

Chapter Ten

Determining the Diet of Predators in the Field.

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Introduction

Integrated pest management (IPM) programs require an estimate of the level of mortality naturally occurring predators cause to pest populations. However prey consumption by generalist predators is difficult to measure quantitatively in the field (Knutson & Gilstrap 1989, Mills 1997). There is little or no evidence left of consumed prey and the interaction between a predator and its prey is brief and hard to observe in the field (Mills 1997). Despite these difficulties a number of techniques have been utilised to investigate predation with varying degrees of success. For a full description of techniques available for evaluating natural enemy impact see Seymour and Jones (1991), Mills (1997), Luck *et al.* (1988), and Sunderland (1988).

Laboratory based feeding tests

Feeding trials of predatory arthropods involve the predator being confined with prey in a limited search arena for a set period of time. Results from no-choice feeding experiments are often used to determine maximum prey consumption per unit of time, or to generate functional response curves (Johnson 1999). They can provide a great deal of data and are relatively cheap and time efficient in comparison to fieldwork. The main problem with these tests is stated by Greenstone (1999): “Since a starved spider in a small, featureless arena is apt to attack any but the most unsuitable (noxious, venomous, too large or too well armoured) arthropod placed before it, it is hardly surprising that such spiders usually feed, and sometimes consume large numbers of prey.” This statement can be applied to the majority of predators, not just spiders. The results of such tests have limited relevance to a field situation, even when a prey choice is provided, or a larger more complex search arena is used.

Direct observation in the field

The direct observation of feeding events in the field has provided a great deal of data on the dietary range of some predators (Greenstone 1999). Whitcomb (1967) released second-instar bollworm larvae on cotton and observed the predators that attacked the larvae. The author comments that the work “proved to be much slower and more tedious than expected”. Of the 480 larvae released the first year predators consumed only 35 percent, and of the 1,125 larvae released in the second year 50 percent were eaten. A total of 207 feeding observations, most of which involved ants and spiders, were recorded over two years. Of the 330 *Nabis kinbergii* Reuter (damselflags) observed by Wade *et al.* (2002) in cotton during eleven days only ten feeding events were recorded. This predator was recorded feeding for only two percent of the

observation period. Small, fast moving predators can be very difficult to observe in the field and the presence of an observer may alter behaviour of some predators (see Wade *et al.* 2003).

Exclusion experiments

The majority of studies that evaluate natural enemy impact do so indirectly by using exclusion experiments. There is one population of the pest in which the natural enemy is present, and another in which the natural enemy is excluded through the use of a cage or other barrier technique (see Chapters nine). When the resulting densities and mortalities in the populations are compared, the differences are attributed to the actions of the natural enemy (Bellows *et al.* 1992). Caution is required when designing and interpreting cage experiments because of confounding factors. Exclusion studies assume that predator mortality is irreplaceable and does not take into account mortality agents that work in concert with predators (Jones 1982). The cage itself may alter the microclimate (light intensity, wind speed, humidity and temperature) to which prey are exposed to within the cage (Mills 1997, Luck *et al.* 1988). This can cause confounding cage effects that make accurate conclusions regarding predator impact difficult (Titmarsh 1992). Cages restrict the movement of the predators and prey resulting in artificially high densities within the cage (Kidd & Jervis 1996). If the conditions within the cage are similar to those found in the field then the results of the experiment may be of some relevance to the field situation (Seymour & Jones 1991).

An alternative to cages is the use of insecticides to reduce the numbers of natural enemies in an area (see Chapter six). Test plots are treated with an insecticide, which eliminate or reduce the natural enemies, and the control plots are left untreated. Repeated applications of insecticide are used to prevent immigrating natural enemies from having an impact (Kidd & Jervis 1996). With this method, the confounding effects of microclimate changes due to cages are not a problem. Another advantage is that larger experimental plots can be used that may bear more relevance to natural field conditions. Insecticide drift onto control plots may confound the results obtained. Furthermore, the use of an insecticide may actually increase pest numbers via hormoligosis (James & Price 2002).

Life table analysis

Life tables are built to explain the observed numbers of individuals of different ages or life stages in a population (Room *et al.* 1991). They can be used to determine how specific

mortality factors, such as a natural enemy, affects the pest population dynamics (Kidd & Jervis 1996). They are useful in pest control because they can indicate which life stage of the pest should be targeted to have the most impact on the population (Room *et al.* 1991). Life table studies represent a detailed ecological study of a single species, and as such are time-consuming. Furthermore, identifying the true cause of observed mortality in each life stage can be difficult. Despite these constraints life table analyses have been completed for a number, although not a lot, of important pests. Titmarsh (1992) found that parasites, pathogens, and predators were relatively unimportant in mortality of early *Helicoverpa* spp. developmental stages. The majority of egg loss was attributed to plant host characteristics such as leaf waxiness or hairiness. Jones (1982) used life table data of the pest combined with cage and insecticide exclusion techniques to determine that naturally occurring predators and parasitoids impart irreplaceable mortality on the cabbage looper, *Trichoplusia ni* (Hubner). Van De Berg and Cock (1995) demonstrated the importance of integrating exclusion methods and life table studies. Life table studies include predation as the unknown mortality, and exclusion experiments rarely reveal the impact of predators in relation to other pest mortality agents. By combining the two techniques the irreplaceable role of predators can be demonstrated in the context of total mortality of the pest.

Post-mortem gut analysis

Post-mortem gut analysis using molecular techniques (serological and DNA based) can directly measure the proportion of predators feeding on a particular prey in the field without the confounding effect of structures such as exclusion cages. Molecular techniques take into account the effect of the alternative prey present within a field and offer a way to rapidly screen predators and assess their potential as biological control agents (Johnson 1999).

Serological methods make use of antibodies that have been raised in a vertebrate against antigens of the target pest. Prey antigens in the guts of predators can then be detected by tests employing the antibody (Sunderland 1988). In immunoassays (e.g. ELISA, immunodot) the antibody is labelled with bioluminescent compounds or enzymes that can be used to detect the pest antigen in the gut contents (Greenstone & Morgan 1989, Stuart & Greenstone 1990, Greenstone & Trowell 1994). The amount of label detected can be used to quantify the amount of pest antigen in the sample and estimate the amount of prey consumed by the predator (Sopp *et al.* 1992). Sigsgaard (1996) assayed predators in a pigeonpea field by ELISA using monoclonal antibodies for helioithine eggs. This data was used to identify the

main predators and examine the pattern of predation over the season. The immunodot method was used to detect *H. armigera* antigen in predators collected from a cotton field where *H. armigera* eggs had been 'seeded' into the crop at known density (Johnson 1999). Harwood *et al.* (2001a) examined the gut remains of Linyphiidae spiders in wheat using ELISA techniques. Of the spiders tested, 26 percent contained aphid protein in significant quantities. When the availability of collembola was high in the field, the concentration of aphid protein in individual spiders was reduced. Unfortunately the production of monoclonal antibodies against a particular target is expensive and time-consuming (Zaidi *et al.* 1999), which limits the widespread adoption of these techniques.

Alternative methods of prey detection based on DNA have recently become available (see review by Symondson 2002). These techniques involve the development of PCR assays specific for the DNA sequence of particular prey species to detect prey remains in the gut of a predator. This technique relies on the ability of PCR to detect and amplify a target sequence of prey DNA that has resisted digestion in the predator gut.

