers maintain or encourage the regeneration of native vegetation on their cotton farms (Roth Rural, 2014). Native vegetation is often found in riparian zones on farms. Most cotton farmers (90%) indicated that they either agreed or strongly agreed when asked if they valued riparian zones on their cotton farms (Roth Rural, 2014). These farmers associated native vegetation with an increase in the number of beneficial insects (90%), fewer overall pests (including non-mirids) (89%), and a reduction in required insecticide sprays for nearby cotton (87%) (Roth Rural, 2014). Despite the association of native vegetation with mirids, the perceived benefits of pest management as a whole by consultants is similar to those of cotton growers (CRDC 2019b).

Several consultants indicated most mirids move onto farms from the west, but a few reported invasions from the east (Table 2.2). Similarly, there have also been occasional reports that mirids move in from the north or east near the Queensland-New South Wales border (Dowling, 2013a), suggesting that mirids may not always invade from the proposed winter sources in western landscapes (Miles 1995, Hereward et al. 2013a). The direction of invasions is likely related to the location of host patches where mirids build up (Table 2.2). It is impossible to verify independently whether mirids do build-up in natural vegetation or move in with storms without an extensive network of quantitative mirid surveys beyond the routine pest surveillance associated with growing cotton. Ideally these surveys would need to represent each cotton district as well as the remote regions west of the primary cotton-growing region. To assess the influence of weather patterns, mirid surveys would not only need broad geographic coverage, but also a sampling frequency that would detect mirid dispersal before and after unpredictable storm events.

The district reports from 9 growing seasons indicated that severe mirid infestations are most frequent in southern Queensland and northern New South Wales (Table 2.5). Severe infestations were never reported from the southernmost districts. All "severe" mirid densities were reported in December, after most initial invasions are expected to have taken place (Table 2.5). The absence of reported severe infestations in the southern regions may be due to the cool climate and shorter growing season relative to the warmer regions in Queensland and northern New South Wales. Mirid growth is slower in cool conditions (Khan 1999), resulting in slower population growth. Alternatively, assessing infestation intensities can be subjective, and what may be a moderate pest level in southern New South Wales may be perceived as a higher severity infestation in central Queensland. To be able to assess the degree to which the subjectivity of "pest pressure" varies between consultants from different districts would require standardized mirid surveys, against which the reports of "severity" can be compared. Nevertheless, the higher pest pressures reported by consultants working in northern districts in this study identifies a direction of future research when assessing mirid numbers across broad geographical landscapes.

While environmental conditions can be highly variable, rainfall in particular, regional patterns of mirid pest pressure across the districts of Darling Downs, St. George, and Border Rivers are discernible. These districts are all within 200 km of each other. When one district has severe mirid pressure, then the other districts also have severe and moderate pest pressures. These three districts may have similar mirid intensities because mirid population growth is similar because of their close proximity and similar climate. Within each district, however, some farms receive substantial rain that promotes crop growth, whereas others are much drier leading to delayed crop maturation or loss (e.g. Dowling, 2016b). This rain-driven mosaic of host availability and potential crop attractiveness (i.e. squaring cotton) may promote mirid dispersal across farms, and potentially between nearby districts. The districts could also have similar intensities through a near-simultaneous invasion (from a long-distance dispersal event). The reasons behind the similar intensity of mirid infestations across these regions could be evaluated further with field studies (mirid counts) in these districts, which would be particularly informative if these surveys took place during both wet and dry seasons as non-cotton host availability fluctuates.

2.4.3 Management and research recommendations

Mirid infestations are a consequence of two processes; insect movement (invasions) across the landscape, and the production of offspring once mirids settle in cotton. It is important to understand whether mirid movement into cotton continues throughout the season or occurs sporadically in response to specific stimuli. Molecular evidence suggests that mirids regularly move between adjacent cotton and lucerne patches (Hereward et al., 2013a), but there is much less data available on large scale patterns of crop invasion.

Mirid invasions are correlated with the presence of squaring cotton, availability of non-cotton hosts, and weather patterns. Green mirids are likely to respond to similar environmental stimuli across their range, but the specific timing of these cues varies across regions within the cotton-growing landscape in eastern Australia. Central Queensland, southern Queensland/northern New South Wales, and the southern cotton districts have different mirid invasion and population growth dynamics.

The reports of consultants between the 1992/93 and 2013/14 seasons are the best historical record of mirid invasions available, but they do not replace the need for long-term quantitative monitoring efforts. To fully understand mirid dispersal routes, subtle mirid-plant interactions in field settings, and the effects of storm events, requires a more refined spatio-temporal resolution. Fortunately, the consultant community regularly quantifies pest numbers and can be a valuable resource for long-term monitoring programs.

Every consultant routinely quantifies the densities of mirids and other pests for the farms that they monitor, relying on a combination of industry guidelines and personal experience. Most consultants check fields at least twice per week during peak growing periods when assessing pest densities (CRDC 2015). If consultants were able to report their pest records using shared protocols, standardized by the cotton industry, then a dataset of pest abundance would be constructed that has a field-scale spatial resolution. This proposed pest-record resource could then be used to assess local and landscape level environment-pest dynamics.

The contents of the following section (Chapter 3) have previously been published. I (Justin Cappadonna) am responsible for the concept and design of the study, and have conducted all statistical evaluations. Gimme Walter and James Hereward contributed editorial revisions to the manuscript.

Chapter 3. Inferring invasion paths into cotton by *Creontiades dilutus* (Hemiptera: Miridae) from arid zone and agricultural sources

Abstract

Managing agricultural pests that use multiple host plant species is a challenge when individuals move between host plants in natural vegetation and agricultural environments. The green mirid (*Creontiades dilutus,* Hemiptera) is endemic to Australia and routinely invades cotton from local uncultivated vegetation, or may originate from remote locations in the arid continental interior. This bug is polyphagous and highly mobile, which contributes to its pest status in cotton systems as well as its persistence in arid environments with sparsely distributed ephemeral host plants. The aim of this study was to evaluate how *C. dilutus* individuals use a variety of host species across remote arid regions and highly managed agricultural landscapes. A set of structured field surveys spanning vast areas across the Simpson Desert in the arid heart of Australia, as well as subcoastal cotton production systems, evaluated host use across environments that share few plant species. High numbers of *C. dilutus* were sampled from *Cullen australasicum* (perennial hosts) and *Goodenia cycloptera* (ephemeral hosts) in the desert following rain. In agricultural environments, *C. dilutus* bugs were mostly on irrigated *Medicago sativa* (lucerne), and to a lesser extent *Melilotus indicus* near rivers. Significantly, bugs were on these plants prior to the planting of cotton across all environments surveyed. Further, these data allow inferences to relate host use, host abundance and insect migration to one another to understand the connection that *C. dilutus* bugs have between arid and agricultural environments.

3.1. Introduction

Green mirids, *Creontiades dilutus* (Hemiptera: Miridae), are polyphagous bugs endemic to Australia (Malipatil and Cassis 1997). They have been recorded from at least 97 plant species (Hereward and Walter 2012), feed on soft plant tissues (Hori and Miles 1993, Khan 1999), and are found across diverse environments in Australia, including the arid continental interior and sub-coastal agricultural environments (Hereward and Walter 2012). Bug numbers in these environments are seasonally inverse, with relatively higher densities in arid regions during winter (Hereward and Walter 2012). Their relative abundance and spatio-temporal dynamics across these environments likely reflects their tolerance of extremely hot and dry climatic conditions, the availability of appropriate host species at the appropriate phenological stages for feeding, and their ability to cover vast distances necessary to colonize suitable host plants (Walter and Hengveld 2014). Aspects of this generalization are investigated in this paper, as explained and justified below.

The Simpson Desert in the arid Australian interior has hot, dry summers with temperatures regularly exceeding 40°C (BOM 2019). This is well above the reported survival threshold of *C. dilutus* individuals (Khan et al. 2009), although a few individuals have been found in this region when temperatures are in the high 40's (J. P. Hereward, pers. comm., 2016). Peak plant growth during summer occurs with grass species following pulses of light rains (Nano and Pavey 2013). In general, these grasses do not host *C. dilutus* bugs (Hereward and Walter 2012). Most hosts are typically forb species (Hereward and Walter 2012), with peak growth during mild winters following isolated heavy rains (Nano and Pavey 2013). Previous surveys of winter hosts have found the highest bug densities on *Cullen australasicum* (Fabaceae) in drainages across the arid interior (Hereward and Walter 2012).

The Simpson Desert and eastern agricultural environments are separated by a semi-arid expanse of floodplains and braided rivers known as the Channel Country. This area is dry for much of the year, with water permanently present only in the deepest channels (Purdie 1984, Lawley et al. 2011). Most of this region consists of grasslands and sclerophyll woodlands, which are not known to support high *C. dilutus* densities (Hereward and

Walter 2012), but occasional hosts may be found scattered along dry river channels and disturbed roadsides.

In contrast, eastern subcoastal agricultural environments receive more rain than the arid interior (BOM 2019), and much of the agricultural landscape is comprised of irrigated crop monocultures (Fitt 1994, Graetz et al. 1995). Despite the relatively greater plant availability in this region (BOM 2018b), not all plants host *C. dilutus* bugs in equal numbers. For example, cotton (*Gossypium hirsutum*) (Malvaceae) routinely hosts these bugs across vast areas of Queensland and New South Wales during summer (Cappadonna et al. 2018, CRDC 2019b), but bug densities tend to be lower on cotton than on nearby lucerne (*Medicago sativa*) (Mensah and Khan 1997) and pigeon pea (*Cajanus cajan*) (both Fabaceae) crops (Lawrence et al. 2007).

Even in relatively low numbers, *C. dilutus* bugs are important cotton pests (CRDC 2019b) that can delay harvest and reduce yield as a result of boll (fruit) loss (Bishop 1980, Chinajariyawong 1988). Effective management of pests requires understanding the ecology of herbivorous pests (Lewis 1981, Walter 2003). Studies of highly mobile pests, in particular, must encompass spatial scales large enough to include both the source and destination of dispersing individuals (Kennedy and Storer 2000). While the technology to track such small-bodied bugs across hundreds of kilometres is not available, the seasonally inverse patterns of abundance (Hereward and Walter 2012), observations by crop consultants (Cappadonna et al. 2018), and evidence of gene flow across vast (Hereward et al. 2013b) all suggest that these bugs move from the arid interior into crops in eastern Australia. It is unclear, however, if these bugs disperse across hundreds of kilometres in a single flight, or if they use stopover locations before arriving in cotton. There is evidence that *C. dilutus* adults are capable of flying long-distances across the Bass Strait to Tasmania (Hill 2013, 2017), but when traveling overland, various host plants may provide sheltering sites, and perhaps are breeding sites.

The association between high numbers of *C. dilutus* and non-crop hosts has been evaluated for only a few plant species (Miles 1995, Khan 1999, Hereward and Walter 2012). The consistency of host use by *C. dilutus* individuals is likely a result of specific relationships with particular plant species. Host plants can be of primary, secondary, or incidental value to insects (Walter and Benfield 1994, Rajapakse and Walter 2007). Primary hosts consistently support high numbers of individuals in the field, including a high number of nymphs. Secondary hosts are irregularly used, and produce relatively few offspring. In contrast, incidental hosts only sporadically host adults, and generally do not host juveniles. These incidental hosts are often included in taxonomic host lists, even though they are unlikely to contribute substantially to the production of offspring (Walter and Benfield 1994). Incidental hosts, however, may aid the dispersal of bugs by providing occasional sheltering and feeding sites as insects cross otherwise inhospitable environments.

The environmental conditions and host availability across the large spatial scales inhabited by *C. dilutus* bugs varies substantially, both seasonally and stochastically. An organism's response to the plants that are available will be determined by its physiological requirements and tolerances, as well as its sensory abilities (Walter and Hengveld 2014). Therefore, evaluating the relationships that are consistent between *C. dilutus* bugs and the hosts that are available across its range will help identify the behavioural and ecological processes that are responsible for the host use patterns that are documented. The identification of consistent insect-host associations may also improve the predictability of forecasting models, which, of necessity, have to deal with vast spatial scales, because it means that all 97 reported host species do not have to be independently evaluated as to their availability.

The aim of this study is to evaluate patterns of host use by *C. dilutus* populations across the area of known gene flow (the Simpson Desert, Channel Country flood plains, and eastern cotton-growing environments) (Hereward et al. 2013b), to identify the primary host plants of this species and thus infer the movement patterns of these bugs. The background detailed above justifies two objectives. The first is to survey crucial parts of the landscape using a standardized technique to allow for comparisons across localities. The second is to identify if there are host species that are used consistently (and in high numbers) within environments containing secondary, incidental, and non-host plant species.

3.2. Methods

Field surveys evaluated *C. dilutus* densities on different plant species across a vast region of central and eastern Australia, spanning 1,700 km east to west, and 1,300 km north to south (Fig. 3.1). Plants were visually located along driving transects, and bugs were collected with sweep nets. Sweep nets are effective in capturing *C. dilutus* (Deutscher et al. 2005, Threlfall et al. 2005, CRDC 2019b), because they can sample foliage close to the ground. Sampling was focused on single-species plant patches, so that insect-host relationships could be assessed. Each plant species at each site (Fig. 3.2) was swept 100 times, with each sweep being unidirectional. Samples in non-agricultural environments were collected prior to the time when cotton was planted in eastern Australia. The survey period in agricultural environments (during summer) overlapped with the cotton season because this coincided with peak plant growth for that region.

There was substantial variation in the spatial distribution of plants across and within each survey region. Surveys were conducted in the midst of a prolonged drought and patches of vegetation were mostly in locations adjacent to water, separated by large expanses without green plants. This patchy plant distribution was also influenced by local environmental factors, each requiring a slightly different sampling design and statistical analysis. These local environments were classified into five groups, with each associated with only one of the broader geographic areas sampled (see below): 1) arid drainages, 2) arid sand dunes and swales, 3) semi-arid braided rivers, 4) roadside riparian zones, and 5) roadside lucerne. Within each local environment the distribution of bugs across different host species was evaluated (see the subsections below). Any plant species sampled at fewer than five sites within an environment were omitted from further analyses. evaluated independently for adults and for nymphs using a generalized linear model (GLM) with quasipoisson errors. The log number of sweeps was included as an offset term to account for search effort.

We hypothesized that there would be at least one plant species that consistently hosted high numbers of bugs in each local environment. If each local environment contained suitable plants to shelter bugs prior to the cotton season, then these locations may be potential invasion sources of pests, or ecologically significant stopover points during

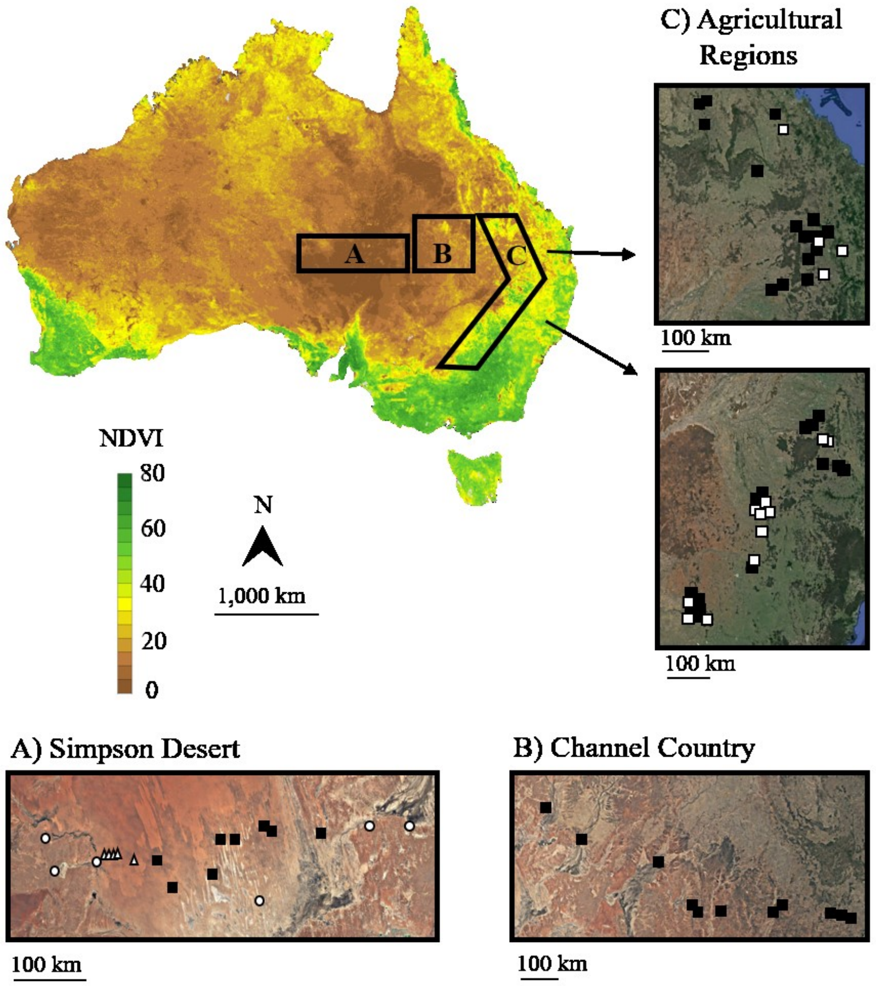


Fig 3.1. Regions surveyed for *Creontiades dilutus* bugs within the context of the normalized difference vegetation index (NDVI). Areas with abundant vegetation are represented in green, and areas with little vegetation, or none, are brown. Survey regions include: A) the Simpson Desert, B) Channel Country, and C) subcoastal agricultural regions. The particular sites with *Cullen australasicum* are indicated by open circles, *Goodenia cycloptera* by open triangles (both in the Simpson Desert and Channel Country), lucerne by open squares (in the agricultural regions), and other less common host plants by closed squares. The base layer of the NDVI is from the (BOM 2018a).

Fig. 3.2. Images of the growth pattern and relative distribution of the plants that were sampled across field sites, including: concentrations of vegetation (including *Cullen australasicum*) at arid drainages (A, B) and diffuse patches (including *Goodenia cycloptera*) on dunes (C) in the Simpson Desert, dry (D) and relatively wet roadside margins near streams in agricultural landscapes (E), and lucerne farms (F).

migration. Also, if these plants consistently supported the production of juvenile bugs, they could be local sources of pests. In addition, we proposed that in environments where plants had access to water (thereby retaining soft tissues on which the bugs feed), bug abundance would be greater than on hosts at drier sites.

3.2.1. Bug numbers across all environments

Bug abundance within each of the five local environments (defined above) was assessed using a generalized linear model (GLM) with a quasipoisson error distribution. The log number of sweeps with a net was included as an offset to account for sampling effort. The statistical significance of bug numbers across all five environments was evaluated against the specific environment that had the fewest bugs (see below for evaluations within each environment). All plant species within particular environments were pooled. All evaluations in this study used the R statistical program (R Core Team 2017).

3.2.2. Arid drainages (Simpson Desert)

Creontiades dilutus bugs were sampled in the Simpson Desert between 14 – 25 August 2014. Plants were visually located by driving a desert track circuit between the small towns of Birdsville in the east and Mt. Dare in the west (450 km apart from one another). The landscape was comprised predominately of sandy and stony soils that were virtually devoid of vegetation, as well as scattered clay-bottomed drainages where most of the plants grew. Plant growth at the drainages was likely a response to the 51.4mm of winter rain that had fallen between April and August (BOM 2019).