Zaidi *et al.* (1999) used PCR directed at the multiple-copy esterase genes in laboratory strains of *Culex quinquefasciatus* Say. The mosquitoes were fed to carabid beetles, *Pterostichus cupreus* L. and left for 28 hours. From one mosquito strain a 263 bp product was detected after 28 hours, however in another strain this target sequence could not be detected five hours after consumption. The authors found a relationship between the size of the target sequence and the chance of detection with increasing digestion time. Shorter target sequences appear to survive digestion longer and can be detected for a greater period after prey consumption. Gut analysis of aphid predators, *Chrysoperla plorabunda* (Fitch) and *Hippodamia convergens* Guerin, was attempted using primers that amplify an aphid mitochondrial COII fragments from 77 bp to 386 bp (Chen *et al.* 2000). The detectability half-life for a 198 bp DNA target from a single aphid was almost four hours for *C. plorabunda* and almost nine hours for *H. convergens*. The detectability half-life was then used to devise a correction method to determine the importance of each predator species. Predators with a longer prey DNA half-life have a greater chance of being detected and may seem to be more important when in fact they have consumed the same number of prey. The COII gene, like the multiple copy esterase genes, has numerous copies per cell that increases the chances of successful amplification (Chen *et al.* 2000). Agusti *et al.* (1999) used sequence amplified characterized region (SCAR) markers that were unique to *H. armigera*. This allowed precise amplification

of the *H. armigera* target DNA after commencement of digestion by *Dicyphus tamaninii* Wagner (Miridae). Detection was possible after four hours using primers that amplified a 254 bp fragment.

A major limitation of molecular assays is that they provide an estimate of predators consuming prey rather than predation rate. Detection of prey remains within the gut of a predator does not confirm that the predator killed the prey because scavenging on dead prey may give a positive test result. Furthermore consumption of diseased or parasitised prey will give a positive result but will not contribute to the predation rate because the prey individual would have died from other causes. Researchers must assess whether the consumption rate is a reasonable estimate of the actual numbers of prey killed by the predator. An additional inadequacy of molecular techniques is that they cannot distinguish between primary and secondary predation. Harwood *et al.* (2001b) tested for secondary predation using monoclonal antibodies in an aphid-spider-carabid system. They concluded that it was unlikely to cause major errors in field studies because secondary predation was detectable only immediately after feeding. Inaccuracies are more likely the result of the many factors that affect the sensitivity of the assay. These include the number of prey consumed, time elapsed since feeding, the temperature at which the specimens were held, predator digestion rate, and predator size (Hagler & Naranjo 1997, Agusti *et al.* 1999).

PCR techniques are superior to monoclonal antibody technology because they give comparable detectability half-lives with lower expense, much shorter development times, and greater accuracy (Chen *et al.* 2000). However, PCR techniques cannot achieve stage or instar level specificity. Techniques involving monoclonal antibodies are currently used more widely than PCR based techniques. Some authors predict that PCR based techniques may displace other techniques in the future due to their versatility (Symondson 2002).

Here I investigate the diet of generalist predators of grain crops (with an emphasis on spiders), and techniques to assess prey consumption in a field situation. Laboratory no-choice tests were conducted on different spider families to determine which groups will consume *Helicoverpa* spp. eggs and larvae in a controlled environment. I then tested if PCR based molecular techniques could be used to detect undigested *H. armigera* DNA in the gut of spiders. Clubionidae (*Cheiracanthium* sp.) or night-stalking spiders were used as the target predator on the basis of the no-choice feeding test results.

10.A No-choice feeding tests in the laboratory

Laboratory based no-choice feeding tests were used to assess the potential of particular spider groups as predators of *Helicoverpa* spp. under ideal conditions. These results, combined with Australian and overseas literature, was used to highlight spider groups that may be significant predators of *Helicoverpa* spp. in the field. The results were used to determine which spiders are best cultured in the laboratory on *H. armigera* diet. This became essential for subsequent feeding experiments (see section 10.B).

Materials and methods

Hunting spiders were collected from lucerne and wheat fields during September and October 2000 using a sweep net. Web-building spiders (Araneidae, Tetragnathidae, and Theridiidae) were not included in the feeding tests because it was unlikely that *H. armigera* eggs and larvae would be captured in their webs in the field. All spider families, and many spider species, collected within these fields were present in soybean fields (Bishop & Holtkamp 1982, Evans 1985). Spiders were stored individually in solo cups and kept cool whilst being transported back to the laboratory. Each individual spider was transferred to a petri dish that contained a wet dental wick. Spiders were stored in a constant temperature cabinet, set at (14:10 light:dark; 24:20⁰C; 65% relative humidity). As the weather began to get warmer the temperature within the cabinet was increased by 2⁰C to reflect natural conditions.

Spiders were starved for a maximum of 14 days prior to the introduction of *H. armigera* eggs or larvae. Single starved spiders in the first group (n = 96) were provided with 20 eggs in a petri dish at 8:00am and the cumulative number of eggs consumed after two, four, six, eight, and 24 hours was recorded. The same protocol was used for a second group of spiders (n = 70) that had been incubated for one day after field collection (non-starved spiders).

A feeding experiment with larvae as prey was conducted using another group of starved spiders (n = 68). Ten *H. armigera* first instar larvae were placed into the petri dish with a spider and sealed with plastic cling wrap and sticky tape. Dishes were examined two, four, six, eight, and 24 hours after introduction of the larvae and number of larvae consumed recorded. Control dishes (n = 9) containing ten larvae but no spider were used to assess the number of larvae that died or escaped from the petri dish. The loss rate in the controls was used to correct the results recorded in the feeding experiment.

No spider was used in more than one feeding experiment. Hunting spiders from six families, all ages and both sexes were used for the egg feeding experiments. The collection of spiders used reflected the spider families and age structure present in the crop at a single point in time. This resulted in unequal numbers of spiders in each family for each feeding test.

Results

Oxyopidae and Pisauridae were the most abundant families collected by sweep netting for the egg feeding experiments (table 1) allowing the composition of hunting spiders in the starved and non-starved spider groups to be similar. A similar family composition was sampled for the starved spiders used in the larvae feeding experiment, with Oxyopidae and Thomisidae being the most abundant. Pisauridae consisted mostly of immature spiders, where as Oxyopidae had individuals from both age groups and sexes.

The spiders generally did not readily consume eggs in the laboratory. After eight hours 15 percent (± 3.1 standard error) of the available eggs had been consumed by the starved spiders and 25 percent (± 3.8) after 24 hours. Non-starved spiders consumed 6 percent (± 2.4) of the available eggs after eight hours and 12 percent (± 3.3) after 24 hours. Spiders in the family Clubionidae consumed the greatest number of eggs; 18.4 (± 1.5) eggs per starved spider and 8.2 (± 3.9) eggs per non-starved spider after 24 hours (table 2). The starved Pisauridae (0.7 ± 0.3) and Lycosidae (4.0 ± 2.3) consumed some eggs. Spiders in the Salticidae, Thomisidae and Oxyopidae consumed few or no eggs after 24 hours. Most of the eggs consumed were eaten between eight and 24 hours after the start of the experiment. The experiment commenced in the morning and the first eight hours were during the day, suggesting that a number of spiders did not consume eggs until dusk.

All spiders readily consumed larvae in the laboratory. Starved spiders consumed 63 percent (± 3.4) of the larvae after two hours and 91 percent (± 1.7) after 24 hours. There were no non-starved spiders for comparison in this experiment. Spiders belonging to all families consumed around nine larvae in 24 hours (table 3).

Table 1. Family composition of the spiders collected from lucerne and wheat fields using a sweep net and used for no-choice feeding experiments in the laboratory on *A.* eggs and *B.* larvae of *H. armigera*. Dash indicates family not tested.