Cullen australasicum is a putative primary host for *C. dilutus* bugs (Hereward and Walter 2012), and grew in most drainages that were surveyed. The specific composition of vegetation varied across sites. Since *Cu. australasicum* was already considered to be a putative primary host, this set of analyses compared bug numbers found on this host species against the alternative plant species within the drainages. Vegetation patches varied in their taxonomic composition. Some drainages had a mix of *Cu. australasicum* and alternative plant species growing near each other (within 100m). In contrast, some drainages contained only a single species (either *Cu. australasicum* or an alternative plant species) that grew at least 300m from any other patches of plants, with no hosts growing between them. These patch types were called "mixed" and "isolated," respectively.

The plant species that were evaluated (besides *Cu. australasicum*) included the native species *Ptilotus polystachyus* (Amaranthaceae) and an unidentified *Senecio* sp. (Asteraceae), as well as the introduced weed *Lactuca serriola* (Asteraceae). These alternative plant species are documented hosts for *C. dilutus* bugs (Hereward and Walter 2012), and were present in sufficient numbers to allow statistical evaluations. These alternative species were patchily distributed, however, and not all sites had each of the three alternative host species (*P. polystachyus, Senecio* sp., and *L. serriola*) growing near *Cu. australasicum*. These non-*Cullen* species were pooled into a single "alternative host" group for analyses.

Total mirid numbers (adults and nymphs) per sample were statistically evaluated using a generalized linear mixed model (GLMM) with Poisson errors, performed in R 3.4.3 (R Core Team 2017) using the "lme4" package (Bates et al. 2015). Host plant identities (*Cu. australasicum* or the alternative host group) and the proximity of different host types (mixed or isolated) were included as fixed effects. The "sampling site" term was included as a random effect to avoid pseudoreplication. The log-transformed number of sweeps was included as an offset term to account for sampling effort.

3.2.3. Arid sand dunes and swales (Simpson Desert).

Surveys at the sand dunes and swales were conducted at the same dates as the drainage surveys (above). In general, plants that grew on dunes and swales were sparse, and separated by vast expanses of sandy soil without vegetation. The exception was a 30km section of the driven transect that had high densities of small herbs and forbs flourishing. They were in flower after receiving 51.4mm of localized rain within six months prior to the survey (BOM 2019), with extensive areas of flowering *Goodenia cycloptera* (Goodeniaceae), *Craspedia* sp. (Asteraceae), and *Polycalymma stuartii* (Asteraceae).

The abundance of plants in this extensive patch of vegetation across dunes and swales contrasted markedly with that of the relatively isolated plant patches surveyed in the drainage surveys. Individual plants mostly grew in single species clumps, with clumps separated from one another by a few meters of bare sand (Fig. 3.2). This allowed an 18km transect with six sampling sites, 3km apart, to be established without *a priori* knowledge of the composition of the plant species at any of these preselected localities. At each site, plants were surveyed within a 100m radius. Since these plants were not previously identified as primary host species, differences in bug numbers were evaluated without an *a priori* prediction of which plant species would host higher mirid densities. Mirid numbers were statistically evaluated independently for adults and nymphs using a generalized linear model (GLM) with quasipoisson errors. The log number of sweeps was included as an offset term to account for sampling effort.

3.2.4. Semi-arid braided rivers (Channel Country).

Surveys were conducted during 26 – 28 August 2014 and 4 – 8 October 2015. The aim was to collect mirids in natural environments before that season's cotton had been planted (in October and early November). The region, however, was extremely dry (BOM 2018c) and few green plants were located. Statistical evaluations were not possible because too few plants and bugs were found. Despite the scarcity of host plants, the conditions of this region between the known wintering sites and cotton fields in which bugs likely pass through are described, and details about the captured bugs are also given.

3.2.5. Roadside riparian zones (agricultural landscapes).

Roadside surveys were conducted from late September through mid-February in 2014 and 2015. This coincided with the growing period of local cotton crops, and the typical arrival of *C. dilutus* pests in cotton (Cappadonna et al. 2018). Sweep net surveys targeted weeds, crop species that had escaped cultivation, and herbaceous native species growing along roadsides and waterways. Samples were treated as independent replicates if conspecific plant species were more than 1km apart, and within 30km of cotton farms.

Plants growing near rivers tended to have more vigorous growth than conspecifics at drier sites. Plants were considered to grow at "wet sites" if they were within 100m from water, and "dry sites" were at least 400m from water (no samples were collected between these sites). Flowering plants with green leaves were checked for bugs if the size of vegetation patches allowed for at least 30 sweeps with a net. The plant species evaluated at these sites were *Melilotus indicus* (Fabaceae), *Medicago polymorpha* (Fabaceae), *Ammi majus* (Apiaceae), and *Rapistrum rugosum* (Brassicaceae). Bug numbers across host species at wet and dry sites were compared statistically with a generalized linear model with negative binomial errors and adjusted for zero inflation.

3.3.6. Roadside lucerne (agricultural landscapes)

To assess whether mirids are associated with volunteer lucerne growing on roadsides to the same extent as they are with irrigated lucerne, both were checked for mirids. When lucerne was surveyed along roadsides, the nearest cultivated lucerne (within 20km) was also checked for mirids. These localities were surveyed within 1h of each other. Mirid densities from lucerne across two environments were contrasted using a student's t-test.

3.3. Results

3.3.1. Bug numbers across all environments

The most bugs/sweep of the net were in the drainages of the Simpson Desert where *Cu. australasicum* grew, and at patches of lucerne growing in the agricultural environment (Table 3.1). High numbers of bugs/sweep were also found along the dunes and swales of the Simpson Desert. Relatively few bugs/sweep were collected in the Channel Country and along agricultural roadsides. These environments had substantially different numbers of sites with green plants from one another, and this was reflected in the differential in the number of sites checked for bugs across these environments.

3.3.2. Arid drainages (Simpson Desert)

Samples from 14 drainages across the desert were suitable for statistical evaluation. Four sites had a mix of *Cu. australasicum* and various alternative plant species, and five had only *Cu. australasicum*. An additional five drainage sites had the suite of alternative host species and no *Cu. australasicum* plants. All other drainages were devoid of vegetation, or supported small patches of plant species found only at a single site.

Table 3.1. The numbers of *Creontiades dilutus* bugs captured/100 sweeps of vegetation (not specified as to plant species) in each environment (mean ± 1 SE), and number of sites sampled (n). Statistical significance was evaluated using a GLM (number of bugs ~ environment + offset (log (number of sweeps)), quasipoisson). Statistical comparisons were made relative to the agricultural roadside environment.

Environment	**Bugs/100 sweeps**	**Sites sampled (n)**	**t-value**	**P-value**
Roadsides (agricultural landscapes)	2.51 ± 0.58	155	–	–
Channel Country	3.04 ± 1.44	33	0.023	NS
Sand dunes and swales (Simpson Desert)	9.40 ± 2.81	21	3.52	<0.001
Arid drainages (Simpson Desert)	15.32 ± 5.91	20	5.07	<0.001
Lucerne (agricultural landscapes)	15.67 ± 4.18	38	5.26	<0.001

Cullen australasicum hosted significantly more bugs than the alternative plant species, regardless of the distance that *Cu. australasicum* grew from the other plants (GLMM, $p<0.01$, Fig. 3.3). The statistical interaction between host plant species and the distance that plants grew from each other was not significant. A total of 494 mirids was collected at these 14 sites, with 70% of them nymphs. Of the nymphs, 311 individuals were from *Cu. australasicum*, and 35 individuals from alternative hosts. Of the adults, 119 individuals were from *Cu. australasicum* and 29 individuals were from alternative hosts.

3.3.3. Arid sand dunes and swales (Simpson Desert)

A total of 260 mirids was collected across all hosts. *Goodenia cycloptera* hosted a significantly higher number of adults than the alternative hosts (GLM, $p<0.05$, Fig. 3.4). Most bugs were adults, but all hosts supported nymphs. As with adult numbers, *G. cycloptera* hosted the most nymphs.

3.3.4. Semi-arid braided rivers (Channel Country)

A total of 85 mirids was collected. Most were hosted on diffuse patches of *Scaevola parvibarbata* (Goodeniaceae) (22 mirids) in drier regions collected from four sites, and from patches of green plants (at 3 sites) within 30m of river channels that still retained water (12 mirids). The vegetation near water could not be identified because it had been trampled by cattle. The remaining mirids were found in low densities (1-2 per patch) associated with roadside vegetation.

3.3.5. Roadside riparian zones (agricultural landscapes)

The numbers of bugs on the 16 most common plant species along roadsides were evaluated (Table 3.2). Four of those plant species grew at sites near and far from rivers with water (Fig. 3.5). The plants at wet sites were greener and grew with more vigour than those at dry sites (assessed visually). Overall, *C. dilutus* densities were highest at wet sites, but the contrast was statistically significant only for *Mel. indicus* (Fig. 3.5).

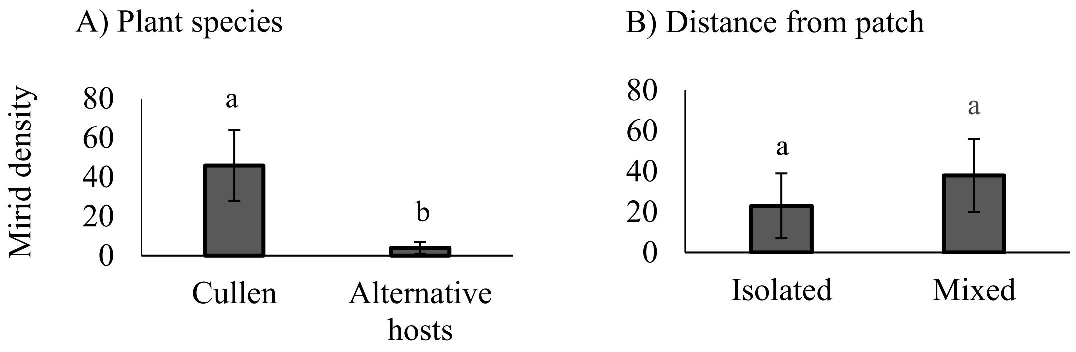


Fig. 3.3. *Creontiades dilutus* densities ($\bar{x}$ ±1 SE /100 sweeps) from plant patches containing *Cullen australasicum* and alternative host plant species (GLMM, different lowercase letters within each bar diagram indicate statistical significance ($p < 0.01$), n = 14). Fig. A) bug densities for each host group (*Cu. australasicum,* various alternative host species). Fig. B) bug densities for each host group in "isolated" patches (with a single species growing more than 300m from alternative host plant species), and "mixed" patches (with multiple species growing within 100m from one another).

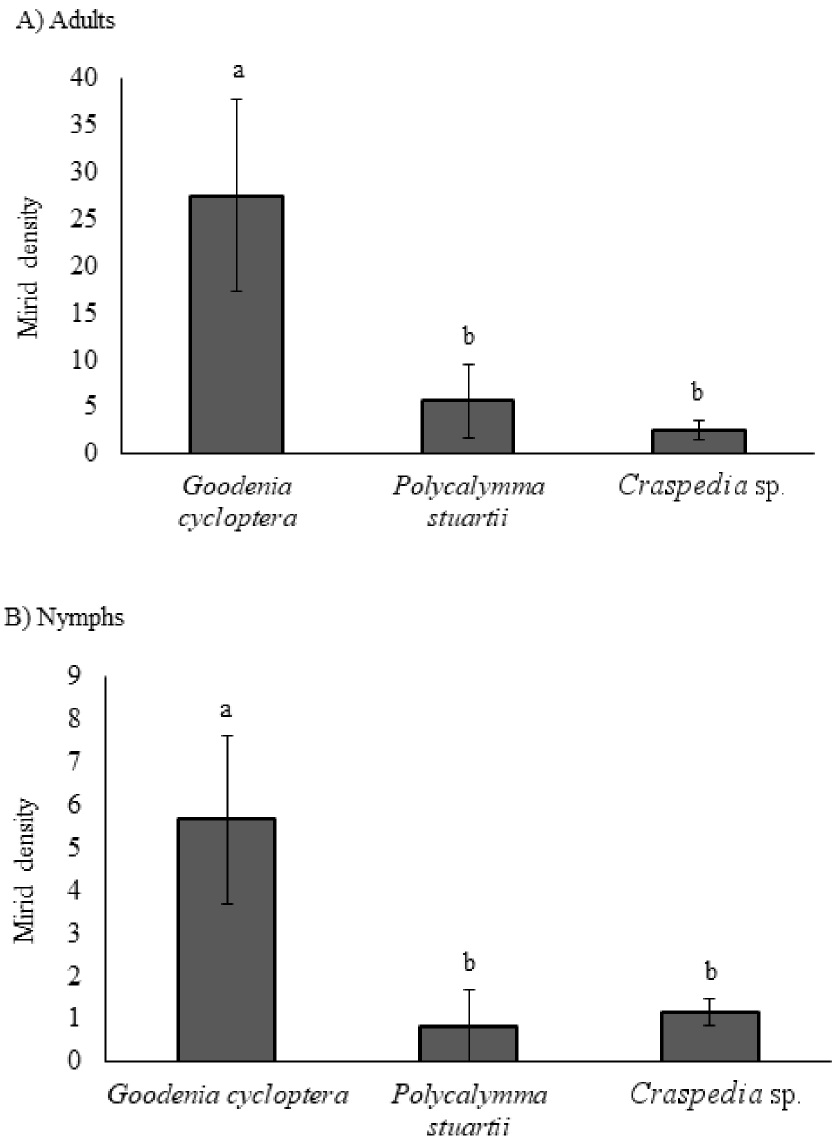


Fig. 3.4. *Creontiades dilutus* densities ($\bar{x}$ ± 1 SE /100 sweeps) collected from three plant species growing on sand dunes. Differences in bugs numbers were independently evaluated for A) adults and then for B) nymphs (GLM, different letters within each age class indicate statistical significance at $p < 0.05$, $n = 8$).

3.3.6. Roadside lucerne (agricultural landscapes)

Creontiades dilutus bugs were found on irrigated lucerne crops (on farms) and non-irrigated volunteer lucerne along road margins. The mean density of *C. dilutus* (adults and nymphs combined) on farms was 38.7 ± 14.4 SE individuals/100 sweeps, which was about three times as many as found along roadsides (11.3 ± 4.8 SE, see Table 3.3). Relatively few volunteer lucerne patches were found along roadsides (n = 13), and there were significantly more nymphs at farms than at roadside sites (Table 3.2). Although *C. dilutus* densities on lucerne across the two environments were quite different, this was not statistically significant, the variance in each being substantial. The greater numbers of *C. dilutus* bugs on farms, and the 15:1 ratio of nymphs across the two environments suggest that lucerne crops (which also cover a relatively larger area) are more productive hosts for *C. dilutus* bugs than is roadside lucerne (Table 3.3).

3.4. Discussion

Creontiades dilutus bugs use different environments at different times in Australia (Miles 1995, Hereward and Walter 2012), and they move long distances across landscapes (Hill 2013). Suitable conditions for host plants (and the bugs that feed on them) in arid environments are highly variable across years (van Etten 2009, Nano and Pavey 2013). In agricultural systems, crops provide some degree of consistency in host availability (e.g. BOM 2018b, CRDC 2019a), but the alternate hosts outside of crops are highly variable (Table 3.2). Understanding the interaction of these insects with arid and agricultural landscapes is therefore crucial to understanding the timing of their appearance and likely abundance in crops.

Table 3.2. *Creontiades dilutus* densities ($\bar{x}$ ± 1 SE /100 sweeps) on roadside hosts in agricultural landscapes, the proportion of nymphs on each host species, and the proportion of sampled sites with *C. dilutus* individuals (for each plant species). Hosts are listed in sequence, from highest density to the lowest. Several hosts were evaluated further in relation to their proximity to water (superscript "a") (see Fig. 3.5). Volunteer lucerne located along roadsides was further evaluated with respect to bug density against lucerne crops (superscript "b").

Host species	**Family**	***C. dilutus* density**	**% nymphs**	**% sites with bugs**	**n**
Tribulus micrococcus	Zygophyllaceae	39.4 (±18.8)	19	86	7
Melilotus indicus [a]	Fabaceae	11.9 (±4.0)	31	46	13
Medicago sativa [b]	Fabaceae	11.3 (±4.8)	37	54	13
Ammi majus [a]	Apiaceae	7.1 (± 4.5)	50	56	9
Verbena bonariensis	Verbenaceae	5.6 (± 2.4)	17	50	14
Rapistrum rugosum [a]	Brassicaceae	5.5 (± 3.1)	7	22	41
Tribulus terrestris	Zygophyllaceae	4.8 (± 2.4)	54	43	7
Crotalaria dissitiflora	Fabaceae	2.9 (± 1.9)	27	29	7
Sorghum bicolor	Poaceae	1.9 (± 0.7)	6	50	12
Solanum elaeagnifolium	Solanaceae	1.6 (± 1.2)	23	40	5
Helianthus annuus	Asteraceae	0.6 (± 0.3)	3	50	8
Medicago polymorpha [a]	Fabaceae	0.1 (± 0.1)	0	3	29
Tithonia diversifolia	Asteraceae	0.1 (± 0.01)	2	14	7
Lactuca serriola	Asteraceae	0.0	–	0	26
Vicia sativa	Fabaceae	0.0	–	0	15

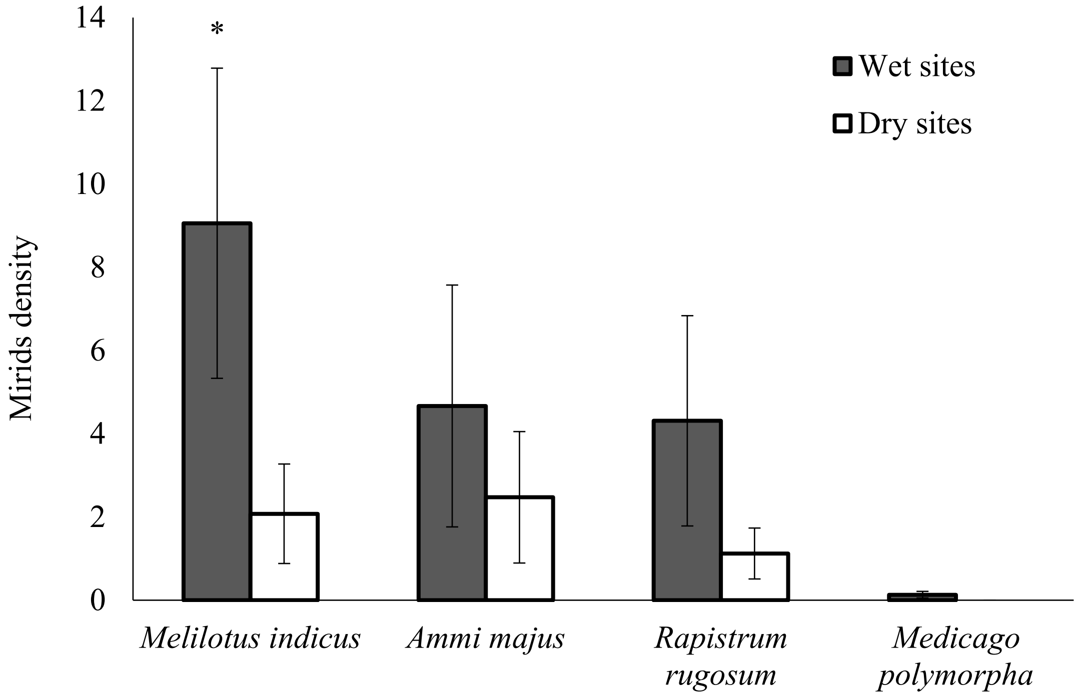


Fig. 3.5. *Creontiades dilutus* densities ($\bar{x}$ ± 1 SE /100 sweeps) from different hosts growing at wet sites (defined as sites within 100m from a water source) and dry sites (more than 400m from a water source). The number of sites sampled was as follows: *Melilotus indicus* (wet = 6, dry = 7), *Ammi majus* (wet = 3, dry = 6), *Rapistrum rugosum* (wet = 12, dry = 29), and *Medicago polymorpha* (wet = 12, dry = 17). Bug numbers were evaluated with a GLMM with negative binomial errors. An asterix indicates statistical significance ($p < 0.05$) across wet and dry sites for a host species.