A. Fed eggs

	Starved spiders				Non-starved spiders			
	No. of spiders	% Female	% Male	% Immature	No. of spiders	% Female	% Male	% Immature
Oxyopidae	41	24	37	39	32	13	28	59
Pisauridae	20	5	0	95	16	0	0	100
Thomisidae	17	24	29	47	10	50	0	50
Clubionidae	13	15	31	54	6	0	0	100
Salticidae	2	0	0	100	6	33	17	50
Lycosidae	3	33	33	33	-	-	-	-
Total	96				70			

B. Fed larvae

	Starved spiders			
	No. of spiders	% Female	% Male	% Immature
Oxyopidae	28	29	21	50
Thomisidae	15	13	27	60
Pisauridae	14	7	0	93
Clubionidae	8	13	13	74
Salticidae	3	33	66	0
Total	68			

Table 2. Average number of *H. armigera* eggs eaten by spiders belonging to different families at 24 hours after introduction of eggs. Each spider was offered 20 eggs in a no-choice test in a limited search arena. A total of 96 starved spiders were tested and 70 non-starved spiders. Numbers in brackets indicate standard error. Dash indicates family not tested.

	No. eggs eaten/spider	
	Starved	Non-starved
Oxyopidae	0.73 (0.26)	0.19 (0.08)
Pisauridae	9.70 (1.44)	6.88 (1.96)
Thomisidae	0.29 (0.19)	0.00
Clubionidae	18.40 (1.53)	8.17 (3.92)
Salticidae	0.00	0.00
Lycosidae	4.00 (2.31)	-
All Spiders	5.00 (0.76)	2.36 (0.67)

Table 3. Average number of *H. armigera* first instar larvae consumed by spiders belonging to different families at 24 hours after introduction. Each spider was offered 10 larvae in a no-choice test in a limited search arena. A total of 68 spiders were tested. The number in the brackets indicates the standard error.

	No. of larvae eaten/spider
	Starved
Oxyopidae	9.10 (0.26)
Thomisidae	9.46 (0.14)
Pisauridae	9.64 (0.20)
Clubionidae	9.63 (0.26)
Salticidae	9.67 (0.33)
All spiders	9.37 (0.12)

Discussion

Laboratory feeding tests suggest that the assemblage of spiders collected may potentially be able to consume between two and five *H. armigera* eggs per day per spider (10-25% of those available) depending on level of starvation. Insect predators are known to feed on varying numbers of *Helicoverpa* spp. eggs under similar conditions. For example, *Solenopsis invicta* Buren (red imported fire ant) ate 1.5 eggs (± 0.2) in 24 hours (Agnew & Sterling 1982), *Dicranolaius bellulus* (Guerin-Meneville) (red and blue beetle) fed on an average of 34.4 (± 3.6) eggs and *Nabis kinbergii* Reuter (damselfly bug) fed on 51.3 eggs (± 4.6) in 24 hours after being offered 100 (Johnson 1999). If spiders are in a state of food limitation they may potentially be able to consume nine *H. armigera* first instar larvae per day per spider (90% of those available). In the field many factors such as area and complexity of search arena, prey movement and escape behaviours, predator species interactions, and weather will impact on predation rates of spiders (Johnson 1999). Until such factors have been investigated further these results will have limited application to field situations. The results presented here may be better used to highlight spider families that should be targeted for further study.

Spider families that show significant predatory potential against *Helicoverpa* spp. or other soybean pests are discussed below. The majority of the literature found on the diet preferences of individual spider species and spider groups in agroecosystems came from overseas studies. This literature is included in the discussion, however caution must be taken against translating this work directly to Australian agroecosystems.

Lycosidae

These are ground dwelling hunting spiders that often build burrows in the soil between the crop rows (Humphreys 1975, Pyke & Brown 1996, see Chapter five). All authors note this group as being known predators of *Helicoverpa* spp. larvae. In the laboratory feeding tests they consumed an average of four *H. armigera* eggs over 24 hours however very few individuals were tested ($n = 3$). Lycosidae were not tested in the larvae feeding experiments but Bishop (1978) observed Lycosidae feeding on *Anomis flava* (Fabricius) (cotton looper larvae) and *Earias huegeli* Rogenhofer (rough bollworm larvae) in cotton. Agnew & Smith (1989) estimated that almost eight percent of the Lycosidae diet consisted of Lepidoptera in Texas peanut fields.

Pest control researchers generally overlook Lycosidae because they are not sampled on plants during the day and are difficult (for non-taxonomists) to identify to genus. Pitfall traps are the most effective method for capturing these spiders (Chapters two and five). They do climb into the soybean foliage but are rarely captured using a vacuum sampler or sweep net. Ballooning may be the primary source of very young colonizers, but the adults are capable of travelling great distances by walking (Pearce & Zalucki 2002a). They are most abundant at the start of the season before crop canopy closure (Agnew & Smith 1989, Pearce & Zalucki 2002b). Further investigation into the feeding activity of Lycosidae is warranted particularly because they are abundant early in the season when other predators are rare.

Clubionidae

Cheiracanthium sp. has been observed at night feeding on *H. armigera* eggs on cards in soybean, and will readily eat eggs and larvae in the laboratory (Scholz *et al.* 2000, Pearce & Zalucki 2002a). Bishop (1978) recorded *Helicoverpa* spp. in the diet of *Cheiracanthium* sp. in cotton fields, as well as *A. flava* and *N. viridula*. My feeding test shows that Clubionidae can potentially eat 18 eggs per day if starved and eight eggs per day if not starved. This is a higher estimate than that of Room (1979) who estimated five eggs per day if starved and one egg per day if not starved under comparable conditions. Despite this discrepancy it is clear that this family has significant potential as predators of *Helicoverpa* spp. eggs in soybean.

Araneidae

Most species in the Araneidae are capable of constructing large orb webs (Grisworld *et al.* 1998). The prey species captured by this group of spiders is dependent on web location and structure. Many of the larger species collected in soybean fields (*Neoscona theisi* (Walckenaer) and *Argiope trifasciata* (Forskoel)) were capable of constructing webs between rows of 75cm spacing. It is generally thought that these webs are capable of capturing adult Lepidoptera including *Helicoverpa* spp. Araneidae spiders were not included in the no-choice feeding tests and there are very few Australian studies that record prey captured in Araneidae webs in cropping areas (Bishop 1978 is one exception). Overseas studies have recorded other spiders, small Diptera, Cicadellidae, Formicidae, Coleoptera as well as adult Lepidoptera in orb webs (Nyffeler *et al.* 1989). Aphids (35-90%) were the most abundant prey for five orb weaving spiders in Texas cotton (Nyffeler *et al.* 1989). Homoptera (36%), Diptera (21%) and Coleoptera (24%) were the most abundant prey caught in *Argiope* sp. and *Neoscona* sp. webs (Nyffeler 1999).

Linyphiidae

Money spiders (Linyphiidae) are easily overlooked due to their small size and inconspicuous sheet-webs. They constitute a significant proportion of the ballooning spider fauna and the ground dwelling spider fauna. Both adults and immatures have been recorded ballooning (Pearce & Zalucki 2002a). These spiders build horizontal sheet webs on the ground, around the bases of the plants and between clumps of dirt. Little information exists about the diet range of this family of spiders. Due to their size their diet would consist mainly of smaller arthropods that are present in the lower vegetation and ground surface. In European and American studies Linyphiidae diet consisted of Collembola (48%), Homoptera (33%) and no Lepidoptera (Nyffeler 1999), and in Great Britain studies 60% of prey captured in Linyphiidae webs were Collembola (Harwood *et al.* 2001c). Collembola, followed by aphids and thrips were the most abundant prey caught in Linyphiidae webs (Sunderland *et al.* 1986a,b). Linyphiidae have been recorded feeding on *H. virescens* eggs in cotton (Nyffeler *et al.* 1990). Whilst it appears that this group, despite their abundance, may have little impact on *Helicoverpa* spp. in soybean their potential as aphid predators should be investigated further.

Theridiidae

Theridiidae build untidy tangle-webs within the soybean foliage and around the base of the plants. Most of the species collected are small in size, and like the Linyphiidae, would capture only smaller sized prey. Bishop (1978) recorded *A. flava*, *E. huegeli* and aphids as their prey in cotton. In the laboratory *A. veruculata* has been recorded eating *Helicoverpa* spp. larvae (Room 1979). In overseas studies, Homoptera (26%), Hymenoptera (32%) and Diptera (15%) were the most abundant prey caught in webs (Nyffeler 1999).

Salticidae

Known as jumping spiders, Salticidae are sighted diurnal hunters within the soybean canopy. In the feeding tests Salticidae (as well as Thomisidae and Oxyopidae) consumed few or no eggs after 24 hours. Some hunting spiders lie in ambush rather than actively hunting down prey; attacking only when the prey moves very close (Foelix 1996). Mechanical vibrations, or immediate contact with prey may induce catching behaviour. Such cues would not be present when the prey item is a stationary egg. Salticidae (and some Lycosidae) have well developed eyes and use visual cues to orient themselves towards prey, even motionless prey. They may still require prey movement to illicit an attack (Foelix 1996). Salticidae, Thomisidae and Oxyopidae spiders have been recorded feeding on *Helicoverpa* spp. eggs in

cotton fields (Nyffeler *et al.* 1990), suggesting that these spider families should not be discounted as predators of *Helicoverpa* spp. eggs until field studies have been conducted. Overseas studies show that the diet of Salticidae consists mainly of Miridae (26%), other Araneae (21%), Diptera (15%) and Lepidoptera only 6 percent (Nyffeler *et al.* 1994).

Oxyopidae and Thomisidae

Lynx spiders (Oxyopidae) and crab spiders (Thomisidae) were commonly collected in the soybean foliage. Like the Salticidae they are hunting spiders. Oxyopidae generally do not eat *Helicoverpa* spp. eggs in the laboratory, but did eat on average nine larvae per spider per day. Room (1979) showed that *Oxyopes* sp. ate no eggs in no-choice feeding tests, and consumed less than two larvae per spider per day. Bishop (1978) recorded *Oxyopes* sp. feeding on *Helicoverpa* spp. larvae in cotton fields. Studies from Texas have shown that predaceous arthropods made up 42 percent of the diet of *Oxyopes salticus* in cotton (Nyffeler & Benz 1987, Nyffeler *et al.* 1992). Thomisidae do not eat *Helicoverpa* sp. eggs in the laboratory but will readily consume larvae. Bishop (1978) recorded them eating aphids and small lepidopteran larvae in cotton. What proportion of their diet consists of beneficial arthropods is unknown.

It is clear that certain spider groups found within soybean are capable of feeding on *Helicoverpa* spp. eggs and larvae in the laboratory and may do so in the field. The impact of spiders on *Helicoverpa* spp. populations in the field must be quantified before they can be fully incorporated into IPM programs. The mortality that they impose on predaceous arthropods is still unknown and may negate some of their potential as biological control agents.

10.B Molecular methods to detect *Helicoverpa* spp. remains in the guts of Clubionidae spiders

Here I investigate if PCR based molecular techniques could be used to detect undigested *H. armigera* DNA in the gut of spiders. Clubionidae (*Cheiracanthium* sp.) or night-stalking spiders were used to investigate the effectiveness of PCR based molecular techniques in detecting undigested *Helicoverpa* spp. DNA in the gut of spiders. Clubionidae will readily eat *H. armigera* eggs and larvae in the laboratory (see above) and have been observed consuming *H. armigera* eggs on cards in the field (observation by S. Pearce). Spiders feed by injecting fluid through the fangs into the captured prey. This venom contains digestive enzymes that liquefy the prey, which is then sucked up by the spider (Brunet 1994). This form of external digestion is very different to that of chewing predators. To my knowledge this is one of the first studies in which molecular techniques have been used to assess predation by spiders. Serological techniques have been used by Harwood *et al.* (2001a,b) in studies on Linyphiidae spiders. Predators belonging to the Miridae, Anthocoridae, Coccinellidae, Chrysopidae and Carabidae families have been tested using molecular techniques (Symondson 2002).

Materials and methods

Spider collection and feeding

Spiders were collected during June to August in 2001 and 2002 from The University of Queensland Gatton campus. Adult male and female *Cheiracanthium* sp. were collected during the day from their retreats on weeds and winter crops. The spiders were kept cool whilst being transported back to the laboratory. They were maintained in petri dishes with a moist dental wick in a temperature cabinet (14:10 light:dark; 24:20°C; 65% relative humidity) and starved for seven to 15 days. After the starvation period the spiders were fed a variety of prey depending on the treatment (summarised in table 4). Each spider was weighed, sexed and placed in a small screw top vial with the prey item. The vial was placed in a dark box and checked every hour. When the prey was consumed the spider was killed or returned to a petri dish depending on the treatment. Spiders were killed by freezing or by placing in a vial of 100 percent ethanol. The spiders that were left for up to 48 hours post-feeding were placed in a petri dish with a moist dental wick and returned to the temperature cabinet. The *H. armigera* and *H. punctigera* eggs and larvae were obtained from a laboratory culture and other prey items were collected from lucerne crops at Gatton using a sweep net.

DNA extraction

Prey DNA was extracted from individual spiders using a microwave heating method (Cheyrou *et al.* 1991, Goodwin & Lee 1993, Saini *et al.* 1999). If the specimen was stored in ethanol the spider was left to dry until there was no ethanol residue left. Dissecting instruments were sterilised between dissections. It was not possible to completely dissect out the spiders digestive organs so the entire cephalothorax and abdomen were used in the extraction. The legs, palps and chelicerae (if male) were removed and returned to the ethanol. The remaining cephalothorax and abdomen was transferred to a 1.5 ml polyethylene tube. A Kontes micro-pestle was used to crush the spider in the tube. If the spider was frozen they were placed on a chilled petri dish on dry ice. The sterilised dissection instruments were freeze chilled using liquid nitrogen. The cephalothorax and abdomen were placed in a chilled polyethylene tube on dry ice. Chilled Kontes micro-pestles were used to crush the spider. For both types of specimens 300µl of TE buffer (10mM Tris HCl, 1mM EDTA pH 8.0) was added to the tube. A hole was put in the top of the tubes and they were irradiated in a 700 watt microwave oven for 3 minutes. The hole in the top of the tube was sealed and the tubes centrifuged at 22000g for 20 seconds. After the samples were originally tested in a PCR reaction the extraction tubes were incubated in a 65⁰C water bath for 20 minutes to destroy any enzyme activity. Extractions were initially stored at 4⁰C and later aliquots of each extraction were transferred to -20⁰C for storage.

The chelex method was occasionally used for DNA extraction (Scott & Graham 2001). The frozen specimens were dissected as above and placed in chilled polyethylene tubes on dry ice. A chilled Kontes micro-pestle was used to crush the spider. A 5 percent chelex solution (5% chelex 100 resin, 10mM Tris HCl pH 8.0, 1mM EDTA, 1ng/mL RNase) was boiled on a magnetic heating block with rapid mixing. One millilitre of the boiling chelex solution was transferred to the polyethylene tube using a truncated pipette tip that allowed resin to be transferred as well. The tubes were placed on a 100⁰C heating block for 15 minutes. The pressure was released by opening the tubes occasionally. Tubes were centrifuged at 22000g for 5 minutes and stored at 4⁰C.

Primer design

Primers were designed to amplify small segments of *Helicoverpa* spp. DNA in the ITS2 region of the genome. The following primers, designed for a *Helicoverpa* spp. diagnostic service, were used:

ITSN2 (5'-TTCTCGCATCGATGAAGAACG),

ITSB (5'- TCCTCCGCTTATTGATATGC).