Table 3.3. *Creontiades dilutus* numbers ($\bar{x}$ ± 1 SE /100 sweeps) collected from lucerne crops (on farms, irrigated) and volunteer lucerne (not irrigated) growing along roadsides. The same number of sites on farms and along roadsides was sampled (n = 13 each). Bug densities were statistically evaluated (Student's t-test with unequal variances) independently for adults and nymphs (adults, t-stat = 1.23, df = 17; nymphs, t-stat = 2.61, df = 13). NS = statistically non-significant.

	Farms	**Roadsides**	**Statistical significance**
Adults	23.4 (± 9.8)	10.0 (± 4.7)	NS
Nymphs	15.3 (± 5.3)	1.3 (± 0.8)	$p = 0.02$

3.4.1. Host use across different environments

In arid environments, bugs were most abundant at the drainages of the Simpson Desert (Table 3.1). High numbers were also at the sand dunes and swales of the desert, but unlike those concentrated at drainages and crops, these bugs were distributed in diffuse ephemeral patches across the landscape. Few bugs were found in the drought-stricken Channel Country prior to cotton planting. These tended to be on plants growing at the few sites near rivers that retained water. Relative to agricultural roadsides during the early and mid-cotton season, fewer plants were available to survey in the Channel Country, but both environments had similar capture rates of bugs (Table 3.1). The situation in wetter years is likely to be different. There may be more bugs invading cotton as a result of a greater abundance of reproductive hosts closer to cotton, and shorter distances between hosts that provide shelter for adults migrating from arid regions.

Plants that were included in the desert drainage analyses were all known host species for *C. dilutus* bugs (Hereward et al. 2013b), but there were consistently higher numbers on *Cu. australasicum* than on the surrounding vegetation (Fig. 3.2). In contrast to the importance of *Cu. australasicum,* in particular, the composition of the alternative host plant patches (e.g. isolated single species, mixed species) had little influence on bug abundance. The presence of bugs on *Cu. australasicum* may be a result of transient individuals temporarily sheltering on these hosts, but the high proportion of nymphs indicates that these hosts are also good reproductive host plants, and thus a source of bugs in the environment.

Cullen australasicum is a relatively persistent plant with a long-tap root that can access deep ground water several months after heavy rains (Grimes 1997, Suriyagoda 2011). This contrasts with the phenology of ephemeral, shallow-rooted plant species that grow on the sandy soils of dune slopes immediately following rain (Nano and Pavey 2013). Historical records demonstrate that consistently the most common ephemeral plant in the dune systems after rain is *G. cycloptera,* which can cover vast swaths of the desert in lush flowering vegetation (Crocker 1946, Eardley 1946, Boyland 1970, Fatchen and Barker 1979, Purdie 1984, Gibson and Cole 1988). Since *G. cycloptera* yielded a high proportion of nymphs across all the sampling sites in the dune system (Fig. 3.4), this host is likely a primary source of bugs across the arid environment following sufficient rain, and a likely source of bugs that invade cotton.

In agricultural landscapes *C. dilutus* bugs use a variety of host species (Table 3.2). Unlike hosts in arid environments, it was possible to examine bug abundance on particular host species relative to their proximity to water (i.e. riparian zones) or irrigated fields (roadside and crop lucerne). Bug abundance was higher on *Me. indicus* near water and irrigated lucerne than on those same species growing at drier sites (Fig. 3.5, Table 3.3). Other hosts had similar, but statistically non-significant, correlations between wet and dry sites.

In general, plants at wet sites were growing with greater vigour than those at dry sites. Well-hydrated plants may host higher bug numbers than do water-stressed plants, because hydrated hosts may persist for longer in the environment (e.g. Anjum et al. 2017), and their soft tissues may facilitate feeding. The rupture-and-flush feeding mechanism of mirids, used to imbibe liquified plant contents (Backus *et al.*, 2007), may be most effective with host tissues that have relatively high water content. This contrasts with those heteropterans that feed directly from the phloem, in which the extraction of nutrients relies on a differential osmotic gradient to move food contents (Sharma et al. 2014), and thus allows feeding on relatively more dehydrated plants (e.g. Tariq et al. 2012, Bestete et al. 2016, Tan et al. 2017).

3.4.2. Proposed dispersal patterns

Many pest managers perceive that *C. dilutus* invasions originate from arid regions west of cotton farms, and are associated with particular weather patterns that come from west to east (Cappadonna et al. 2018). This study identified specific hosts in the Simpson Desert that produce bugs in high numbers when autumn rainfall is sufficient, and this would be the likely source of such invasions. However, directly tracking individual bugs across the landscape was beyond the scope of this study. There was no evidence of high numbers of bugs in the regions between the Simpson Desert and cotton farms. Infestations of farms by *C. dilutus* pests in the cotton season following the surveys of arid environment reported here (e.g. Roughley et al. 2015), does suggest, therefore, that these bugs may migrate directly across these intermediate areas.

Possibly, though, many of the bugs did not move eastwards across the Channel Country. The pest pressure on cotton farms during the 2014/15 season was reported to be lower than previous seasons (Roughley et al. 2015), so the dry conditions in the Channel Country may have acted as a barrier that hindered long-distance dispersal. This suggests that a proportion of bugs may use hosts as sheltering sites during migratory flights in wetter seasons, when hosts are more readily available.

That is, they may disperse independently of one another, rather than together in large numbers. Attempts to evaluate the movement of *C. dilutus* bugs within agricultural environments did not detect the arrival of bugs in large pulses (Miles 1995, Pearce and Zalucki 2005, Macfadyen et al. 2015). Even when *C. dilutus* pests infested crops were harvested, forcing emigration, no corresponding influx of arrivals was detected in neighbouring crops (Miles 1995, Mensah and Khan 1997, Pearce and Zalucki 2005), which explains some of observations of initial infestations at different times and locations on cotton farms (Cappadonna et al. 2018).

3.4.3. Conclusions and future research

The way in which animals in arid Australia persist between wet periods has been well investigated for a few vertebrate species (e.g. Tyndale-Biscoe 2005, Free et al. 2015, Jordan et al. 2017). Similar investigations about invertebrate persistence are less common (Morton et al. 2011, Molyneux et al. 2018), so conducting surveys during drought conditions was fortuitous because it provided insight about the persistence of bugs during prolonged dry periods. Animal survival under such conditions is often attributed to their use of refugia (e.g. Free et al. 2013, Tischler et al. 2013), and dramatic fluctuations in population size can be explained by a sudden change in food availability following rain (e.g. Greenville et al. 2012, Greenville et al. 2013, Pedler and Lynch 2016). This is likely also true for *C. dilutus* populations, with productive refuges at desert drainages and near those few water sources that do persist, and booming populations on ephemeral plants in areas of sufficient rain. Clearly, movement across extensive areas provides a basis for desert persistence of *C. dilutus* populations.

Future research should identify the specific timing and pathways used by bugs as they move onto farms. This may require a network of intercept traps (e.g. light traps (Hill 2013)), similar to those used to track *Helicoverpa* spp. (Lepidoptera) pests as they move from the arid interior into cotton (Fitt et al. 1995, Gregg et al. 1995). These traps should be established in relation to the geographic areas, environmental situations, and by hosts that have been identified in this study. It may also be possible to model the phenology of these hosts in relation to the rainfall that stimulates the growth of soft tissues fed upon by bugs, which may help predict the severity of pest pressure in agricultural environments, but more precise tests of the interpretations offered here are needed.

Chapter 4. Assessment of the differential use and movement of green mirids (*Creontiades dilutus*) across cotton and pigeon pea

Abstract

The distribution and relative abundance of insect herbivores across different hosts is influenced by the movement patterns of individuals, as well as their relative attraction to and retention on these hosts. Understanding these movement patterns is crucial to interpreting the host associations of insects and designing effective management strategies, but this does present methodological difficulties. In Australia, *Creontiades dilutus* bugs (Miridae) are hosted in low numbers on cotton (*Gossypium hirsutum*) (Malvaceae), but even low numbers can cause substantial crop damage. In contrast, bugs are consistently more abundant on pigeon pea (*Cajanus cajan*) (Fabaceae), which, in Australia, is routinely planted near cotton (as a resistance management tool for bollworms (*Helicoverpa* spp.) (Noctuidae). The frequency and direction of the movement of *C. dilutus* bugs across the two crops are poorly understood, as is the relative use they make of each. For instance, bugs may move away from cotton and onto pigeon pea, thereby reducing pest pressure in cotton, or pigeon pea refuges may produce bugs that eventually feed on cotton. This study is the first assessment of the movement by *C. dilutus* adults across cotton and pigeon pea hosts using a series of field surveys and molecular gut content evaluations. Pigeon pea is a more productive host for *C. dilutus* bugs than is cotton, but bugs move back-and-forth between both crops across hundreds of metres (at least) while feeding. These results suggest that pigeon pea may be a local source of *C. dilutus* pests. The implications of host use and movement by *C. dilutus* bugs are discussed.

4.1. Introduction

Ecology is primarily concerned with understanding the dynamics underlying the distribution and local abundance of organisms across the environment, a large part of which is understanding their movement within their environment (Andrewartha and Birch 1954, Walter and Hengeveld 2000). In agricultural systems, effective and environmentally acceptable pest management of herbivorous insects requires an intricate understanding of the ecology of each species (Lewis 1981, Walter 2003), with movement patterns often being a particularly challenging aspect to understand (Stinner et al. 1983, Irwin 1999). Assessing the movement of insects further complicated for species that fly at night, are passively transported by winds, feed on multiple host plant species, or have invasions that originate from several localities (Stinner et al. 1983). These points are not mutually exclusive, so all may apply in any situation.

In Australia, green mirids (*Creontiades dilutus*) are important pests of cotton (*Gossypium hirsutum*) (McColl et al. 2011, Wilson et al. 2018), and understanding their ecology faces all the difficulties outlined above. These bugs are nocturnal (Cappadonna et al. 2020), have long-distance dispersal associated with wind (Hill 2013, 2017), routinely use multiple host plant species (Hereward and Walter 2012), and arrive at cotton hosts from distant inland sources each season (Miles 1995, Cappadonna et al. 2018). Invasions from the arid continental interior may also be aided by the presence of alternative host species growing at irrigated farms and riparian zones across agricultural landscapes in subcoastal eastern Australia (Chapter 3).

Sticky traps that intercept flying individuals (Khan 1999) and molecular gut analyses (Hereward and Walter 2012, Hereward et al. 2013a) have provided evidence that *C. dilutus* adults frequently move back-and-forth across different host species. When cotton and lucerne (*Medicago sativa*) (Fabaceae) are cultivated near each other, 35% of bugs collected from cotton, and 18% from lucerne (also known as alfalfa), had gut contents that contain both host species (indicating bidirectional movement across hosts) (Hereward et al. 2013a). This bidirectional movement is notable because lucerne consistently hosts significantly more bugs (including nymphs) than does cotton (Miles 1995, Mensah and Khan 1997, Khan 1999), suggesting that individuals move both towards and away from hosts supporting high bug densities.

Explicit tests of the movement by *C. dilutus* bugs across cotton and alternative host species (other than lucerne) are relatively rare. Movement of *C. dilutus* adults has typically been inferred by the differential numbers and the timing of substantial infestations of bugs across different hosts (Lawrence et al. 2007, Cappadonna et al. 2018, Cappadonna et al. 2019). Pigeon pea (*Cajanus cajan*) (Fabaceae), for example, consistently hosts *C. dilutus* bugs in significantly higher numbers than does cotton (Lawrence et al. 2007), but it is not known whether pigeon pea: attracts bugs away from cotton, supports a relatively higher production of bugs than cotton, is associated with the bidirectional movement of individuals to and from cotton (as described above with respect to lucerne), or some combination of these aspects. Understanding these movement patterns is crucial to understanding the spatio-temporal dynamics of *C. dilutus* bugs, and is important for pest management efforts, because in Australia pigeon pea is routinely planted within 2km of transgenic *Bt* cotton (which is toxic to lepidopteran pests) as refuges for *Helicoverpa* bollworm *Bt* resistance management (Wilson et al. 2013, CRDC 2019a). These refuges are required to be irrigated through the cotton season, so they produce non-resistant bollworms to mate with insecticide resistant individuals from *Bt* cotton, and thus minimise the production of offspring that are homozygous for resistance (CRDC 2019a). An unintended result of establishing these pigeon pea refuges, however, is that they may also act as a nursery crop that is a source of *C. dilutus* bugs that move into cotton.

This study is the first explicit evaluation of the movement of *C. dilutus* pests across host plant species in the field, in this case across cotton crops and nearby pigeon pea refuges. There were two objectives. The first was to determine if bugs restrict feeding to either cotton or pigeon pea, and the second was to assess the relative frequency at which bugs move between these host species. This method is applicable to other generalist herbivore bugs and the results relevant to pest management, as discussed later.

4.2. Methods

4.2.1. Field surveys

Field surveys were conducted on cotton farms across the Australian states of Queensland and New South Wales during the 2014-15 and 2015-16 cotton seasons. *Creontiades dilutus* (adults and nymphs) were collected with sweep nets from pigeon pea refuges and unsprayed Bollgard II cotton. Half the surveys were conducted during November and December when most cotton was squaring (developing flower buds), and the remaining surveys were in January (the end of the season) when mature cotton began to senesce (with relatively few green leaves and other soft tissues).

Each sample consisted of 100 sweeps, with multiple samples collected at each farm. Pigeon pea refuges were much smaller than surrounding cotton fields, so typically had two samples (per farm) separated from one another by at least 200m. Surveys of cotton were taken "near" pigeon pea refuges (within 300m), "far" from refuges (500-600m), and "without pigeon pea" (more than 2km from refuges). The flexibility of pigeon pea allowed nets to be swept through rows of plants more easily than rows of cotton that had rigid, woody stems. This likely resulted in a difference in the capture rates of bugs between the two crops, so the search effort (number of samples) in cotton (n = 97) was increased relative to pigeon pea (n = 45).

The number of adults collected from the field was statistically tested with a generalized linear model (GLM) using the glmmTMB package in the R statistical software (Brooks et al. 2017). Model terms included the distance from the nearest edge of the pigeon pea field (the explanatory variable), the log-transformed number of sweeps with a net (a term added to the linear predictor, and which represents search effort at each location), and a negative binomial error distribution. The abundance of adults on senescent cotton and late season pigeon pea was not statistically assessed because of low collection rates at the end of summer. Also, the proportion of nymphs relative to adults in collections (a measure of productivity) was recorded, but not statistically evaluated because of low numbers on cotton.

4.2.2. Gut content analyses

The gut contents of *C. dilutus* adults were evaluated from the bugs collected in the field surveys. Captured bugs were immediately stored in 100% ethanol. The relevant host plants had been grown in a glasshouse to provide reference samples to confirm the molecular identity of food items among the gut contents. Chloroplast intergenic spacers *trnC-trnD* were amplified using polymerase chain reaction (PCR) from both bugs and host plants. DNA was extracted from plants using a CTAB protocol, and from *C. dilutus* adults using a DIY spin column method (Ridley et al. 2016). The *trnC-trnD* intergenic spacer was amplified using primers *c* (B49317: CGAAATCGGTAGACGCTACG) and *d* (A49855: GGGGATAGAGGGACTTGAAC (Taberlet et al. 1991). PCR was performed in 25µl reactions containing 0.5 units of MyTaq (Bioline), 1× buffer, 0.2 µm of forward primer, and 0.2 µm of reverse primer. PCR cycling conditions consisted of an initial denaturation of 95°C for 10 minutes, this was followed by 42 cycles of 95°C denaturation for 30s, 60°C annealing for 60s, and extension at 72°C for 60s, the annealing temperature was dropped by one degree per cycle for the first 15 cycles, and the remainder of the PCR had an annealing temperature of 45°C.

Gel electrophoresis of the PCR-amplified *trnC-trnD* regions was conducted for the gut contents of bugs collected from pigeon pea and cotton. In this way, bugs were checked to determine if their gut contained food items with *trnC-trnD* intergenic spacer PCR product lengths that matched those of the plant species from which they had been collected. Also, plant reference samples were used to confirm that each host species produced PCR product of the *trnC-trnD* intergenic spacer with a different length from one another, such that this could be readily discriminated using gel electrophoresis. PCR products from plants may be detected for up to 48h after feeding (Hoogendoorn and Heimpel 2001, Gariepy et al. 2007, Fournier et al. 2008, Muilenburg et al. 2008), so the presence of bands means that feeding must have occurred within two days of collection.

It is expected that bugs that fed only on cotton or pigeon pea would have gut contents with band lengths similar to those from the corresponding reference samples. If bugs fed on both cotton and pigeon pea, or on any other alternative plant species in the vicinity, there would be multiple bands of different lengths. Alternative plant species may possibly have similar band lengths as cotton and pigeon pea, but since the collection sites were dominated by cotton and pigeon pea monocultures, the likelihood of this occurring in substantial numbers of bugs is unlikely.

The number of *C. dilutus* adults collected from pigeon pea and squaring cotton was statistically evaluated using GLMs to determine if bugs fed upon a different host species than the one from which they were collected. Bugs collected from each host species were independently evaluated using the "stats" package in the R statistical software (R Core Team 2017). The explanatory variable was the PCR band length (with factor levels based on the length of gel bands: those individuals with band lengths similar to that of the pigeon pea reference samples only, those with band lengths similar to the cotton references only, or those with bands of multiple lengths). The presence of multiple band lengths indicates that bugs were feeding on multiple host species, but this method could not directly identify if these bands belonged to pigeon pea, cotton, or another plant species. All statistical evaluations included a binomial error distribution. Pairwise comparisons across different band lengths were then evaluated with a Tukey's post hoc test with multiple comparisons (R package "multcomp," Hothorn et al. 2008).

4.3. Results

4.3.1. Field surveys

Pigeon pea hosted more bugs (per 100 sweeps with a net) than cotton, regardless of the distance between these two crops (Fig. 4.1). A total of 284 bugs was collected from pigeon pea, whereas only 41 bugs were collected from squaring cotton (across all sites). About 48% of bugs from pigeon pea were nymphs. Relatively high proportions of nymphs were also found in cotton within 300m (40% nymphs) and 500-600m (43% nymphs) from pigeon pea. In contrast, only 18% of bugs were nymphs in cotton growing more than 2km away from pigeon pea.