This primer pair amplified a 420 bp band in the starved and fed spiders, a 800 bp band in *H. armigera* control and a 830 bp band in *H. punctigera* control. The 420 bp spider ITS2 band was sequenced and compared to *Helicoverpa* spp. and other DNA sequences using a BLAST analysis (NCBI web site: <http://www.ncbi.nlm.nih.gov>).

The *H. armigera* and *H. punctigera* ITS2 sequences were used to design a set of three nested primers which all used the same forward primer.

ITSN2 (5'-TTCTCGCATCGATGAAGAACG) common forward primer,

HaITS (5'- CACACACACACGAGTGTTTAT) specific to *H. armigera*,

HpITS (5'- CACGAGTGATCACTCACTTGT) specific to *H. punctigera*,

Ha+pITS (5'- CTCGCGCAATGTGGCATTGT) common to both.

The primers were synthesised by Genset Oligos and were originally tested on a range of prey species (without spider consumption). Each PCR reaction (carried out in a 12.5µL reaction volume) contained: 2.0µL of extracted DNA, 1.25µL 10×reaction buffer (100mM Tris-HCl pH 8.3, 500mM KCl, 15mM MgCl₂), 0.25µL dNTPs (10mM, Fisher BioTech), 1.0µL of both primers (10mM, Genset Oligos), 0.5µL *Taq* DNA polymerase (5 unit/µL, Qiagen), and 6.5µL of distilled, autoclaved water. Amplification was carried out on a Corbett Research PC-960 cooled thermal cycler and consisted of 40 cycles of 20 s at 94⁰C, 20 s at 57⁰C and 30 s at 72⁰C. A final cycle of 5 min at 72⁰C and 2 min at 25⁰C followed. Amplification products were resolved electrophoretically in a 1.5% agarose gel.

The Ha+pITS primer amplified a 180 bp segment in *H. armigera* and *H. punctigera*. The HaITS primer amplified a 200 bp segment in *H. armigera*. The HpITS primer amplified a 190bp segment in *H. punctigera*. The two *H. armigera* primers (HaITS and Ha+pITS) were used in further tests.

Amplification of *Helicoverpa* spp. DNA remains in spiders

The primers were initially tested on fed and starved spiders using the same PCR protocol used for positive controls above. Amplification was unsuccessful and a nested PCR protocol utilising the nested primer design was tested.

The first PCR reaction (PCR-1) (carried out in a 12.5µL reaction volume) contained: 4.0µL of extracted DNA, 1.25µL 10×reaction buffer (100mM Tris-HCl pH 8.3, 500mM KCl, 15mM

MgCl₂), 0.25µL dNTPs (10mM, Fisher BioTech), 1.0µL of ITS2 primer (10mM, Genset Oligos), 1.0µL of HaITS primer (10mM, Genset Oligos), 0.5µL *Taq* DNA polymerase (5 unit/µL, Qiagen), and 4.5µL of distilled, autoclaved water. Amplification was carried out on a Corbett Research PC-960 cooled thermal cycler and consisted of 40/45 cycles of 20 s at 92°C, 20 s at 57°C and 30 s at 72°C. A final cycle of 5 min at 72°C and 2 min at 25°C followed.

The PCR products from PCR-1 were diluted in distilled, autoclaved water at 1:1000. The diluted samples and undiluted controls were then used in the second PCR. The second PCR reaction (PCR-2) (carried out in a 12.5µL reaction volume) contained: 2.0µL of diluted DNA, 1.25µL 10×reaction buffer (100mM Tris-HCl pH 8.3, 500mM KCl, 15mM MgCl₂), 0.25µL dNTPs (10mM, Fisher BioTech), 1.0µL of ITS2 primer (10mM, Genset Oligos), 1.0µL of Ha+pITS primer (10mM, Genset Oligos), 0.5µL *Taq* DNA polymerase (5 unit/µL company, Qiagen), and 6.5µL of distilled, autoclaved water. Amplification was carried out on a Corbett Research PC-960 cooled thermal cycler and consisted of 45 cycles of 20 s at 92°C, 20 s at 57°C and 30 s at 72°C. A final cycle of 5 min at 72°C and 2 min at 25°C followed. The amount of diluted PCR-1 product used in PCR-2 had to be increased occasionally.

The PCR products were loaded onto a 1.5 percent agarose, 1 × TBE gel (SeaKem® LE agarose, BioWhittaker Molecular Applications) and electrophoresed at 6V/cm for one hour. The gel was visualised with ethidium bromide (20µL of 10mg/mL stock) under UV light.

Testing of protocol with field collected spiders

From December 2001 to May 2002 Clubionidae (*Cheiracanthium* sp.) adults were collected from soybean and adjacent lucerne fields at Gatton. The spiders were collected along with other arthropods using a vacuum sampler. Ten row metres of soybean at five locations and 10m² of lucerne at five locations were vacuumed at each sampling date. The collection bags were placed in the freezer overnight to kill the arthropods. The Clubionidae adults were removed from the other arthropods and placed in individual vials of 100 percent ethanol. The presence (larvae per metre) of *Helicoverpa* sp. in the samples was recorded. The *Helicoverpa* sp. pressure was very low in these and many other crops during the season. Often, no or very low numbers of larvae were collected in the vacuum samples. The Clubionidae were analysed using the PCR protocol previously described and scored as positive or negative for *H. armigera* remains. The PCR was repeated twice on some individuals to confirm the result.

The Poisson model of predation was used to estimate the number of prey eaten (Nakamura & Nakamura 1977, Sigsgaard *et al.* 2002) within the detection period:

$$\text{Mean number of eggs killed per predator} = -\ln(1-p)$$

Where p is the proportion of positives after a PCR reaction.

Table 4. Treatments used in nested PCR protocol.

Treatment/prey	Stage of prey	Length of treatment	Number of spiders
Starved	no prey	7 days	34
Starved	no prey	15 days	5
<i>Helicoverpa armigera</i>	1 egg	0 hours	17
<i>H. armigera</i>	1 egg	8 hours	12
<i>H. armigera</i>	1 egg	24 hours	18
<i>H. armigera</i>	1 egg	48 hours	12
<i>H. armigera</i>	10 egg	0 hours	10
<i>H. armigera</i>	1 first instar larvae	0 hours	6
<i>H. armigera</i>	5 first instar larvae	0 hours	6
<i>Helicoverpa punctigera</i>	1 egg	0 hours	5
<i>H. punctigera</i>	1 first instar larvae	0 hours	5
<i>Austroasca alfalfae</i>	adult	0 hours	6
<i>Spodoptera litura</i>	1 second instar larvae	0 hours	6

Results

Spider ITS2 region

The spider ITS2 region sequenced (GenBank accession number AY143427) was very different to that of *H. armigera* and *H. punctigera*. The BLAST alignment showed that this region of Clubionidae DNA was very different to other spider species. The following spider species produced good alignments at the start of the target sequence:

Eidmanella pallida (AF003822)

Nesticus valentinei (AF003821)

Nesticus tennesseensis (AF003820, 819, 818)

Nesticus stupkai (AF003816, 815, 814)

Nesticus reclusus (AF003813)

Nesticus silvanus (AF003812)

These spiders all belong to the family Nesticidae that generally live in caves and dark, damp habitats. Further into the sequence (in the ITS2 region) no significant alignments were found. The BLAST database contains very few DNA sequences from spider species so these alignments are unlikely to be the only significant ones.

Sensitivity of nested PCR protocol

The nested PCR protocol was able to successfully amplify *H. armigera* DNA (eggs and first instar larvae) from the spiders (fig.1). Spiders that had been starved for seven and 15 days (21 spiders in total) did not produce bands when tested using the nested protocol. One of the starved spiders was chosen for a negative control in further tests. A single *H. armigera* first instar larvae was placed in an polythelyene with a starved spider prior to extraction. The sample produced a positive band after a PCR reaction and was used as a positive control in further tests. Both *H. armigera* and *H. punctigera* larvae (extracted using chelex) were also used as positive controls. The nested PCR protocol was highly sensitive to even trace quantities of *Helicoverpa* sp. DNA. This meant that negative controls (i.e. those with no DNA) sometimes produced positive bands. Improved sterilisation techniques were used to avoid false positives.

Species specificity

The nested PCR protocol was initially tested on prey species without spiders. The chelex method was used to extract DNA from a section cut from a single *H. armigera* and *H. puntigera* larvae. The microwave procedure was used to extract DNA from a single

Austroasca alfalfae adult and *Spodoptera litura* larvae. All species were tested with all three primer combinations (fig. 2). These results followed what was anticipated given the design of the primers. No bands were detected using *A. alfalfae* or *S. litura* with any primer combination. Bands were detected using *H. armigera* larvae with the Ha and Ha+p primer combinations, and using *H. punctigera* larvae with the Hp and Ha+p primer combinations. The same prey types were fed to spiders and tested using the Ha primer in PCR-1 and Ha+p primer in PCR-2 (fig. 3). No bands were found for spiders fed *A. alfalfae* or *S. litura*. Spiders fed *H. armigera* eggs or larvae produced positive bands. Contrary to expected results, bands were observed in some of the spiders fed *H. punctigera* eggs or larvae. The primers were not specific to a single *Helicoverpa* species once digestion commenced. The primers may be specific within the Heliiothinae sub-family. Further testing with closely related species is necessary to confirm this result.

Detectability time

The remains of *H. armigera* within the guts of the spiders could be detected many hours after feeding (fig. 4). Eight hours after feeding on one *H. armigera* egg 50 percent of the assayed spiders tested positive. At 48 hours post feeding eight percent of the spiders tested positive. The detectability curve decreased exponentially over time. A logistic regression showed there was a significant relationship between the PCR result and time since feeding ($P < 0.01$). Detectability at 48 hours post feeding was close to zero. Fed spiders would not reliably produce positive bands after 48 hours using the nested PCR protocol, therefore this can be considered the maximum detection period.

Protocol testing using field collected spiders

Seven of the 23 Clubionidae spiders collected tested positive (table 5). This means that at least 30 percent of the collected spiders consumed *Helicoverpa* sp. eggs or larvae in the previous 48 hours. The proportion of spiders testing positive is expected to change with the numbers of *Helicoverpa* sp. prey within the crop. The spiders that tested positive mostly came from lucerne fields (table 5). Not enough samples were taken to determine trends between *Helicoverpa* sp. abundance in the crop and proportion of spiders testing positive for *Helicoverpa* sp. remains. Of the spiders that tested positive, four came from samples in which *Helicoverpa* sp. larvae were collected at low levels. Two of the spiders were female and were collected from breeding nests that contained egg sacs with spiderlings. Observations on Clubionidae breeding nests have revealed that they are always closed and the female remains

inside to guard the eggs and developing young for up to three months (Austin 1986, 1984, Wolf 1990). It is unlikely that the mother will leave the egg sac during this period to hunt for prey, therefore the positive results obtained in this study are unusual. The final positive spider came from a sample in which no *Helicoverpa* spp. larvae were detected in the field. This does not mean they were not present within the field at the time, but rather that they were at such low levels that they were not sampled using the collection method employed. To completely validate this PCR protocol in the field spider collections from crops with a range of *Helicoverpa* spp. densities are required.

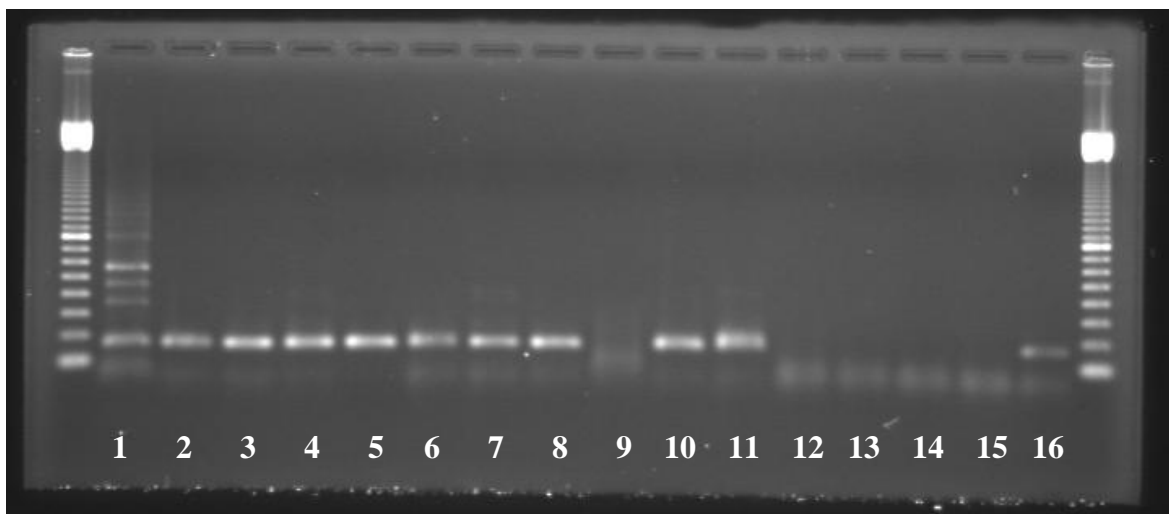


Figure 1. Amplified region of undigested *Helicoverpa* spp. ITS2 from the guts of Clubionidae spiders. Lanes 1 and 2, spiders fed one *H. armigera* egg then killed. Lanes 3 and 4, spiders fed one *H. armigera* first instar larvae then killed. Lanes 5 and 6, spiders fed five *H. armigera* first instar larvae then killed; Lanes 7 and 8, spiders fed 10 *H. armigera* eggs then killed. Lane 9, spider starved for 15 days then killed. Lane 10, a *H. armigera* first instar larvae extracted with starved spider. Lane 11, *H. armigera* larvae positive control in first PCR. Lane 12, 13 and 14, negative controls (no DNA) in first PCR. Lane 15, negative control in second PCR. Lane 16, *H. armigera* larvae positive control in second PCR. 100 base pair molecular marker in the first and last lanes.