No *C. dilutus* adults or nymphs were collected from senescing cotton at the end of summer. Few bugs were collected from pigeon pea during this period (17 individuals), with nymphs comprising about 35% of the samples. Insecticides had not been applied to any field prior to sampling. Some late-season cotton was sprayed with defoliants one week prior to sampling, and the leaves were starting to turn brown.

4.3.2. Gut content analyses

Molecular assessments provided evidence that *C. dilutus* adults had been feeding primarily on pigeon pea (band lengths of about 500bp), regardless of the host from which they had been collected (Fig. 4.2). This included bugs with only 500bp band lengths in their gut contents, as well as those that had fed from multiple hosts. Most of the bugs collected from cotton had been feeding on multiple host species, whereas only 10% had evidence of them feeding from cotton alone. Those individuals from cotton that had fed only on cotton, had been collected from sites that were over 2km from the nearest pigeon pea. Collectively these results suggest that *C. dilutus* adults move frequently between crops, despite pigeon pea consistently hosting a much greater number of bugs than cotton in the field and being much more productive in reproduction terms.

4.4. Discussion

The results described above demonstrate three important aspects of the relationship between *C. dilutus* bugs and their cotton and pigeon pea hosts. The first aspect is that pigeon pea hosted over six times as many bugs as cotton planted at the same time and location (Fig 4.1). Second, cotton supports the production of relatively fewer nymphs than does pigeon pea (Section 4.3.1). Nearly half the bugs collected from pigeon pea were nymphs, and about 40% of bugs collected from cotton within 600m of the border with pigeon pea were also nymphs. In contrast, only 18% of bugs from cotton planted more than 2km from pigeon pea were nymphs. Taken together, these relative proportions suggest that nymph production on cotton was enhanced by the cotton being near to pigeon pea. This suggests that pigeon pea enhances the reproductive output of bugs (relative to cotton), and if these bugs move to cotton, they produce relatively more offspring than do bugs that had been only on cotton. That is, the distant cotton patches had received relatively few mirids that immigrated from alternative hosts. Thirdly, despite the greater densities of bugs in pigeon pea, these bugs nevertheless move from pigeon pea into cotton (Fig. 4.2a). This combination of field surveys and molecular techniques suggests these bugs do not restrict their movement to pigeon pea (as

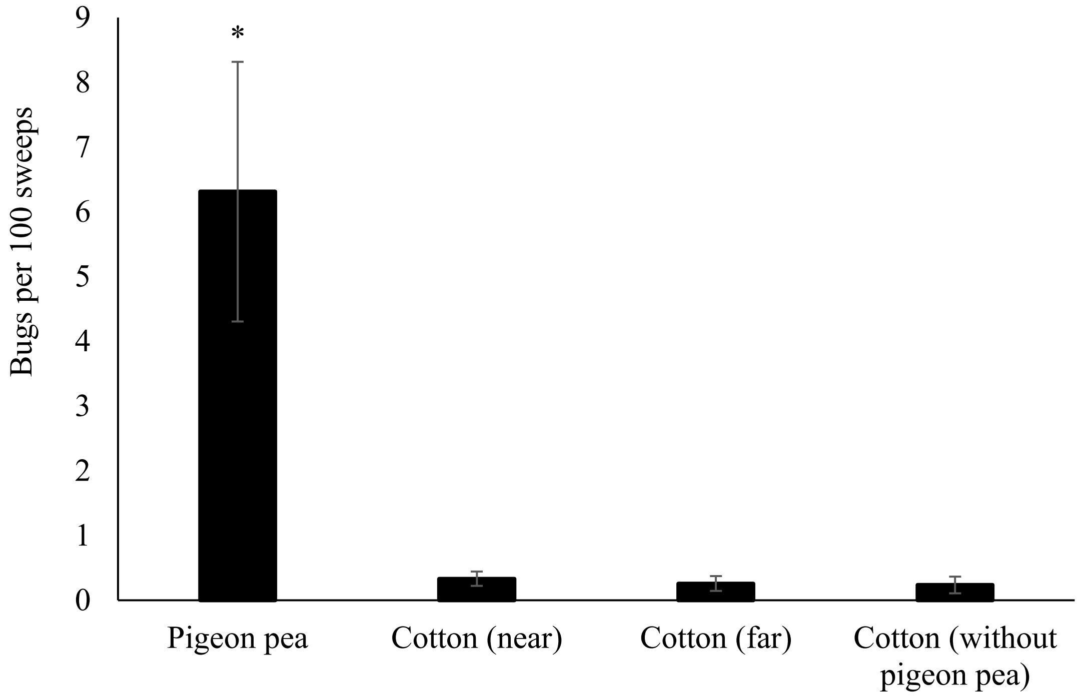


Fig. 4.1. The mean number of *Creontiades dilutus* (adults and nymphs) collected per 100 sweeps from pigeon pea refuges (n = 45 surveys), cotton within 300m of refuges ("near," n =30 surveys), cotton within 500-600m of refuges ("far," n = 27 surveys), and cotton more than 2km from refuges ("without pigeon pea," n = 40 surveys). Statistical significance was evaluated with a generalized linear mixed-effects model, and "*" is significance at $p < 0.05$.

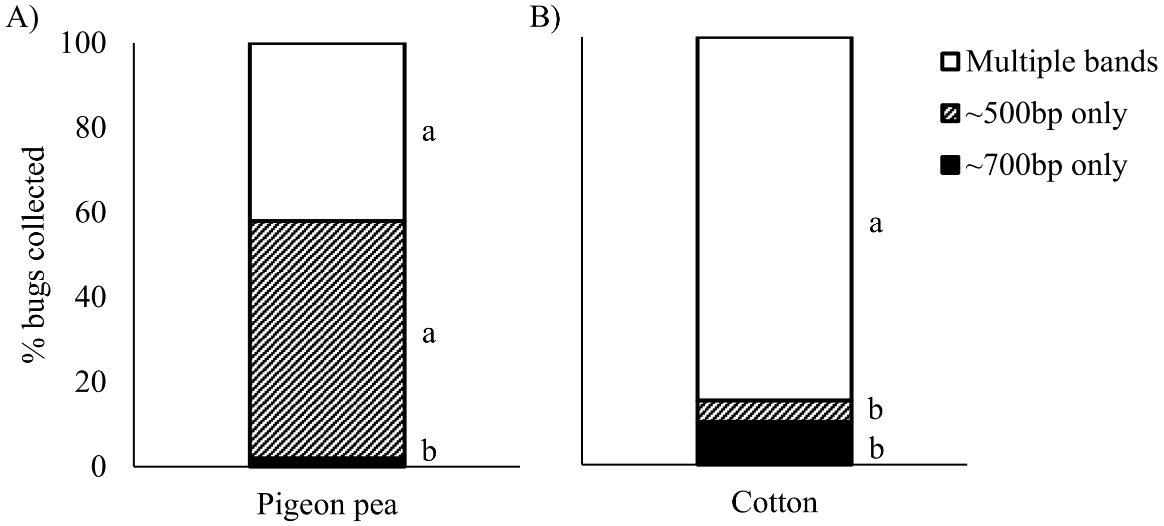


Fig. 4.2. Gut contents of *Creontiades dilutus* adults were evaluated by PCR amplification of chloroplast DNA, designed to identify pigeon pea and cotton, based on different band lengths returned by the *trnC-trnD* intragenic spacers. Bugs were collected from: A) pigeon pea and B) cotton (n = 41 individuals per host). Pigeon pea reference leaves had band lengths of about 500bp, and those of cotton were about 700bp in length. Statistical significance was evaluated with generalized linear models, followed by Tukey's post hoc multiple comparisons. Bands with different lower-case letters indicate differences of significance within a column at $p < 0.01$.

suggested by Lawrence et al. (2007)), and that pigeon pea is likely a local source of *C. dilutus* pests of cotton.

Worldwide, cotton is apparently a poor host for mirids. For example, a meta-analysis of *L. hesperus* densities, across 17 different cotton and alternative host combinations, found that the presence of alternative hosts either increased bug densities in cotton or had a variable influence on bug numbers (Meisner et al. 2017). The only consistent decline in bug densities was from cotton plants growing within cotton monocultures, suggesting that bug numbers in cotton are highly dependent on immigration from non-cotton hosts. Cotton that is developing flower buds (squares) or have open flowers tend to be associated with higher mirid densities than on cotton with only vegetative structures (e.g. Efil and Bayram 2009, Carrière et al. 2012, Pan et al. 2013). Reproductive cotton may be more attractive for some mirid species, but there is no evidence that *C. dilutus* adults are differentially attracted to cotton or pigeon pea (Cappadonna et al. 2020, also Chapter 5). The evidence presented above demonstrates that adults feed on both cotton and pigeon pea, and is inconsistent with the hypothesis that bugs move primarily towards more “attractive” host plants. It is likely the bugs move around in the environment, for reasons we do not fully understand, alight on different plants, and may be differentially arrested by plants of some species. For *C. dilutus* bugs, pigeon pea may be such a host, as well as the native hosts of *Cullen australasicum* and *Goodenia cycloptera* (Cappadonna et al. (2019), also Chapter 3).

Mirids feed on soft plant tissues (Wheeler 2001, Allen et al. 2018), so low densities of bugs in non-flowering cotton may be a consequence of plants having fewer “fleshy” reproductive structures, and so do not support a good rate of nymphal production. Rearing experiments have found that *C. dilutus* nymphal survival is highest when they feed on cotton squares (Chinajariyawong 1988) or lucerne ovules (within flower buds) (Hori and Miles 1993). Nymphs reared solely on vegetative structures fail to develop into adults (Chinajariyawong 1988, Hori and Miles 1993).

We did not explicitly evaluate the influence of host phenology on bug densities, but several previous studies have collected the first *C. dilutus* nymphs of the cotton season about four weeks after the initial invasions into cotton by adults during mid-summer (typically around December) (Cappadonna et al. 2018, also Chapter 4). The highest bug densities, including adults and nymphs, are present an additional 3-4 weeks later, and correspond with the peak flowering period of cotton (Cappadonna et al. 2018, Chapter 4). Individual flowers remain open for only one day (Beasley 1975, CRDC 2019a), and growers tend to manage cotton so that plants develop uniformly, and therefore are ready to be harvested together (CRDC 2019a). This results in pulses of cotton with soft tissues (e.g. squaring and flowering plants) across particular farms. After the peak flowering period, plant growth regulators are applied to maximize cotton yield (reducing the availability of soft new growth and thus suitability for bugs), and defoliants are sprayed at the end of the season in preparation for harvest (CRDC 2019a).

In contrast to cotton, pigeon pea refuges that are managed to maximize the production of *Helicoverpa* spp. are required to be irrigated so that the plants maintain soft tissues throughout the cotton season (CRDC 2019a). Pigeon pea in refuges typically flowers about 75 days after planting, and continues to do so until after all cotton has been harvested (typically from December to the end of February) (CRDC 2019a). Relative to cotton, this longer period in which pigeon pea retains soft tissues suitable for nymphal production likely contributes to why pigeon pea hosts a greater number of bugs.

Consistently higher numbers of adult *C. dilutus* bugs in pigeon pea (Fig. 4.1) suggests that cotton may not retain *C. dilutus* for as long as pigeon pea does. The arrestment of *C. dilutus* adults to cotton and pigeon pea in the field has received little attention by researchers (but see Cappadonna et al. (2020) and Chapter 5 for behavioural tests in the laboratory). There is, however, evidence that the retention period (i.e. arrestment) of *Lygus* mirids (relatives of *C. dilutus*) is much lower on cotton than on various alternative host plants. For example, *L. hesperus* bugs in cotton disperse across longer distances than those individuals in fields of alfalfa (= lucerne, *M. sativa*) and blackeye beans (*Vigna sinensis*, both Fabaceae) (Bancroft 2005). These results complement those from a behavioural study of *L. hesperus* adult bugs introduced into cotton fields immediately emigrated away, but those introduced into alfalfa tended to remain on those hosts (Sevacherian and Stern 1975). Similarly, *L. lineolaris* bugs visited cotton only briefly after they had been displaced from various alternative host species growing alongside cotton when the alternative hosts were destroyed (Fleischer et al. 1988).

To manage generalist pests in cotton, its crucial to understand the production of juvenile insects on significant alternative hosts (e.g. the primary hosts of these species), and also the extent of adult retention on the primary host. Host plants that retain a high proportion of pests may be planted alongside cotton as trap crops, with the intent to divert pests away from the more valuable cotton crops (Hokkanen 1991, Shelton and Badenes-Perez 2006). One of the most important aspects that influences the success of trap crops is the ability of these plants to retain herbivorous insects (Potting et al. 2005, Shelton and Badenes-Perez 2006, Holden et al. 2012). Care must be taken, however, that the presence of alternative hosts do not act as a local source of pests (Hokkanen 1991, Fitt 2000). In the case of pigeon pea, planted as a refuge crop to produce non-resistant *Helicoverpa* moths that should move across the cotton landscape and mate with any resistant moths produced in cotton to "dilute" the resistance allele (Wilson et al. 2013). The establishment of unsprayed refuge crops targeting moths, however, may have the unintended consequence of creating local sources of *C. dilutus* individuals. These bugs do move into cotton and reproduce there, despite cotton being a relatively poorer host with respect to *C. dilutus* retention and reproduction. A detailed understanding of how and why these bugs move across cotton landscapes will help pest managers decide on what trap and refuge crop system works best for their farms.

The contents of the following section (Chapter 5) have previously been published. I (Justin Cappadonna) am responsible for the concept and design of the study, and have conducted all statistical evaluations. Gimme Walter and James Hereward contributed editorial revisions to the manuscript.

Chapter 5. Diel activity patterns and arrestment behaviour in host associations of green mirids (*Creontiades dilutus*)

Abstract

Many plant bugs (Miridae) are generalist herbivores that feed on multiple host species. The reasons these bugs move across hosts, and the behavioural mechanisms responsible for their retention at specific hosts remain elusive. The green mirid (*Creontiades dilutus*) is endemic to Australia. These insects are important pests of cotton (*Gossypium hirsutum*) and even in low numbers can cause substantial damage to crops. These bugs are also present in relatively much higher numbers on pigeon pea (*Cajanus cajan*) planted alongside cotton fields, and evidence shows they move across these crops in both directions. Observations of these cryptic, highly mobile, and disturbance-sensitive insects in the field is challenging, but indirect evidence suggests that they may be nocturnal. Three experiments were conducted evaluating: 1) the temporal activity of bugs in relation to the presence of potted host plants, 2) their attraction to, and arrestment near potted host plants, and 3) the attraction and arrestment of bugs to leaf discs within Petri dish arenas. The results suggest that *C. dilutus* bugs are typically most active early in the evenings, after remaining largely motionless during the day (unless disturbed). The movement of bugs at night was arrested by host-associated olfactory cues prior to their landing upon plant substrates. The tests yielded no evidence to suggest these bugs are attracted by volatiles beyond 2cm. These outcomes demonstrate that insect behaviours need to be investigated within their typical activity periods (which at times may be difficult to establish). They also suggest that arrestment cues possibly play a central role in the host finding process of generalist *C. dilutus* and probably, therefore, other mirid species.

5.1. Introduction

The family Miridae is a large group of bugs with over 11,000 species (Cassis and Schuh 2012). Several mirid species are highly polyphagous herbivores (Allen et al. 2018), and these tend to be agricultural pests (Wheeler 2000, Allen et al. 2018). Their ability to feed on multiple host species makes predicting invasions difficult (Kennedy and Storer 2000). Nevertheless, effective pest management requires the species-specific behavioural mechanisms that underpin their host associations, and drive their movement, to be understood (Walter 2003).

The most important functional aspect of host use by herbivorous insects, in general, is the way in which the insects recognize and localize plants upon which to feed and oviposit. Host recognition not only is likely to influence the movement of insects across crops, but is crucial to understanding the use of multiple host species by organisms that are called generalists (Bernays 2001, Walter 2003). Despite the generally acknowledged importance of host recognition, the functional aspects of the foraging ecology of many mirid species (and polyphagous pests in general) remains elusive (Walter 2003).

One challenge for testing the ability of insects to recognize and process information is interpreting what information is detected as the insect approaches a plant (Bernays 2001). Insects may be attracted towards hosts if they detect particular stimuli, or their movement may be arrested when chance upon host plants (Kennedy 1978). These responses are not mutually exclusive for any particular insect species, so each needs to be investigated for the relative role of these behavioural processes. The role of particular stimuli in the host localization of the subject insect herbivore is influenced by distance from its source, at which it can be detected by the insect (Bernays and Chapman 1994).

Bugs use visual and olfactory cues to locate hosts prior to alighting upon specific plants (Bernays and Chapman 1994). In general, visual stimuli provide information about the location of host plants, and as a result they may attract insects towards plants (Kennedy 1978). Several insect species, however, will land on either host or non-host substrates in equal frequencies when substrates are of similar colour (Finch and Collier 2000). This suggests that visual cues alone are not used to discriminate among host species. Olfactory cues from a particular plant species may comprise a blend of stimuli that insects can use in the recognition and localization of an appropriate host (Bernays and Chapman 1994, Rajapakse et al. 2006, Bruce and Pickett 2011), but in the field, turbulent airflow may make it difficult for insects to achieve this successfully (Cardé and Willis 2008). The influence of olfactory cues may increase in relative importance after insects arrive within several centimetres of a host (e.g. Döring 2014), at which point olfactory stimuli may arrest insects before they leave the vicinity of the plant (Kennedy 1978).

The design of experiments that examine behavioural responses to olfactory stimuli must be able to discriminate among behaviours that result in the movement of insects towards the source of an odour (attraction), the retention of insects within the odour field (arrestment) (Kennedy 1978), as well as the movement of individuals in response to the flow of air across their mechanoreceptors (i.e. anemotaxis) (Hardie et al. 2001). The most frequently used tests of olfactory responses by insects to airborne odours involves pumping odour-laden air past insects confined in an olfactometer apparatus or wind tunnel (Barbosa-Cornelio et al. 2019). It can be difficult for observers to determine if insects are responding to the stimulation of their chemoreceptors by the presence of an odour source, their mechanoreceptors by the movement of air, or by both in combination (Hardie et al. 2001). There is a general tendency to interpret the movement, or the lack of movement, primarily in relation to their attraction to odour sources (e.g. Kennedy 1978, Barbosa-Cornelio et al. 2019). While these tests that evaluate the attraction of insects towards a source of olfactory stimulus can be informative, it is also important to design experiments that explicitly evaluate their arrestment responses as well.

The behaviours through which bugs evaluate hosts after alighting on them are relatively far better understood than those relating to arrestment. With respect to mirids, *Lygus* individuals evaluate the chemical composition of substrate surfaces by repeatedly tapping their antennae briefly onto the surface where they stand (Hatfield et al. 1983, Backus 1988). They also "dab" their mouthparts onto substrates to apply saliva that breaks down plant cells (Backus 1988). When they imbibe the resulting saliva-plant slurry they are able to evaluate the internal chemical composition of host tissues (Miles 1972, Avé et al. 1978, Sharma et al. 2014). Once a potential feeding site has been identified in this way, the *Lygus* individuals conduct a series of test probes with their stylets into the plant tissue before settling into feeding (Miles 1972, Backus et al. 2007). These relatively stereotyped responses have been observed across several studies for several *Lygus* species (e.g. Hatfield et al. 1983, Backus 1988), which suggests that chemosensory mechanisms are central to the host evaluation process. How universal this process is across mirid species in their host acceptance behaviours after their arrival at plants remains unclear.

Creontiades dilutus is an endemic Australian mirid that is hosted by several crops, including cotton (*Gossypium hirsutum*) and pigeon pea (*Cajanus cajan*) (Lawrence et al. 2007, Wilson et al. 2018). The bugs typically feed on soft terminals and reproductive tissues (flowers and young bolls), and even low numbers of these bugs on cotton can cause substantial crop loss (Bishop 1980, Chinajariyawong 1988). Most cotton-growers in Australia now also plant pigeon pea as a refuge crop as part of an insecticide resistance management strategy targeting lepidopteran pests (Doyle and Coleman 2007, Wilson et al. 2013), and these refuges are required to be planted within 2km of transgenic cotton (CRDC 2019b). It is relatively common for cotton and pigeon pea plants to be within one metre of each other at the edge of fields planted next to each other.

The movement of *C. dilutus* adults back-and-forth across cotton and pigeon pea is often inferred (e.g. Lawrence et al. 2007), but the degree to which they do so, and the mechanisms by which they recognize and settle on host plants have not been investigated. When evaluating insect movement patterns and their associated host localization behaviour, assessments should be made at the time of day during which the subject insects are typically active. However, reports about the typical activity period of *C. dilutus* adults in the field are contradictory. Active surveys for *C. dilutus* are typically conducted during the day, with most bugs collected from hosts during mid-morning and mid-afternoon (Bodnaruk 1992). Traps that intercept flying individuals, by contrast, catch most bugs at night (Lowor et al. 2009b, Hill 2013). Therefore, any assessment of movement patterns must first clarify the diel period during which *C. dilutus* adults move about the environment.

The general objectives of this study are, therefore, to identify the activity period of *C. dilutus* adults, for this is when host localization would typically take place, and then to determine the degree to which they are attracted to, and arrested by, olfactory stimuli from cotton and pigeon pea hosts. The specific hypotheses tested, based on what is known about the host-associated behaviour of these bugs (as reviewed above) are as follows: (i) individuals are most active at night, and (ii) attraction and arrestment olfactory cues are used to recognize and localize host plants.

5.2. Methods

The behavioural responses of *C. dilutus* individuals to cotton and pigeon pea hosts were evaluated in three related experiments. The first experiment evaluated the temporal activity of bugs in relation to the presence of potted host plants. The second evaluated the attraction to, and arrestment of bugs by olfactory cues, when visual stimuli associated with potted host plants were obscured. These bugs had unrestricted movement within a cage, and were given enough room to fly or walk to host substrates. The third experiment evaluated the attraction and arrestment of bugs, at close range, to leaf discs of each host within Petri dish arenas. The size of these arenas limited the distance available for flight, but the walking path of individuals could be monitored in detail (see below). These last two experiments were conducted during the period of peak bug activity identified in the first experiment. Each whole plant (experiments 1 and 2) were used once, returned to the glasshouse for a three-week "recovery" period, before the leaf discs were cut from the plant (experiment 3).

The aims of these experiments were to evaluate the temporal activity patterns, response types (attraction and arrestment), as well as the spatial scale over which bugs respond to stimuli. Examining the relative strength of attraction and arrestment across cotton and pigeon pea by *C. dilutus* individuals was beyond the scope of this study. Both male and female bugs were used in this experiment, because *C. dilutus* populations typically have a 50:50 sex ratio and both sexes feed on plants (Khan et al. 2009.

5.2.1. Temporal activity and host associations

To determine if *C. dilutus* bugs are more active during the day or night, and whether the presence of cotton and pigeon pea influences this activity, adult bugs were introduced, individually, into cages (47.5×47.5×93cm) containing either cotton, pigeon pea (both flowering), or control treatments (without hosts, see below). Host treatments included a potted plant placed in the middle of the cage. The plants touched the top of the cage, but not the sides. Each plant within a cage was enclosed by a commercially available sleeve of nylon mesh netting (Diamond Econetting, Melbourne, Victoria) (4mm^2 aperture) that was specifically designed to stop insects from entering the foliage of fruit trees. In this experiment the netting was used to prevent bugs from becoming hidden to the observer. Bugs could, nevertheless, feed on leaves by inserting their stylets through the apertures in the mesh, and they readily did so. For an individual to be judged as feeding, the insertion of their mouthparts into the plant tissue had to be directly observable. Control treatments did not contain host plants. Instead, steel ring stands enclosed in netting were included to provide bugs with a substrate about the size of the net-covered hosts, to assess their behaviour in response to the physical structure alone. In all treatments, individuals could stand either on the cage itself or on the central netting that enclosed the hosts or ring stand.

Creontiades dilutus individuals to be used in experiments were collected from lucerne crops, because these insects cannot yet be reared continuously in large numbers in the laboratory. They were fed sweet corn prior to the start of experiments. Ten *C. dilutus* adults were introduced per cage for each trial, with three trials per treatment, and bugs were used only once. They were introduced at 1400h, and monitored hourly from 1500h (10-minute observation periods) until 2400h (inclusive). This monitoring period (1500-2400h) was chosen because it encompassed periods associated with published reports of high capture rates of *C. dilutus* adults in the afternoon (Bodnaruk 1992) and evening (Lowor et al. 2009b).

Cages were located within a laboratory maintained at room temperature (about 20°C) and illuminated by natural light from windows. Cages were set up away from walls, which allowed the observer to view all sides of the plant without disturbing the bugs. The cage was lit by ambient sunlight through a window during the day, and the room darkened naturally as the sun set (between the 1800h and 1900h observations). The lights in the room were not used at all.

At each observation period, the location of each bug (on the cage, or central netting), and activity (motionless, walking, or flying) was recorded. Walking was scored only if they covered more than 1cm at the time of assessment, because this amount of adjustment in the location of individual bugs was unambiguous across the hourly observation periods, and it was similar to the scale used for the Petri dish observations (see below). Flight was infrequent and lasted less than 1 second when it occurred, so was not included in the analysis. For bugs on hosts, any insertion of their stylets into plant tissues through the mesh, which was readily observable, was also recorded. At night, observations were aided by a handheld red LED lamp. Preliminary observations detected no changes in bug behaviour in the presence of red light.

The numbers of bugs walking on the cage were statistically evaluated using a generalized linear mixed model (GLMM) using binomial errors. Binomial responses were recorded as either a 1 (walking) or 0 (not walking), and statistically evaluated using the R statistical software (R Core Team 2017) following the guidelines of Crawley (2013). Host treatments (cotton, pigeon pea, and control), time of day, and their interactions were all included as fixed effects. The same individuals in each cage were monitored at each observation period (repeated measures), so the specific cage used in each trial was included as a random effect. Similar GLMM evaluations were conducted for the number of individuals on the central netting.

Additional *post hoc* evaluations were conducted to assess if the observed bug behaviours were associated with the daytime periods of observations or those at night. Observations conducted prior to sunset (1500h through 1800h) were illuminated by natural sunlight, whereas after sunset (1900h through 2400h) the bugs were in near darkness. To help assess if *C. dilutus* adults were more active in the dark, a secondary GLMM evaluation was conducted in which the “time” factors were replaced with “darkness.” *Post hoc* multiple comparisons (Tukey's honest significance tests) were used for pairwise evaluations of each host treatment.

5.2.2. Host attraction and post-alightment behaviours

Creontiades dilutus adults were individually introduced into cages (475×475×475mm) containing either a single cotton or pigeon pea plant, or cages with both plant species (1 plant of each). Each flowering potted plant touched the top of the cage, but not the sides. No netting was placed around the host plants. A single bug was gently released, from an aspirator tube, onto the bottom of the cage (n = 30 per treatment). For individuals to reach the foliage they would have to fly or walk upwards. Alternatively, they could walk upwards on the sides of the cage or up the plant stem.

The observation room was in complete darkness, except for a red LED lamp. Each individual was observed continuously for 20 minutes after release. It was not possible to take a video recording of such small bugs in the large cages in the dark, but the of specific behaviours (e.g. antennal waving, walking) of each bug, as well as its position relative to the host plants were noted by the observer. When two plants were present, the identity of the plant species with which it made physical contact was also noted.

To test if bugs physically touched the plant (e.g. landed on a leaf) more than expected by chance, independent statistical evaluations were completed for each of the three treatments. The first two treatments in which bugs were introduced to a single host plant were evaluated by Exact Binomial Tests (with one-directional hypothesis testing). These evaluations tested the prediction that there would be a greater proportion of bugs that initially touched host plants, relative to those that did not physically contact the plants. The third treatment, with both cotton and pigeon pea plants, was evaluated by a Chi-square goodness-of-fit test (with a Yates correction factor). This tested if there were differences in the proportions of bugs that touched cotton, pigeon pea, or that did not contact either host.

5.2.3. Behaviour in the close vicinity of hosts

The previous experiment qualitatively assessed bug responses to cotton and pigeon pea, but the large size of the arena made it difficult to quantify the movement path of each bug when it was near the host plant. Therefore, bug responses to host leaf discs were observed in smaller arenas, so that movement patterns could be evaluated at a spatial scale appropriate for assessing whether: 1) *C. dilutus* individuals respond to plant leaves by changing the orientation of their movement when they are near different host species, and 2) the bugs remain longer near leaves of particular host species. Even though *C. dilutus* individuals can feed on leaves or cotton squares (buds), only their responses to leaves were tested because cotton squares could not fit into the Petri dish arena.

Responses to host plant leaf discs by *C. dilutus* adults were evaluated by introducing one individual per observation period into arenas. The top of the arena consisted of a large inverted Petri dish (14cm diameter, 2cm height). The bottom comprised of fiberglass fly screen (5mm aperture) overlaying a laminated grid (2×2cm cells on white paper) to help record the precise location of the bug as it moved about the arena. A host treatment consisted of two leaf discs (one cotton, one pigeon pea, each 1.8cm diameter) placed 5.5cm apart between the fly screen and the laminated grid beneath it.

Leaf discs were cut from mature (but non-senescent) leaves, immediately prior to the start of observations. The fly screen separating bugs from leaves allowed for the transmission of olfactory cues, but obscured visual and tactile cues. Individuals could touch the leaf discs by inserting their mouthparts through the screen. Control treatments did not contain plant material. Responses in control treatments were evaluated in relation to “focal cells,” which were defined as the same grid cells as those with leaf discs in host treatments.

Individual bugs were released into an arena and observed continuously for 20 minutes. All trials were conducted in the dark, with observations aided by a red LED lamp. It was not possible to capture bug responses with video because they moved from its field of view (e.g., when they moved from the bottom to the top of the arena). Bug responses were therefore recorded in real time by quietly dictating observations into a voice recorder with a built-in timer.

Several hypotheses were independently evaluated in this experiment (Table 5.1). Hypotheses 1 through 4 addressed differences in behaviours of *C. dilutus* individuals across host and control treatments (i.e., leaf discs and the corresponding control focal cells). The exact distances walked by individuals could not be measured, so the extent of exploration was measured as the number of grid cells visited and the time spent at each cell. The term “walking” was preferred over “host searching,” because walking implies movement to new locations without attributing motives to the insects. Hypotheses 5 and 6 evaluated the relative differences in behavioural responses to each host when cotton and pigeon pea leaf discs were presented simultaneously. The number of bugs that made physical contact with leaf discs was relatively low, so Hypothesis 5 was further examined by evaluating whether bugs initially arrived within 1cm of cotton or pigeon pea (using an exact binomial test), even if they did not initiate feeding.

The specific statistical evaluations used are also included in Table 5.1. All hypotheses except number 4 (labial dabbing) evaluated differences in responses across treatments regardless of the relative direction of change in the magnitude of responses. In contrast, labial dabbing is how several mirid species evaluate the gustatory cues of host plants (Backus 1988), so there was no *a priori* reason to expect an increase in labial dabbing when host stimuli were absent. Each treatment used to test hypotheses 1- 4 had 30 replicates. Not all bugs made physical contact with leaf discs, so hypotheses 5 and 6 had 20 replicates.

Leaf discs likely release a different suite of volatiles than do intact plants, and the volatiles may saturate the relatively small Petri dish arenas to a greater extent than the larger mesh cages. Additionally, leaf discs likely have lower turgor pressure than do leaves that are still attached to the plant and may alter the typical probing behaviour of bugs. To help overcome the limitations of leaf disc trials, the proposed conceptual model of host localization by *C. dilutus* bugs (in the discussion) will integrate the observations from all three experiments described above (which include whole plants covered in netting, whole plants without netting, and leaf discs only).

5.3. Results

5.3.1. Temporal activity and host associations

Creontiades dilutus individuals monitored between 1500h and 2400h (inclusive) were clearly and consistently most active after sunset, and were significantly more active in control treatments (across all time periods) relative to either host plant species (Fig. 5.1a, Table 5.2). When data from the day and night periods were pooled, no statistically significant interaction effects with host treatments were detected (Table 5.2). Diurnal bug activity was low overall with relatively high bug activity only in the pigeon pea treatment (Table 5.2), and reflects an increase in the number of bugs walking immediately before sunset (Fig. 5.1a).

Relatively few bugs arrived on the central netting that covered host plants or the control structures (Fig. 5.1b) (compare against Fig. 5.1a). The numbers of bugs that landed, and remained on the netting, continued to increase from 2000h until the end of the observation period at 2400h. There were significantly more bugs on the netting with host plants than on the control structures when the time period data was pooled for statistical evaluations (Table 5.2).

Table 5.1. Comparisons of *Creontiades dilutus* behaviour in the presence of cotton (which hosts relatively low bug numbers in the field), pigeon pea (which hosts relatively high bug numbers), and a control treatment (without hosts).

Hypothesis	**Statistical test**	**Prediction**
Comparisons across control and leaf disc (with both cotton and pigeon pea) treatments.		
1) Bugs differ in the distance that they walk (the number of grid cells visited) when near host leaves.	Wilcoxon rank sum	Host-associated cues may: 1) stimulate an increase in walking by bugs as they increase their search effort, or 2) the absence of cues may encourage bug movement to a location near appropriate hosts.
2) Bugs differ in the time spent walking near host leaves.	t-test (two-tailed)	Bugs likely spend more time walking in the presence of host-associated cues, relative to bugs in the control treatment.
3) Bugs differ in their turning frequency (90º or greater) when near host leaves.	Wilcoxon rank sum	Increased turning may arrest bugs within the vicinity of host plants.
4) Bugs spend more time dabbing with their labium when near host leaves.	t-test (one-tailed)	Labial dabbing is a stereotypical evaluation of feeding sites by heteropterans, so dabbing will likely increase near plants that consistently host higher numbers (pigeon pea) relative to cotton.
Comparisons across cotton and pigeon pea when presented to bugs simultaneously.		
5) Bugs initially contact leaves from a particular host species more often than the other.	Exact binomial test	Bugs may be more strongly attracted to pigeon pea, which hosts more individuals than does cotton, so bugs may initially contact pigeon pea more often.
6) Bugs spend longer on leaves from a particular host species than on those of the other.	t-test (two-tailed)	Bugs may spend more time on pigeon pea than on cotton, because they are arrested on pigeon pea to a greater extent than they are on cotton.

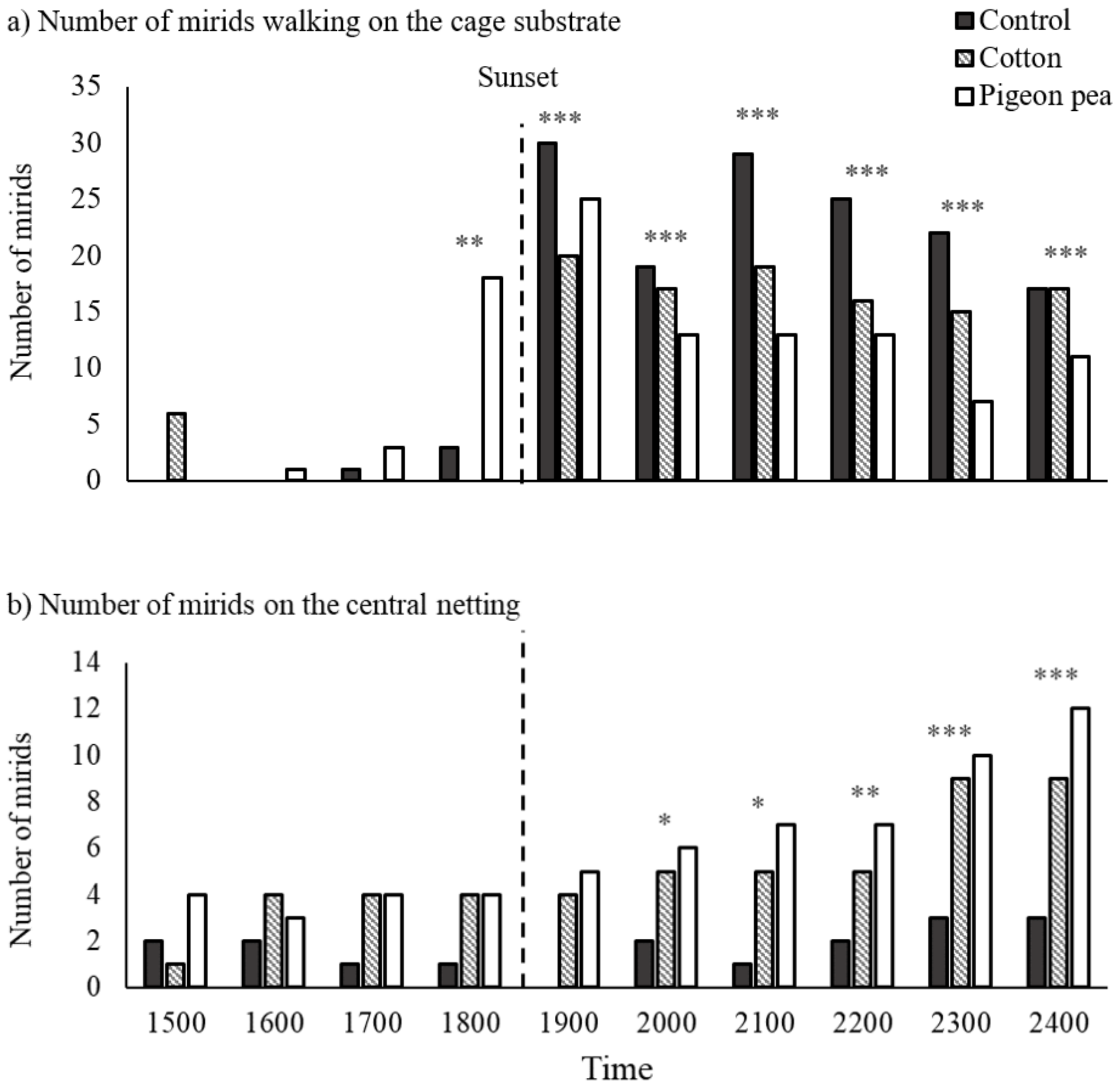


Fig. 5.1. a) Hourly observations of the number of *Creontiades dilutus* individuals walking on substrates (cage surfaces, central netting) in control, cotton, and pigeon pea treatments. b) The number of individuals landing on central netting around the host plants or the steel stand (in the control treatment). Cages were exposed to natural light with the dashed vertical line indicating the time of sunset (1820-1840h). No additional light was provided after sunset. Asterisks represent statistical significance for time periods compared against the initial number of individuals responding (walking or on netting) at 1500h (p-values: *** = 0.001, ** = 0.01, * = 0.05; GLMMs (described in text); n = 30 per host treatment).