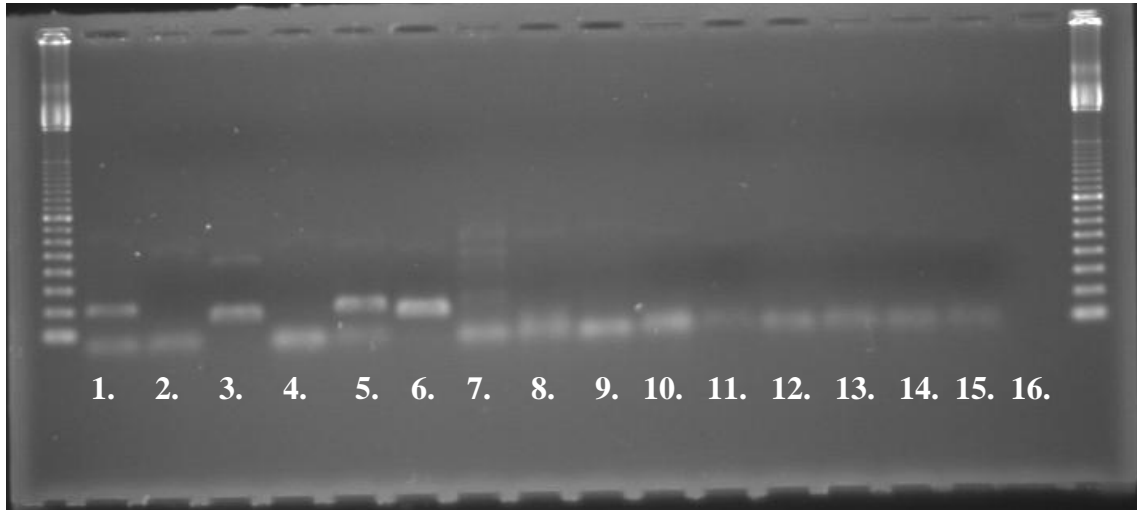


Figure 2. Amplified region of *Helicoverpa* spp. ITS2 from different prey species. Lanes 1, 2, 3, *H. armigera* larvae amplified using Ha primer (1), Hp primer (2) and Ha+p primer (3). Lanes 4, 5 and 6, *H. punctigera* larvae amplified using Ha primer (4), Hp primer (5) and Ha+p primer (6). Lanes 7, 8 and 9 *S. litura* larvae (cluster caterpillar) amplified using Ha primer (7), Hp primer (8) and Ha+p primer (9). Lanes 10, 11 and 12 *A. alfalfae* (lucerne jassid) amplified using Ha primer (10), Hp primer (11) and Ha+p primer (12). Lanes 13, 14 and 15 negative controls. Lane 16 no sample. 100 base pair molecular marker is in first and last lanes.

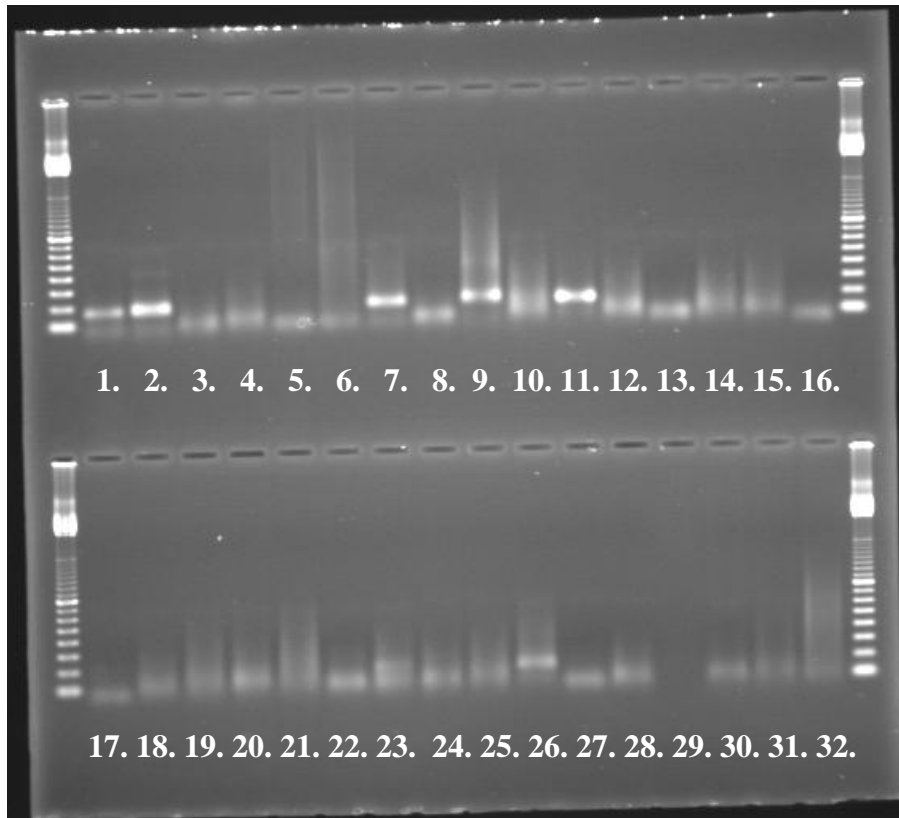


Figure 3. Amplified region of undigested *Helicoverpa* spp. ITS2 from the guts of Clubionidae spiders fed different prey species. Lanes 1, 2 spiders fed one *H. armigera* egg then killed. Lanes 3, 4, 5, 6, and 7, spiders fed one *H. punctigera* egg then killed. Lanes 8, 9, 10, 11 and 12, spiders fed one *H. punctigera* first instar larvae then killed. Lanes 13, 14, 15, 16, 17 and 18 spiders fed one *A. alfalfae* (lucerne jassid) then killed. Lanes 19, 20, 21, 22 and 23, spiders fed one *S. litura* larvae then killed. Lane 24, one *A. alfalfae* adult. Lane 25, one *S. litura* larvae. Lane 26, one *H. armigera* first instar larvae positive control for first PCR. Lane 27, one *H. punctigera* first instar larvae positive control. Lane 28, spider starved for 15 days then killed. Lane 29, no sample. Lane 30, 31 and 32, negative controls in first PCR. 100 base pair molecular marker in the first and last lanes.

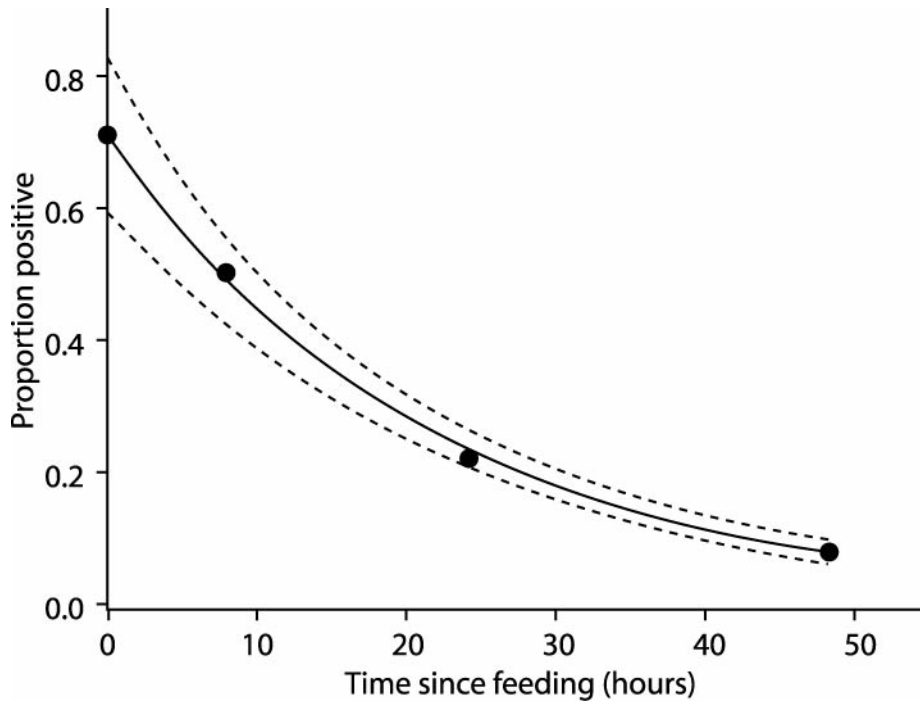


Figure 4. The proportion of spiders that displayed a positive band after feeding on one *H. armigera* egg and being killed after a period of time. 17 spiders were assayed at zero hours, 12 spiders at eight hours, 18 spiders at 24 hours and 12 spiders at 48 hours. The solid line represents the fitted exponential curve and the dashed lines represent the 95% confidence interval.

Table 5. Collection records for Clubionidae spiders that were tested for the presence of *H. armigera* in their guts using a molecular protocol. A positive result indicates that the animal has fed on *Helicoverpa* spp. in the last 48 hours.