Table 5.2. Proportions of *Creontiades dilutus* individuals responding to host plant treatments (blank control, cotton and pigeon pea) in relation to daylight (1500-1800h) or night time (1900-2400h) periods. Statistical significance was independently evaluated for: a) the number of bugs walking in the cage, and b) the number of bugs retained on the central netting by using a GLMM for repeated measures (glmer (bug response ~ host treatment * time period + (1|sample), family=binomial)). Differential responses by bugs were judged against values from control treatments run during daylight. There were 3 trials per host plant treatment, each with 10 bugs (a total of 30 bugs per treatment). Statistical significance: p-value < 0.05 (*), p-value < 0.01 (**), p-value < 0.001 (***), and non-significant (ns).

a) Bugs walking in cage

Period	**Host**	**% of individuals**
Daylight	None (control)	2
Daylight	Cotton	4^{ns}
Daylight	Pigeon pea	17^{**}
Night time (dark)	None (control)	80^{***}
Night time (dark)	Cotton	59^{***}
Night time (dark)	Pigeon pea	46^{*}

b) Bugs on central netting

Period	**Host**	**% of individuals**
Daylight	None (control)	5
Daylight	Cotton	11^{ns}
Daylight	Pigeon pea	12^{ns}
Night time (dark)	None (control)	6^{ns}
Night time (dark)	Cotton	20^{*}
Night time (dark)	Pigeon pea	25^{*}

Individuals that landed fed on cotton and pigeon pea fed on leaves through the netting, were inferred to have remained on the central netting if they were found within 1 cm of the same location on the netting during successive checks. Two bugs were even observed at their same feeding sites after 9 hours, until dislodged at the end of that night's trial. In contrast, the bugs on control netting were usually walking slowly, and were almost never observed at the same location during the following observation period.

5.3.2. Host attraction and post-alightment behaviours

Creontiades dilutus adults displayed seven distinct behaviours when introduced to cages containing cotton and pigeon pea (when plants were not covered by netting) (Table 5.3). The duration of specific behaviours was not recorded, but most movement to new locations involved the insects walking rapidly, or intermittently taking a few steps, and then dabbing their labium on the substrate. When dabbing behaviours were viewed in profile, the reingestion of the droplet of saliva was clearly visible. Flying was relatively rare.

There was no evidence that bugs were actively attracted by olfactory stimuli to either host species. In single host treatments, only 27% of bugs touched cotton plants, and 13% touched pigeon pea (both Exact Binomial Tests were non-significant, n = 30 per treatment). Only 40% of individuals introduced to treatments that had both host species in the same cage made physical contact with plant substrates. Bugs in the two-host treatment did not touch either host more frequently than expected (cotton, 23%; pigeon pea, 17%, df = 2, $\chi^2 = 4.48$, n = 30, NS). The experiment that simultaneously presented both host plant species to bugs was designed to test if one host was relatively more attractive than the other host, so no attempt was made statistically to compare treatments with a single host (cotton or pigeon pea only) against the treatment with two hosts.

All individuals continuously tapped their antennae on cage substrates while walking rapidly, and dabbed their labium when walking broad zig-zag trajectories. Flight was rare and, when it occurred, bugs landed on cage substrates (never onto the plant). When bugs did arrive within 2cm of leaves, they paused momentarily, reoriented towards leaves, then almost immediately stepped onto the plant. Across all treatments (cotton, pigeon pea, both hosts simultaneously), each individual that dabbed its leaf substrates, then inserted their stylets into

Table 5.3. Behaviour of *Creontiades dilutus* individuals in cages containing either cotton, pigeon pea, or both plants together.

Bug behaviours
Rapid walking. The bugs walked quickly forward with few substantial changes in direction. Antennae repeatedly moved up-and-down, with the tips tapping the substrate immediately in front of the insect.
Labial dabbing. The tip of the labium was repeatedly touched to the substrate. Droplets of saliva were secreted onto the substrate, then immediately re-ingested. Dabbing typically occurred with slow walking (one "dab" per 2-3 steps) and frequent turning, but the antennae did not touch the substrate.
Antennal waving. Antennae held erect and waved in a circular motion without touching the substrate. This behaviour never occurred when the bug was walking.
Probing. Insertion of stylets into host plant tissues. Initial probing involved repeated shallow insertions of stylets, sometimes into several nearby leaves. This was followed by deeper probing, when more than 50% of the stylet length was inserted into plant tissue. The stylet was repeatedly inserted and withdrawn from the plant tissues for prolonged periods.
Flying. Short flights, invariably preceded by antennal waving, raising the head and thorax and lowering the abdomen by extending the front legs immediately before take-off.
Grooming. The bugs used their legs to clean their antennae and wings.
Motionless. No antennal or body movement.

the plant tissue (27%, n = 90). Most individuals remained on the leaf with which they initially made contact, but on two occasions bugs moved to a nearby leaf. All individuals on leaves began pumping their bodies up-and-down, while their stylets remained inserted into hosts.

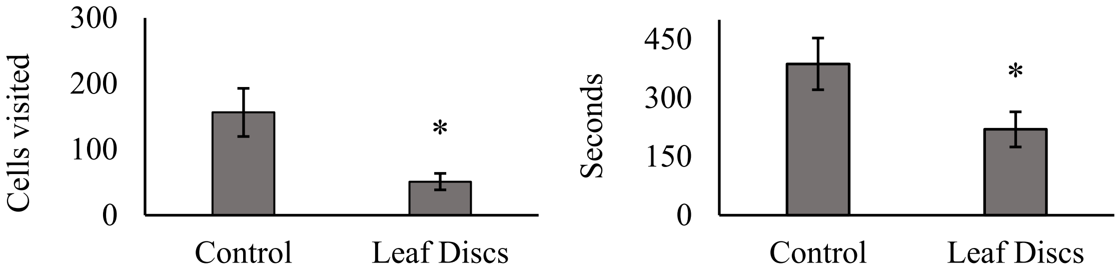
5.3.3. Behaviour in the close vicinity of hosts

In respect to the hypotheses described in Table 5.1, substantial differences were evident in the behaviours of *C. dilutus* individuals exposed to leaf discs relative to those in control treatments without leaf discs. Bugs exposed to cotton and pigeon pea walked shorter distances ($W = 203.5$, $p < 0.05$, Wilcoxon rank sum test) (Hypothesis 1) and spent less time walking ($t = 2.10$, $df = 1$, $p < 0.05$, two-tailed t-test) (Hypothesis 2) than those bugs in control treatments (Fig. 5.2a, b). In general, bugs in control treatments tended to continue walking in the same direction, with only slight turns as they approached the vertical side of the Petri dish. Individuals in leaf disc treatments, in contrast, would turn at least 90°, and change their direction of travel relatively frequently ($W = 303.5$, $p < 0.05$, Wilcoxon rank sum test, Fig. 5.2c) (Hypothesis 3). The presence of leaf discs was also associated with an increase in the time spent labial dabbing by bugs, relative to those in control treatments ($t = -1.90$, $df = 1$, $p < 0.05$, t-test, Fig. 5.2d) (Hypothesis 4). This relative increase in dabbing was even observed for individuals that never touched leaf substrates.

Stylet insertion into leaf tissues was rare (three individuals for pigeon pea, two for cotton), so no statistical evaluations comparing the relative number of bugs probing each host were possible (Hypothesis 5). The low frequency of physical contact between bugs and hosts was similar to results of the experiment that evaluated bug responses to whole plants (see previous section). Twenty bugs, including those that probed hosts, came within 1cm of cotton or pigeon pea leaf discs. There was a statically significant difference in the identity of host species that bugs initially approached, with 90% of individuals first arriving within 1cm of pigeon pea leaf discs ($\chi^2 = 11.25$, $p < 0.001$, exact binomial test). There was no evidence that bugs spent more time at either cotton or pigeon pea leaf discs (hypothesis 6).

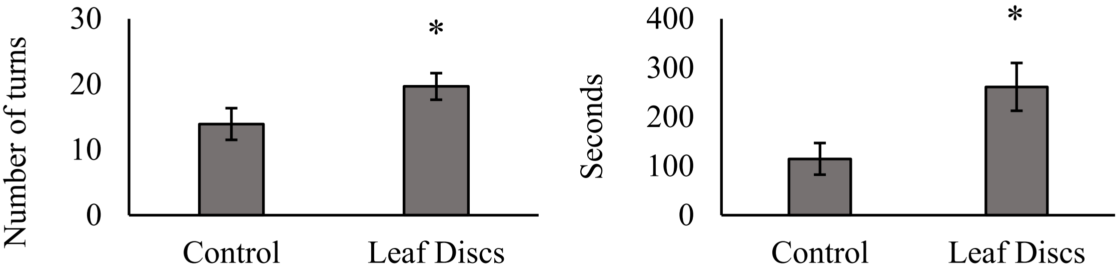
a) Walking distance

b) Time spent walking



c) Number of turns

d) Time spent dabbing



e) Walk path (control)

f) Walk path (leaf disc treatment)

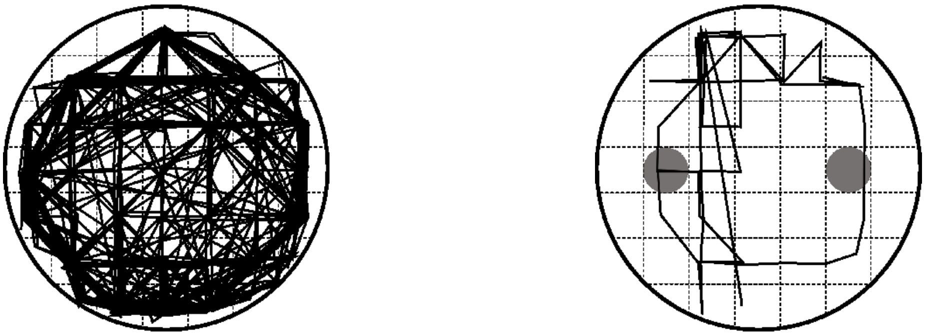


Fig. 5.2. Mean measures of behaviours ($\overline{x} \pm 1$ SE) of *Creontiades dilutus* individuals within Petri dish arenas containing leaf discs (cotton and pigeon pea) or a control treatment (without leaf discs) within a 20-minute observation period. The behaviours evaluated included: (a) walking distance (number of cells covered, see text), (b) time spent walking, (c) turning frequency (changes in body orientation of at least 90°), and (d) time spent dabbing at the surface of the dish. Each behaviour was statistically evaluated separately with a Wilcoxon rank-sum test (a, c), a two-tailed t-test (b), and a one-tailed t-test (d) ($n = 30$, p-value < 0.05 (*)). Typical examples of the walking paths of individual bugs (solid line) were included for the control (e) and leaf disc treatments (f) (where the grey circles are the locations of the leaf discs (see text)).

5.4. Discussion

Experiments that included either whole plants or leaf discs each had fewer bugs walking around their enclosure, relative to the corresponding control treatments without plants (Figs. 5.1 and 5.2, Table 5.2). In contrast to the observations of non-oriented movements by bugs, there was no evidence that olfactory cues attracted bugs to whole plants under netting, whole plants without netting, or leaf discs until bugs were within 2mm of the plants. The results presented above provide the basis for developing an initial conceptual model for the host localization behaviour by *C. dilutus* bugs for subsequent testing. Core to this model is that: (1) bugs are more likely to respond to host plants during the diel period in which they are typically active, (2) host-associated attraction (insect movement towards plants) using olfactory cues, which may be typical of most herbivorous insects, may play relatively little role in the localization of particular host plants by *C. dilutus* individuals, and (3) the responses of individuals with respect to their host associations may include non-oriented movement (arrestment) in respect to specific plants.

5.4.1. Host localization

Insect herbivores respond to host stimuli when they are able to perceive and recognize specific plant-associated cues (Hardie et al. 2001, Walter 2003, Bruce et al. 2005). Both their visual and olfactory sensory systems are crucial for localizing specific hosts (Prokopy 1986, Bernays and Chapman 1994). Visual cues may attract individuals towards hosts that reflect specific colours or UV wavelengths (e.g. Döring and Chittka 2007), because the visual acuity of most insects is greatest within several centimetres (Wehner 1981). This suggests that non-specific visual cues (e.g. the reflectance of green wavelengths) may encourage insects to approach plants, even though visual stimuli may not be used to discriminate among host species beyond 1m (Wehner 1981).

In general, olfactory cues are important components of the host finding process for insects (Bernays and Chapman 1994, Bruce et al. 2005), but interpreting specific behaviours remains elusive. Insects may move towards relatively higher concentrations of volatiles within an odour plume, resulting in their arrival at a host plant (i.e. attraction). To this end, frequent turning by insects may be a mechanism that keeps individuals within the odour plume, thereby allowing them to orient towards regions of higher volatile concentrations near hosts (Bernays and Chapman 1994, Cardé and Willis 2008). Odour plumes, however, may not have distinct boundaries, and turbulent airflow likely makes it difficult for insects to orient in respect to gradients of volatiles (Bernays and Chapman 1994). Alternatively, plant volatiles may increase an insect's turning rate, but do not contribute to the orientation of individuals towards hosts (i.e. arrestment) (Kennedy 1978). Arrestment cues would retain insects near host plants, but their attraction towards specific plants would require additional cues (Kennedy 1978).

In the field, visual and olfactory cues do not operate in isolation, and the presence of visual stimuli likely increases the readiness in which insects respond to olfactory stimuli (Finch 1984). For example, few *L. hesperus* mirids started to walk within an olfactometer apparatus when exposed only to host-associated volatiles, but a relatively higher proportion of individuals started to walk when the apparatus contained these same volatiles but was also illuminated by green light (Blackmer and Cañas 2005). Notably, relatively few *L. hesperus* individuals that entered the olfactory arm containing the source of volatiles walked all the way to the odour source (Blackmer and Cañas 2005), suggesting that they may initiate movement when volatiles and green light are present, but these bugs may not use only these cues to orient towards higher volatile concentrations.

The readiness with which insects respond to visual and olfactory cues is also influenced by their circadian rhythms (Hardie et al. 2001, Saunders et al. 2002). The time of day and night influences the internal physiological state of individuals, which, in turn, restricts host localization behaviours (and movement, in general) to specific periods (Hardie et al. 2001). The results presented in this study reflect behavioural experiments conducted at night when *C. dilutus* individuals are most active in the field (Fig. 5.1, Table 5.2), while minimizing the influence of visual stimuli (experiments conducted in the dark and/or with mesh netting covering plant substrates). This allowed interpretations of movement patterns by *C. dilutus* individuals that distinguished between non-directional arrestment responses, and directional responses that lead to the attraction towards host plants.

5.4.2. Conceptual model of host localization by Creontiades dilutus

Creontiades dilutus bugs frequently moved about their cages and Petri dish arenas at night, but there was no evidence of oriented movement beyond 2cm towards whole flowering plants (Fig. 5.1, Table 5.2), host attraction and post-alightment experiments), or leaf discs (Fig. 5.2). The lack of oriented movement towards whole cotton plants by *C. dilutus* bugs has also been reported by Khan (1999), who noted that it took 2-3h for caged individuals to land on potted cotton hosts before laying eggs. In the results presented above, bugs walked farther and for a longer time in treatments without plants (the control treatment) relative to host treatments (with cotton and pigeon pea). They also turned more frequently and dabbed at substrates with their mouthparts more often in host treatments than in the control treatment. The host plants in each experiment were visually obscured by netting and/or dark conditions, so the behavioural differences observed across control and host plant treatments were likely a response to olfactory cues by the bugs.

Creontiades dilutus bugs are found across arid Australia (Hereward and Walter 2012, Hereward et al. 2013b) where vegetation is sparse for much of the year (Nano and Pavey 2013). The increased movement of bugs in the absence of plant volatiles (Fig. 5.2) likely contributes to the dispersal of bugs across the landscape when hosts begin to senesce. These bugs are also found in cotton crops, usually in relatively low numbers, but often in higher densities when these plants have developing fruits (Cappadonna et al. 2018, CRDC 2019b). The relative increase in their turning frequencies in the presence of plant volatiles may arrest bugs near specific host plants at appropriate phenological stages. This may, in part, explain why *C. dilutus* bugs are clustered at particular locations across farms with abundant host species (e.g. cotton) (e.g. CRDC 2019b). The role of arrestment cues on their dispersal patterns, however, has not yet been directly tested in the field.

Typically, short movements (within 1m) that are not clearly oriented towards a specific target are called trivial flights, but this terminology has been criticized for implying that these “trivial” movements are unimportant (Hardie et al. 2001). Individuals of some bug species are known to undertake short flights at intervals, and these may result in bugs landing on multiple plants in the nearby vicinity of one another (Kennedy 1965, Finch 1984, Bernays and Chapman 1994). That mirids do the same comes from observations on *Lygus* spp.

(Miridae) frequently take flights that last less than 5 seconds (Blackmer et al. 2004), and remain within 1.5m of plant foliage (Muller and Stern 1973, Rancourt et al. 2000), but again further tests are required.

Also, diet analysis has shown that *C. dilutus* bugs in the field routinely move back-and-forth across different hosts growing several meters apart (Hereward and Walter 2012, Hereward et al. 2013a). Direct observations in the field are not available, but in laboratory settings, their movement is arrested when exposed to host volatiles within 47cm of host plants (i.e. the width of the cage), but only directly approached leaf substrates without turning away when within 2cm. Collectively, this suggests that the "trivial flights" of *C. dilutus* bugs that bring individuals near multiple plants are commonly occurring components of their host localization process, and likely function in association with their arrestment by plant volatiles.

Once *C. dilutus* individuals touch a surface of a plant or cage, they evaluate substrates in a similar manner to that of *Lygus* spp., which includes tapping their antennae and dabbing their mouthparts onto substrates (Cervantes et al. 2016). These behaviours are associated with the evaluation of feeding sites after mirids alight onto host substrates. Touching substrates with their antennae allows individuals to assess chemical cues associated with the surface of plants (Chapman and Bernays 1989, Chapman 2003), and re-ingesting saliva that has broken down plant cell walls evaluates the internal chemistry of host tissues (Sharma et al. 2014). The clearest evidence of discrimination among different host tissues by *Lygus* bugs has been demonstrated with observations of them once bugs touch plants with their mouthparts (e.g. Avé et al. 1978), but the evaluation of hosts is a multi-stage process that begins before physical contact between insects and plants (Bernays and Chapman 1994, Hardie et al. 2001). It is notable that *C. dilutus* individuals increase the time spent dabbing in treatments with host plants, relative to the control treatment without plants (Fig. 5.2d), which suggests that host evaluation by means of dabbing behaviour is stimulated by the presence of plant volatiles.

5.4.3. Conclusions and research directions

In summary, *C. dilutus* individuals are most active at night, and are not attracted by olfactory cues to pigeon pea or cotton beyond 2cm. In contrast to the relatively short distance that *C.*

dilutus bugs use olfactory cues to orient their movement towards host plants, olfactory cues arrest the movement of these bugs across distances of several centimetres. In the field, the distance at which bugs are arrested by host plants may differ from the distance observed in the relatively confined space of the arenas used in this study, but it is likely that the arrestment and subsequent retention of individuals near host plants may stimulate bugs to further evaluate feeding sites at that location. There was no evidence of bugs discriminating between cotton and pigeon pea hosts using olfactory cues prior to alighting on hosts. The recognition of olfactory cues that change the movement patterns of insects without leading to the oriented movement towards specific plants appears to be characteristic of other phytophagous mirids (particularly with *Lygus* spp.) (e.g. Blackmer and Cañas 2005, Backus et al. 2007, Cervantes et al. 2016). If mirid species, in general, recognize suitable hosts only after arriving at specific plants, this may explain why many mirid species have a relatively large taxonomic host breadth (e.g. Wheeler 2000, Allen et al. 2018) despite being found in high densities only on a relatively few host plant species (Cappadonna et al. 2019).

It is difficult to compare the responses to hosts by *C. dilutus* individuals (discussed above) with published studies of host finding for other mirid species, because arrestment responses, and behaviours associated with trivial flights are relatively under-reported. Also, the sweep net surveys used in this thesis may catch fewer nymphs than adults (Threlfall, et al. 2005), so caution should be used when comparing bug numbers with the results of surveys that use alternative sampling methods. Future investigations should attempt to distinguish between oriented and non-oriented movement, by designing studies that directly test both response types. For tests that specifically evaluate attraction (e.g. olfactometer experiments), the relative rates of “non-responses” should also be reported, because this should provide indirect evidence that olfactory stimuli may be arresting insects as a primary part of their host localization process.

Future studies should investigate the integration of olfactory, visual, and gustatory cues in the host localization and acceptance process of *C. dilutus* bugs. By identifying how *C. dilutus* bugs integrate multimodal cues, researchers could better understand how these bugs localize host plants in the field across different spatial scales. This would help design pest management efforts. For example, trap crops that attract bugs could be placed immediately next to cotton to draw pests away from the more valuable crop. If trap crops arrest insect movement only, however, then they should be planted at a location where they are likely to intercept immigrating bugs before arriving at cotton. This would likely minimize the number of bugs that would inadvertently land on cotton when moving around trap crop patches.

Chapter 6. General discussion – host localization and relative host use in mirid bugs

6.1. Introduction

To investigate the ecology of any species effectively, it is essential to know location and timing of activities that contribute to the life history of individuals within their environment (Andrewartha and Birch 1954). Many herbivorous mirid species are generalists (Wheeler 2000), and their ability to feed from multiple host plants is a result of behavioural, morphological, and biomechanical adaptations (e.g. Cohen 1998, Wheeler 2001). The timing of their host use is also influenced by the phenological stages of various host species across the environment (e.g. Fleischer and Gaylor 1987, Gerber and Wise 1995). The influence of multiple environmental factors on individual bugs across expansive landscapes can be quite subtle, and consequently, tracking their spatio-temporal dynamics has proved to be surprisingly difficult.

Herbivorous mirids in the tribe Mirini feed on meristems, new foliage, and flower buds (Wheeler 2000). This tendency to feed on soft plant tissues may be related to their primary feeding mechanism. Mirids break plant tissues down by exuding saliva onto plant substrates (i.e. pre-oral digestion), and then imbibing the liquified plant matter (Wheeler 2000, Sharma et al. 2014). *Creontiades dilutus* adults (also belonging to Mirini) feed on the leaf terminals of host plants in relatively low densities, but are found in greater numbers on developing flower buds (Chinajariyawong 1988). Nymphs, in contrast, develop only when provided flower buds (Hori and Miles 1993).

The extensive taxonomic list of *C. dilutus* host plants (Hereward and Walter 2012), as well as those of other mirid species (Wheeler 2000), render it extremely difficult, if at all possible, to use host lists as a tool for predicting the spatio-temporal dynamics of bugs across the landscape. The main problem is that not all host plant species are used by insects to the same extent as one another. Primary host species consistently support relatively higher densities of adults and juveniles, secondary hosts produce relatively fewer juveniles, and incidental hosts support adults only intermittently and are generally not used to produce a new generation

(Rajapakse et al. 2006). Several mirid species regularly land on hosts that are good for nymphal production, as well as those that are not suitable for nymphs (Wheeler 2000), but not much is known about the relative retention times of the insects on these different host species. Indirect information on this aspect of host use is now available on *C. dilutus* bugs, however.

Adults frequently land on *Cullen australasicum,* lucerne, and pigeon pea, all of which yield high densities of nymphs, but the adults also move regularly from these plants to less productive hosts (and vice versa), as demonstrated with molecular tools and structured sampling of individual bugs (Hereward and Walter 2012, Hereward et al. 2013a, Chapter 4). This clear shift from highly productive hosts to relatively unproductive hosts indicates that movement patterns of *C. dilutus* individuals are more nuanced than is typically implied in the literature, which usually mentions the host species that are believed to attract bugs, but this is frequently done without direct evaluation of the movement of those insects.

When evaluating the severity *C. dilutus* pest infestations in agricultural systems, the challenge is to distinguish between the influence of the movement of invading adults and the subsequent production of nymphs within crops. Regular pest surveys of cotton can detect when nymphs are produced in crops (e.g. Chapter 2). Direct evaluation of the movement of small-bodied bugs, however, must be inferred from systematic field surveys across vast regions (Chapter 3), long-term trapping efforts (e.g. Hill 2013, Hill 2017), establishing directional intercept traps at the edges of fields (Khan 1999), from gene flow analyses (Hereward et al. 2013b), and assessing their gut contents to assess the plant species from which the sampled individuals have fed (Hereward and Walter 2012, Hereward et al. 2013a, Chapter 4).

A first step in any explanation of how bugs disperse relative to their use of host plant species, begins by addressing the geographic locations, as well as, the timing in which they and their host plants are found across the environment. Spatio-temporal evaluations of host use by generalist herbivores must include the hosts from which individuals emigrate, as well as those at which insects arrive (Kennedy and Storer 2000). Identifying the origin of emigrating mirids, in general, may have to deal with there not necessarily being a single source population for invasions, and simultaneous outbreaks may occur at several widely separated sites when environmental conditions become favourable for insect development (e.g. Kobayashi et al. 2011).

Creontiades dilutus bugs in the cotton systems of eastern Australia are suspected to originate from the arid continental interior, because evidence suggests that gene flow occurs over hundreds of kilometres between the arid interior and eastern agricultural environments (Hereward et al. 2013b), seasonal differences are evident in bug densities across regions (Hereward and Walter 2012), and high bug densities are associated with the spatio-temporal patterns of flushing hosts (Chapter 3). Also, many crop consultants that monitor bug infestations (who routinely the same fields across several years across eastern Australia) perceive that early invasions occur over prolonged periods, and often are initially detected at different sections of their farms each year (Cappadonna et al. 2018, Chapter 2). These perceptions are consistent with the immigration of bugs that originate from different localities across the landscape (which may differ in response to changes in the ecological conditions at each site across years), rather than from a single point source that produces bugs every year.

Presented below is the first conceptual model that attempts to explain the host localization process of *C. dilutus* bugs relative to the vast spatial scale of their geographical distribution from the remote arid continental interior, to the cotton production systems in subcoastal agricultural systems. Underpinning this conceptual model is the understanding of the behavioural mechanisms by which individual bugs localize and recognize hosts, which in turn, drives their use of hosts across environments that share few plant species.

The key findings for each of these chapters are summarized in Table 6.1. The conceptual model for host localization is summarized in Fig. 6.1, and is articulated in broad terms so that it applies to bugs across the variety of environments that they inhabit and in which they are found. The influence of particular host plant species on the production and development of nymphs are discussed in greater detail in Section 6.2 (below), and the movement patterns of adult bugs across arid and agricultural landscapes is in Section 6.3.

Table 6.1. Summary of key findings from observations and experiments with *Creontiades dilutus* bugs, as derived from Chapters 2-5.

Chapter	**Findings**
Ch. 2 – Consultant Surveys	• Invasions into cotton occur at different locations and dates in early and mid-summer (Figs. 2.3, 2.4). • Bug numbers in cotton gradually increase as the growing season progresses (Fig. 2.4, Table 2.4). • Invasions are associated with the availability of alternative host species, both locally in agricultural systems (particularly legume crops and riparian vegetation), and also in the arid interior, by consultants familiar with the pest dynamics in cotton (Tables 2.2, 2.3).
Ch. 3 – Landscape Surveys	• In arid environments, bugs are consistently hosted in high numbers by *Cullen australasicum* and *Goodenia cycloptera.* Vast distances (100's of km) without green vegetation intervene between plants that host bugs (Figs. 3.3, 3.4). • In agricultural environments, high numbers of bugs are hosted by irrigated lucerne. Non-irrigated lucerne along roadsides and uncultivated riparian vegetation also consistently host bugs, but in significantly lower numbers (Fig. 3.5, Table 3.3).
Ch. 4 – Cotton and Pigeon Pea Surveys	• Pigeon pea plants host relatively higher densities of adults and nymphs than do adjacent cotton plants, despite the lesser area covered by pigeon pea (Fig. 4.1). • Bugs move back-and-forth across these crop types, and feed from both host species (Fig. 4.2).
Ch. 5 – Behavioural Experiments	• Bugs are nocturnally active, and are predicted to localize host plants at night in the field (Fig. 5.1, Table 5.2). • No evidence suggests these bugs use olfactory stimuli (in the absence of visual stimuli) to discriminate across cotton and pigeon pea (Fig. 5.2). • Olfactory stimuli from cotton or pigeon pea do not attract bugs beyond 2cm, but there was evidence of arrested movement within 0.5m (Figs. 5.1, 5.2).

Fig. 6.1. Proposed host localization model for adult *Creontiades dilutus* bugs. The presentation of this model starts with nymphs on their natal hosts and continues until adults arrive at new hosts to feed and oviposit. See the text of the corresponding sections for further details and justification.

Survival and production of bugs across different environments (Section 6.2)

- Nymphs develop on suitable host plant species that maintain soft tissues throughout their development.
- The development and survival of nymphs requires the average air temperature to remain between 17-38°C (the lethal extremes for adults are unknown).

Leaving hosts and long-distance dispersal (Section 6.3.1)

- We suspect that adult bugs are likely to leave host plants when host-associated stimuli no longer retain individuals to the vicinity of the plant.
- Individuals may move long-distances on the wind with little control over the horizontal direction of their movement.
- They can, to some extent, control their vertical movement within the air column, and visual cues likely help bugs land on vegetation.

Activity patterns and response to host-associated stimuli near plants (Section 6.3.2)

- Bugs reside on particular plants during the day, unless they are disturbed.
- At night they fly back-and-forth, potentially landing on several different host species.
- Olfactory cues arrest bugs within a few metres of appropriate host species without providing information about the specific location of plants.

Post-alightment host evaluation (Section 6.3.3)

- Bugs evaluate hosts by landing on plant substrates.
- They tap their antennae, and excrete then re-ingest saliva (i.e. dabbing) to evaluate the chemistry of the plant substrates.
- They probe the tissues with their stylets, before either accepting or rejecting a host for feeding and oviposition.

6.2. Survival and production of *Creontiades dilutus* bugs in the field

Adult *C. dilutus* individuals have been collected from 96 host plant species, yet only 62% of these plant species host nymphs (Hereward and Walter 2012). In addition to feeding from soft terminals and developing flower buds (e.g. Chinajariyawong 1988), adult females lay their eggs only within soft plant tissues (Miles 1995, Khan 1999). Observations in laboratory settings suggest that eggs may get crushed as the surrounding plant tissues begin to desiccate and shrink (Khan 1999). Also, eggs and nymphs do not survive at sustained temperatures below 17°C and above 38°C (Khan et al. 2009).

Nymphs do not fly, so their persistence on particular plants and in particular locations is strongly dependent on local conditions. Nymphs survive to adulthood only if temperatures remain the range 17-38°C, and eggs were deposited into appropriate plant species that maintain soft tissues for the duration of the bug's development period. At 25°C, it takes 3-4 weeks for eggs to develop into adults (Khan et al. 2009). This implies that patches of primary hosts that maintain fresh growth for about one month in warm conditions are potentially substantial sources of bugs.

In arid environments, densities of *C. dilutus* are lower in summer than in winter (Hereward and Walter 2012), presumably because summer temperatures frequently exceed survival threshold of nymphs (BOM 2019), and few host plants have fresh growth in these extremely hot and dry conditions (Hereward and Walter 2012, Nano and Pavey 2013). In contrast, winters are milder, with sporadic rainfall associated with the peak growth of short-lived forbs (Nano and Pavey 2013). These forbs include several hosts for *C. dilutus* bugs (Hereward and Walter 2012).

Some of these forbs, such as *Goodenia cycloptera,* are ephemeral and persist only for several months following rain but support high densities of reproductive bugs (Fig. 3.4). The highest production of bugs in the arid environment was, however, on *Cullen australasicum* (Fig. 3.3). This is a perennial species that maintains soft tissues during prolonged dry periods throughout the year (Grimes 1997, Dear et al. 2007), so is unlike the other primary host in the area. The relatively long flushing period of the two most prominent host species recorded to date may allow for more than one generation of *C. dilutus* to develop per year. While *C. australasicum* plants were found at only a few remote drainages in arid landscapes during this study (Chapter 3), they have also been previously recorded from across semi-arid landscapes in wetter years (Grimes 1997). Unlike any of the ephemeral plant species, high densities of *C. dilutus* bugs are hosted on *C. australasicum* across several generations (Miles 1995, Hereward 2012, Chapter 4). This temporal consistency of host use on this particular plant species likely contributes to the long-term survival of bugs in harsh arid environments.

In subcoastal agricultural systems, cotton is a relatively unproductive host that consistently hosts relatively low densities of *C. dilutus* bugs (e.g. Miles 1995, Mensah and Khan 1997, Chapter 4). Despite the low bug densities on particular plants, cotton monocultures cover vast regions (Fitt 1994), and the overall abundance of these hosts can collectively produce a large number of bugs. The low densities of *C. dilutus* bugs may, in part, be a result of the relatively short temporal period in which the plants maintain the soft tissues needed by these bugs to feed. On cotton plants, *C.* bugs feed primarily on the squares (developing flower buds) and young growing tips (Bishop 1980, Chinajariyawong 1988), with the highest bug densities corresponding with the peak squaring period from mid-November through December (Cappadonna et al. 2018, CRDC 2019a, also Chapter 2). In late January, growers start applying growth regulators to reduce vegetative growth, and defoliants are applied around February to remove soft tissues in preparation for harvest (CRDC 2019a). This means that cotton can sustain nymphal development for about three months. The specific periods that cotton is most suitable for feeding by bugs at particular sites will likely vary, because farms have slightly different timing requirements for effective crop management.

Lucerne and pigeon pea hosts relatively higher densities of *C. dilutus* bugs than does cotton (Miles 1995, Mensah and Khan 1997, Lawrence et al. 2007, Chapter 4). These plant species also flower for substantially longer than the three-month period of nymphal production in cotton. Lucerne flowers for about 8 months and pigeon pea for 7 months each year in the cotton-growing area of eastern Australia. These legumes, therefore, potentially contribute to relatively higher nymphal production than does cotton, despite the much more extensive area planted to cotton (Miles 1995, CRDC 2019b). Notably, pigeon pea is planted at the same time as, and adjacent to cotton, as part of an insecticide resistance management strategy targeting lepidopteran pests (Wilson et al. 2013). Growing pigeon pea and cotton in such close proximity likely increases the possibility that bugs move from one crop to the other (Section 6.3.3 below).

The braided rivers of the semi-arid Channel Country floodplains, and riparian zones in agricultural landscapes also support low densities of bugs. The specific sites and times in which bug densities increase remain ambiguous for much of the continent (not just the sites surveyed in this thesis), but small patches of vegetation near water courses do support *C. dilutus* individuals during dry periods (Chapter 3). These patches are unlikely to support large numbers of bugs, unless environmental conditions promote the growth of appropriate host species (e.g. Hereward and Walter 2012) with soft tissues that persist for the duration of *C. dilutus* egg and nymph production.

6.3. Conceptual model of host localization for *Creontiades dilutus*

6.3.1. Leaving hosts and long-distance dispersal

Researchers have developed a variety of tools to evaluate the behavioural, physiological, and morphological mechanisms in which herbivorous insects locate new host plants (Hodkinson and Hughes 1982, Bernays and Chapman 1994, Woiwod et al. 2001). It remains difficult, however, to directly test why insects leave the plants on which they were previously hosted. The proposed reasons for why insects leave hosts are typically inferred from field surveys and behavioural experiments. Leaving hosts may be a result of insects that are: dislodged by external factors (e.g. the harvest of crops), escaping inhospitable environmental conditions or predators, and in search of new hosts for feeding and oviposition substrates (Woiwod et al. 2001). This does not, for example, explain why *C. dilutus* individuals leave seemingly good hosts (e.g. Hereward and Walter 2012, Hereward et al. 2013a, Chapter 4). The occasional localized disturbance of host plants will likely result only in the sporadic departures of insects, yet many species consistently, and frequently, move across the environment after leaving what seem to be suitable habitat (Walter and Hengveld 2014). The movement of large numbers of herbivorous insects away from a region is often attributed to the desiccation of host plants (e.g. Fitt 1989), but insects also move away from areas with abundant hosts (e.g. Fitt 1989, Campos et al. 2006).

The reasons that *C. dilutus* bugs leave host plants remains elusive. Occasional predation has been observed, but there is no evidence that predation substantially contributes to the mortality rates or influences the spatial distribution of these bugs, unless there has been inundative introductions of predators against *C. dilutus* pests by managers (Grundy 2007). *Creontiades dilutus* adults are highly mobile (McColl et al. 2011) and frequently move back-and-forth across native plant species (Hereward and Walter 2012), as well as cotton-lucerne (Hereward et al. 2013a) and cotton-pigeon pea boundaries (Chapter 4). In these latter systems both lucerne and pigeon pea crops were abundant and flowering, and thereby suitable for feeding and oviposition (Hori and Miles 1993), so resource limitation was unlikely to drive movement patterns.

When bugs leave host plants, they will remain in the vicinity of these hosts unless they stop responding to the cues that had initially attracted or arrested these bugs near specific plants (Hardie et al. 2001). In general, insects that move relatively long distances into new geographic areas will likely experience changes in their physiological condition that results in them to becoming temporarily less responsive to host-associated cues (Kennedy 1978), or external environmental conditions are such that they are unable to recognize these host-associated cues (Walter and Hengveld 2014).

Creontiades dilutus adults fly across the Bass Strait from the continental Australia to Tasmania every year with the assistance of winds (Hill 2013, 2017), and gene flow across long distance of arid environments with little to no vegetation (Hereward and Walter 2012, Hereward et al. 2013b), suggests that once these bugs leave plant patches, they are able to travel long distances without landing. The possibility that *C. dilutus* adults also use winds to cover long distances from the arid continental interior into subcoastal cotton systems still needs to be investigated, but in general, small insects that disperse with the aid of wind have little control over the direction in which they travel across the landscape, but they can control when they land (Kring 1972, Hardie et al. 2001) and typically land on green plants (Finch and Collier 2000). It is possible that airborne *C. dilutus* immigrants entering cotton systems may descend from relatively strong wind currents and land any available green host plant, even if a particular plant is not a good host (see Section 6.3.2 for responses to cotton as bugs approach plants).

6.3.2. Activity patterns and response to host-associated stimuli near plants

When assessing the behaviours associated with localizing host plants by *C. dilutus* bugs, it is crucial to evaluate their movement patterns within the context of their typical activity periods pest managers typically count the insects that shelter on vegetation by dislodging individuals with sweep net and beatsheet surveys conducted during the daytime (CRDC 2019b). *Creontiades dilutus* bugs sheltering on plants are often captured in the highest numbers during early mornings and late afternoons, with their lowest numbers found midday (Bodnaruk 1992). Flying bugs, in contrast, are captured using pheromone and intercept traps in the highest numbers at night (Lowor et al. 2009b, Hill 2013). In laboratory settings there is a substantial increase in the number of these bugs that move about their cage after sunset (in the dark) (Fig. 5.1, Table 5.2). Collectively, this suggests that most *C. dilutus* adults move about the environment at night.

Insects integrate visual and olfactory stimuli when localizing host plants, and distinguishing appropriate and inappropriate hosts for feeding (Bernays and Chapman 1994, Hardie et al. 2001). For example, the mirids *Lygus hesperus* and *Apolygus lucorum* move towards the source of plant-associated odours more often when the testing arena is illuminated with green light, relative to when they are presented either stimulus independently (Blackmer and Cañas 2005, Pan et al. 2015).

The visual acuity of many insects are unlikely to have the resolution to differentiate between different plant species beyond a few metres (Wehner 1981, Döring 2014), but there is a tendency for insects to land on substrates that are similar in colour to their host plants (Finch and Collier 2000, Döring 2014, Farnier et al. 2014). Several insect species can distinguish between plants with green or yellow foliage and the brown of bare soil (Finch and Collier 2000, Döring and Chittka 2007), and such a distinction may be beneficial for *C. dilutus* bugs that routinely land on isolated plant patches in the arid continental interior (Fig 3.2). The response to visual stimuli by *C. dilutus* adults still needs to be tested in this regard. The experiments presented in Chapter 5 were conducted either before and after sunset, or completely in the dark (often with plants also visually obscured with netting), so that the behaviours of the bugs could be attributed to olfactory stimuli. If *C. dilutus* bugs respond to host-associated colours when alighting on plants, then the strongest responses will likely be to green substrates similar in colour to the plant tissues on which they feed. They are unlikely to respond to the colour of flowers, because the flower colour of their hosts addressed vary substantially. The flowers of the hosts evaluated in this study (Chapters 3 and 4) were white (cotton), purple (*Cullen australasicum* and lucerne), and *Goodenia cycloptera* and pigeon pea (yellow).

Recognizing plant-associated olfactory cues is a crucial aspect of the host localization process by herbivorous insects (Kennedy 1978, Bernays and Chapman 1994, Bruce et al. 2005). Interpreting the responses of insects to plant-associated odours is challenging, and various hypotheses have been proposed to explain the host localization process. Some insects may be attracted to hosts across distances greater than several metres, and will move towards increased concentrations of odours, which results in their arrival at specific plants (Bernays and Chapman 1994, Bruce et al. 2005). Alternatively, insects may be initially attracted to plants by visual stimuli, and respond to olfactory cues only when they are within five metres of plants (Kennedy 1978, Finch and Collier 2000). For these insects, olfactory stimuli likely arrests the movement of insects near appropriate host plants without explicitly providing information about the direction to the plants (Kennedy 1978).

There was no evidence of attraction to cotton or pigeon pea hosts by olfactory cues beyond 2cm in in laboratory settings by *C. dilutus* adults. Bugs did, however, elicit behaviours consistent with arrestment responses when within 47cm of plants (Figs 5.1a and 5.2, Table 5.1a). Arrestment behaviours are difficult to test in the field, but the relative importance of non-oriented movement by bugs in the vicinity of hosts is consistent with the evidence of the high frequencies of their back-and-forth movement across different host species (Hereward and Walter 2012, Hereward et al. 2013a, Chapter 4).

While the relative increase in arrestment may help explain why bugs visit multiple host plant species across short distances, the corresponding relative increase may explain the ecological mechanisms that underpin long-distance dispersal (see Section 6.2.2 above). The relatively increased movement of bugs along with a decrease in turning rate in the absence olfactory cues (Fig. 5.2), may lead to the dispersal of bugs in arid environments when suitable host plants are scarce and separated by large expanses of bare soil. A logical extension to this hypothesis is that, if host-associated arrestment cues are present, but in sufficiently low concentrations as to be undetectable by bugs, then patches of host plant may not retain high numbers of bugs.

6.3.3. Post-alightment host evaluation

Mirids evaluate plant substrates for suitable feeding sites once they have alighted upon a potential host. This post-alightment host evaluation process is best understood for *Lygus* species. Upon landing upon plant they evaluate the chemical composition of substrates by repeatedly tapping their antennae and dabbing saliva onto the surface of the plant (Miles 1972, Backus 1988, Sharma et al. 2014). After evaluating the surface of plants, bugs conduct a series of brief test probes by secreting copious amounts of saliva and lacerating plant tissues with their stylets (Backus 1988). Electropenetrography studies have provided evidence that while test probing behaviours are frequent for *Lygus* species, less than 3% of these probes resulted in prolonged feeding events (Cline and Backus 2002, Cervantes et al. 2016). Collectively, these observations suggest that mirids will frequently sample surface and interior plant tissues on host plants to locate tissues that meet specific requirements for feeding.

The observed antennal tapping, saliva secretion, and insertion of mouthparts into plant substrates by *Creontiades dilutus* adults are similar to those of *Lygus* species (Hatfield et al. 1983, Backus 1988, Cline and Backus 2002). In laboratory settings *C. dilutus* adults rarely fly directly to plants, rather they tend to walk along the surface of cages (Fig. 5.1). While walking, these bugs will repeatedly tap their antennae and dab saliva onto the surface of the cage (Cappadonna et al. 2020). When these behaviours occurred as bugs were standing on plant substrates it was followed by probing tissues with their mouthparts. The observations that antennal tapping and dabbing saliva occurred on both plant and cage surfaces suggests that these are behaviours that routinely occur when bugs are standing upon any new substrate, and probing occurs only after receiving a positive response from these earlier evaluations.

This study did not include electropenetrography experiments to evaluate probing, or chemical assessments of plant tissues to identify which plant characteristics are associated with the acceptance specific feeding sites. Previous evaluations of salivary enzymes have found high concentrations of pectinases (which breaks down plant cell walls) and amylases (which breaks down complex sugar molecules and increases the solubility of sugars in hydrated tissues) (Taylor and Miles 1994). This suggests that once bugs have landed upon plants, these salivary components are the mechanisms responsible for the acceptance or rejection of feeding sites in relation to the toughness and sugar content of host tissues. This, however, would be difficult to mechanically test.

6.4. Concluding remarks

In conclusion, *C. dilutus* populations persist on a variety of host species in diverse environments across Australia. Not all plants host bugs to the same extent. Primary hosts that maintain soft tissues for the duration of nymphal development likely become sources for emigrating insects. Secondary and incidental hosts produce fewer insect generations, but when present may provide shelter to bugs as they disperse long distances.

In addition to the importance of nymph production, the ability of host plants to arrest the movement of bugs across the landscape is crucial to explain their spatial distribution. In general, the arrestment of insects by specific host-associated cues have not received as much attention by researchers as the role of attraction to host plants. The results presented in this study suggests that future studies explicitly addressing both arrestment and attraction would greatly contribute to our understanding of why polyphagous insect move across different host species.

These bugs are cryptic and primarily move across the environment at night. Care should be taken to choose the most appropriate survey methods for the research questions being asked. Counting insects during daytime field surveys remains an effective method to measure the spatial distribution of bugs, but interpreting patterns of movement across hosts will need to incorporate their typical nocturnal movement. Meaning that in most instances, daytime surveys reflect the geographic location in which bugs are sheltering after bugs have localized hosts during the previous night.

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Appendix 5.1. Rearing and transport protocols to improve *Creontiades dilutus* nymph survival using sweet corn

Abstract

Researchers studying green mirids (*Creontiades dilutus*) (Miridae) in laboratory conditions typically rear bugs on green bean (*Phaseolus vulgaris*) (Fabaceae) diets. Rearing bugs on this diet, however, has had mixed success, with population declining after two generations. This study presents an alternative rearing method using sweet corn (*Zea mays*) (Poaceae) to improve the survivorship of bugs. When provided fresh corn cobs bugs readily lay eggs in the cobs, the kernels stay soft for longer than beans, and provide shelter for bugs reared *en masse*. Bug survival is also improved during transit from the field when cobs are secured together with wooden skewers to create a "scaffolding" that prevents cobs shifting while cages are moved (which otherwise would damage these long-legged fragile insects).

A.1. Introduction

Creontiades dilutus bugs have been the focus of much research, including development rate in relation to temperature (Foley and Pyke 1985, Khan et al. 2009), host choice and feeding behaviours (Bishop 1980, Chinajariyawong 1988, Hori and Miles 1993), feeding mechanisms associated with crop damage (Colebatch et al. 2001), and their response to pheromone trapping (Lowor et al. 2009a). These investigations typically involve rearing mirids in captivity, which is difficult because they are delicate and their legs and wings are easily damaged. Also, these bugs suffer high mortality during collection and transport, are susceptible to residual insecticides on vegetable diets, and larger individuals will opportunistically cannibalize smaller nymphs in crowded cages.

Creontiades dilutus bugs are typically reared on green beans (*Phaseolus vulgaris*) (Fabaceae) (Foley and Pyke 1985, Khan et al. 2009, Lowor et al. 2009a). Beans are easily acquired, inexpensive, and mirid bugs readily lay eggs on them, but they may crush eggs as they desiccate (Khan 1999), toughen as they age (which makes feeding difficult for small nymphs) and can retain residual insecticides. Khan (2009) had the most success (about 80% survival) at 26-30°C using washed green beans, but others have had mixed success using similar techniques with beans (Foley and Pyke 1985, Miles 1995, pers. obs.).

An alternative rearing method is required that mitigates the challenges presented by green beans. I, therefore, evaluated the rearing success of *C. dilutus* nymphs when young ears of sweet corn (*Zea mays) (Poaceae)* (hereafter "corn") are included as food items and rearing substrates. Corn was investigated for this purpose because it lasts longer than beans before desiccating, tissues of kernels remain soft (which may improve feeding success, relative to green beans, by small nymphs), and removal of the husks eliminates insecticide contamination. Furthermore, when corn is secured together it provides a physical substrate that shelters mirids during transport and rearing. This is particularly important when the cage is moved during transportation, as it helps prevent accidentally crushing the bugs. Specifically, I addressed mirid survival during transit from the field and in the laboratory, with bugs reared in both small and large cohorts.

A.2. Methods

A.2.1. Insect collection and general rearing

Creontiades dilutus adults and nymphs were collected from flowering lucerne with sweep nets on several occasions between November 2013 – December 2016. Sampling sites were at Gatton (27.54°S, 152.32°E) and Emerald (23.53°S, 148.16°E) in Queensland, as well as Baan Baa (30.59°S, 149.96° E) in New South Wales. Bugs were removed from nets with aspirators, and transferred into nylon mesh cages (475 x 475 x 475mm) prior to transport (see below for details). Bugs were reared at The University of Queensland in Brisbane in a climate-controlled room at 25°C, 70% relative humidity, with a 14:10 light:dark cycle.

A.2.2. Survivorship during transport

Upon collection, insects were transferred randomly to a cage containing green beans or one with corn. Each cage had equal mirid numbers. Green beans were purchased from a supermarket, and soaked in a bowl of water overnight to leach out residual insecticides, then lightly scrubbed with a paper towel and rinsed with tap water. The husks of corn were removed, and the cob rinsed in tap water. To prevent corn from shifting during transport, cobs were cut into 2cm thick discs, and each disc was pierced with a small wooden cooking skewer. The free end of each skewer was inserted into an adjacent corn disc. This resulted in each corn disc being pierced several times and, connected to the others, forming a “scaffolding” that did not shift during transit.

The duration of trips between collection sites and rearing facilities ranged between 1.5-10 hours. Vehicles transporting bugs were maintained at 24-26°C, and cotton sheets loosely covered the top of cages to minimize exposure to direct sunlight. Bug survival during transport for bean and corn treatments were statistically evaluated with a Pearson’s chi-squared test of independence. A total of 242 bugs (adults and nymphs) were collected over 3 trips.

A.2.3. Small cohort rearing

Ten first instar nymphs were transferred from large rearing cages with a soft bristled paintbrush into a plastic container (17 x 11 x 5cm) with mesh-covered ventilation holes. Each container was provided six washed green beans or three corn discs (as above). Containers were checked daily for mirid survival, and dead individuals were removed and the instar at date of death was recorded. Food was replaced as needed, but bugs always had access to non-desiccated food items.

The overall survival of bugs, by instar, to the adult stage in each diet treatment was statistically evaluated with a Pearson’s chi-squared test of independence. A total of 30 nymphs was divided equally across three containers for each diet treatment. Male and female nymphs were reared in the same containers. The rates of development and survival are similar for each sex (Khan et al. 2009).

A.2.4. Mass rearing protocols

Large cages *(*47.5 x 47.5 x 47.5cm*)* were used to rear mirids *en masse* for several generations (typically 4-5 generations in a nine-month period). Bugs were collected on several occasions from October through January (spring and summer), and reared until mid-June (winter). Initially attempts were made to rear bugs on green beans, but populations rapidly diminished after 1 month (about 2 generations) so the diet was supplemented with corn.

The observations from rearing bugs on corn and beans are provided, but there was no statistical evaluation on survivorship of the hundreds of bugs reared. Bug cultures were periodically supplemented with additional newly captured individuals, but most bugs in cultures were produced in captivity. The initial rearing effort using a corn and bean diet was established with only 10 field captured bugs, and the population grew to about 40 individuals before being supplemented with additional bugs from the field.

Corn and beans were prepared as described above, except greater quantities per cage were used. Each cage was provided with four ears of corn cut into discs. Every 3-4 days half the corn was replaced with fresh corn discs (with all beans being replaced every 2 days). The corn that was not replaced was left in the cages for another 3-4 days, when they were replaced. This resulted in each cage having a mix of fresh and older corn at any given time.

Older corn was left in cages, because mirids sheltered on corn discs and adults laid eggs within the kernels. Often bugs would rest in the crevices that formed as kernels began to dry and separate from each other. Nymphs were difficult to see under overhanging kernels, so corn discs were checked for nymphs before removal from the cage.

Food items that were removed from bug cages were transferred to an empty “emergence” cage, and monitored (4-5 days) for newly-hatched nymphs. First instar nymphs were most easily detected if fresh corn discs were placed near the older disc where eggs were laid. Shortly after emergence, nymphs would move to the new discs to feed. After 6 days it was assumed no additional nymphs would emerge, and the corn discs were discarded. The duration of the monitoring period was based on published developmental rates for *C. dilutus* nymphs (Khan et al. 2009), and a decrease in new emergences accordingly took place with time.

A.3. Results

A.3.1. Survivorship during transport

Bug survival during transport was higher for bugs provided with corn (90%) than for those provided with beans (61%) ($\chi^2 = 9.93$, df = 1, $p < 0.01$, performed on raw data). The cause of death was not specifically addressed, but several bugs appeared to be injured when they were captured or during transport. It was not feasible to continuously observe bug feeding during transport, but corn appeared to be fed upon by many bugs within minutes of introduction, whereas there were only three observations of bugs feeding from beans during transit. Corn removed from cages yielded 20 nymphs (indicating eggs were laid during transit), whereas eggs or nymphs were not recovered from beans.

A.3.2. Small cohort rearing

When reared in small cohorts (10 mirids per cage), bug survival was marginally higher, but not significantly so, when provided corn (Table A.1). Causes of morality could not be positively identified, but on three occasions larger nymphs were observed feeding on smaller nymphs, but these may have died prior to being fed upon.

A.3.3. Mass rearing protocols

Bugs were reared in large numbers on corn and bean diets. It was not possible to count every bug in the large cages where bugs would hide in corn cob discs, but in general, the total population doubled in size. Corn required less processing time (e.g. soaking, scrubbing) and its longevity also allowed for discs to be managed in staggered stages (fresh corn addition, retention of old corn discs as a substrate for sheltering bugs, and inclusion in “emergence cages”). This helped design an efficient workflow to rearing efforts. In contrast, desiccated green beans were in poor condition when they were in “emergence cages”, and in general were poorly suited to the “staggered stage” workflow used for corn.

In all instances mirids sheltering on partially dried corn adopted the same posture. Their abdomen was positioned near the centre (interior) of the corn disc with their heads facing outwards. Nymphs

Table A.1. Survivorship (%) of *Creontiades dilutus* nymphs reared on green bean or corn diet (n = 30 per treatment). Statistical evaluations between diet treatments for "all instars" was non-significant (Pearson's chi-squared test of independence).

Instars	I	II	III	IV	V	All instars
Bean	90	85	87	50	90	30
Corn	90	100	85	96	73	53

were small enough to fit completely beneath overhanging kernels, and early instars were difficult to see. Undetected nymphs may have been unknowingly moved to the emergence cage along with the corn, but they were likely recovered in the emergence cages.

The predation of nymphs by adult bugs was observed on several occasions. All observed predation involved adults piercing nymphs dorsally with their mouthparts, and occurred in the open region of the cage. Predation was never observed for nymphs sheltering within corn discs.

A.4. Discussion

Inclusion of corn into diets improved rearing efforts of *C. dilutus* nymphs and adults. This was particularly beneficial for their survival during transport. Much of this improved survival is likely due to a reduction of injury associated with a more structurally stable substrate during transport. Corn also appeared to be more readily fed upon by bugs than beans, and eggs were readily laid upon cobs. Access to the water, sugars, and other nutrients associated with corn may have contributed to improved survival, but this was not explicitly addressed.

When reared in small cohorts, bug survival was similar across corn and bean diets. This suggests there are no intrinsic nutritional or energetic differences across the diets. Food items, however, were replaced before they dried out. Food items with less fluid content or tougher tissues may be more difficult for bugs to feed from (Chinajariyawong 1988, Miles 1995), so older bean or corn may have relatively lower bug survival.

Bugs were reared for nine months (October through June) at ***25°C, which is a temperature that*** is theoretically capable of producing two mirid generations per month (Khan et al. 2009). In general, when provided corn, population growth broadly tracked this theoretical growth, and populations were able to be maintained for the entire nine months. By contrast, bugs fed only beans were difficult to maintain for more than two generations before numbers declined.

When reared in small groups, the survival rate of bugs was similar across bean and corn diets (Table A.1). This suggests that these diets had no substantial intrinsic differences in the nutritional content for mirids. Corn, however, was a better diet during transport and mass rearing efforts. Immediately after capture, typically during hot conditions, the readily accessible water from corn may have contributed to the improved survival. It is also likely that the stability of corn scaffolding during transport, as well as the structural complexity of corn substrates that provided shelter improved survivorship as well. The relatively more complex corn structure may have improved access to feeding sites when cages were crowded, relative to the smaller cylindrical green beans.

In conclusion, green beans remain a viable diet for bugs, but corn is an effective supplement that helps reduce mortality rates. Corn scaffoldings were particularly useful in reducing moralities during collection and transport. When rearing large numbers of bugs, the inclusion of corn diets improves the efficiency of rearing efforts, because maintaining corn diets requires less processing time, relative to bean diets, and provides a practical alternative that can increase insect numbers.