Site	Crop	Number of spiders collected	Number of positives	Proportion positives	Number of <i>Helicoverpa</i> spp. larvae recorded (no./m of row)
Gilbert A	soybean	4	0	0.00	0
Gilbert A	lucerne	5	3	0.60	0.02 to 0.2
Gilbert C	soybean	12	2	0.17	0 to 0.02
Gilbert C	lucerne	2	2	1.00	0 to 0.12
Total		23	7	0.30	0 to 0.2

Discussion

This study has clearly demonstrated that it is possible to detect partially digested DNA in the gut of spiders using a molecular approach. The primers used could not differentiate between *H. armigera* and *H. punctigera* once consumed by spiders. Unrelated Lepidoptera and Hemiptera prey species did not produce bands. The detectability half-life of a single *H. armigera* egg meal in the gut of spiders was estimated at eight hours post feeding, however positive bands were detected after 48 hours. This result is similar to previous studies in which detectability of similar sized fragments decreased rapidly after four hours post feeding (Agusti *et al.* 1999, Chen *et al.* 2000). The exponential decay curve will vary with temperature, meal size, predator hunger level, and if alternative prey has been consumed (Sunderland 1988). All these factors will vary under field conditions and must be taken into account when estimating predation rates from field collected spiders. In comparison to other arthropods, spiders tend to have longer detectability times in ELISA studies. Sopp and Sunderland (1989) found the antigen decay rate in Staphylinidae beetles much faster than in spiders and carabids. The spiders (Linyphiidae) exhibited very long detection periods of up to 13 days, even at high temperatures.

The current protocol can only provide a positive or negative result rather than quantifying the number of eggs or larvae eaten. Several equations have been proposed to estimate the number of prey ingested from such data (Sunderland 1988, Sopp *et al.* 1992). In this study a mean number of 0.36 eggs (or egg equivalents) killed per spider throughout the detection period was estimated using the Poisson model. This model assumes that the encounter of prey by the predator is random and so follows a Poisson distribution (Nakamura & Nakamura 1977). The mean number of Clubionidae spiders collected in a soybean field during the season was 0.09 per one metre of row. This means that on average 0.032 eggs will be eaten per one metre of soybean row within 48 hours. In a field situation *Helicoverpa* spp. moths lay eggs singly and sometimes at low densities. It seems unlikely that a spider will find and consume a large number of eggs or larvae in a 48 hour period because the area that the spider must search is large. It is reasonable to assume that a positive result means the spider has consumed one egg or larvae in the detection period. Dempster's equation (1960) may give a better estimate in this situation because it assumes that a positive reaction in an ELISA test represented one prey item consumed during the detection period. However both these equations give similar estimates of consumption rate. Sopp *et al.* (1992) found that both of these equations underestimated the observed consumption of cereal aphids by generalist predators.

The protocol presented may be improved by exploring different extraction techniques, and PCR cycling conditions. Only the microwave DNA extraction technique was used in these experiments. Whilst this technique provides a fast, low cost extraction that is amenable to high throughput diagnostic testing, it may not be the optimum method for this situation. The nested PCR protocol provides high sensitivity; however reducing the protocol to a single step may reduce the chance of false positives. Hand collection of spiders in the field rather than vacuum samples may reduce the chance of false positives due to external contamination of the specimens. The development of quantitative PCR techniques currently used in viral research (Reischl & Kochanowski 1999) may prove useful for directly measuring the amount of prey consumed by an individual spider. Without quantitative information on prey consumed by predators in the field they cannot be fully incorporated into integrated pest management systems.

Chapter summary

- No-choice feeding tests in the laboratory, using *H. armigera* eggs and larvae as prey, were used to ascertain the predatory potential of spider groups.
- The field-collected spiders tested ate two to five eggs per 24 hours per spider (10-25% of those available) depending on level of starvation. Few spider groups consumed eggs in the laboratory with the exception of Clubionidae spiders that ate eight to 18 eggs in 24 hours.
- Starved spiders consumed nine first instar larvae per 24 hours per spider (90% of those available). All spider groups collected from the field readily consumed first instar larvae in the laboratory.
- One is unable to assume that these results are indicative of predation rates that occur in the field. However the Lycosidae, Clubionidae, Oxyopidae, Salticidae and Thomisidae have the capacity to contribute to control of *H. armigera*.
- A molecular technique was developed to detect *H. armigera* remains in the guts of Clubionidae spiders (*Cheiracanthium* sp.) using nested primers to target the ITS2 region.
- The nested PCR protocol was able to successfully amplify *H. armigera* DNA (eggs and first instar larvae) from the guts of Clubionidae spiders. The assay was specific within the Heliiothine sub-family but not species specific to *H. armigera* or *Helicoverpa punctigera* (Wallengren).
- Eight hours after feeding on one *H. armigera* egg 50 percent of the assayed spiders tested positive using the two-step PCR protocol. *H. armigera* egg remains could be detected for a maximum of 48 hours post feeding in the spiders' guts.
- Clubionidae spiders were collected from field crops and tested using the protocol developed. Thirty percent of the field collected Clubionidae tested positive for *Helicoverpa* spp. remains. Field collections need to be conducted in crops with a range of *Helicoverpa* spp. densities to better estimate the proportion feeding.
- Molecular methods, such as the protocol investigated here, may be used in the future to quantify predation rates of generalist predators in the field without confounding effects.

References

- Agnew CW & Smith JWJ (1989) Ecology of spiders (Araneae) in a peanut agroecosystem. *Environmental Entomology* **18**, 30-42.
- Agnew CW & Sterling WL (1982) Predation rates of the red imported fire ant on eggs of the tobacco budworm. *Protection Ecology* **4**, 151-158.
- Agusti N, De Vicente MC & Gabarra R (1999) Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: A new polymerase chain reaction-based technique for predator gut analysis. *Molecular Ecology*, **8**, 1467-1474.
- Austin AD (1984) Life history of *Clubiona robusta* L. Koch and related species (Araneae, Clubionidae) in South Australia. *The Journal of Arachnology* **12**, 87-104.
- Austin AD (1986) Guarding behaviour, eggmass shape and the eggsac in *Clubiona robusta* L. Koch (Araneae: Clubionidae). *Australian Arachnology, Proceedings of the Australian Entomological Society* (eds Austin AD & Heather NW), Adelaide 87-95.
- Bellows TSJ, Van Driesche RG & Elkinton JS (1992) Life-table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology* **37**, 587-614.
- Bishop AL (1978) *The Role of Spiders as Predators in a Cotton Ecosystem* M.Sc. thesis, The University of Queensland, Brisbane.
- Bishop AL & Holtkamp RH (1982) The arthropod fauna of lucerne in the Hunter Valley, New South Wales. *General and Applied Entomology* **14**, 21-32.
- Brunet B (1994) *The silken web. A natural history of Australian spiders*. Reed New Holland, Sydney.
- Chen Y, Giles KL, Payton ME & Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887-1898.
- Cheyrou A, Guyomarch C, Jasserand P & Blouin P (1991) Improved detection of HBV DNA by PCR after microwave treatment of serum. *Nucleic Acids Research* **19**, 4006.
- Dempster JP (1960) A quantitative study of the predators on the eggs and larvae of the broom beetle, *Phytodecta olivacea* Forster, using the precipitin test. *Journal of Animal Ecology* **29**, 149-167.
- Evans ML (1985) Arthropod species in soybeans in southeast Queensland (Australia). *Journal Of The Australian Entomological Society* **24**, 169-177.
- Foelix RF (1996) *Biology of spiders*. Oxford University Press, New York.
- Goodwin DC & Lee SB (1993) Microwave miniprep of total genomic DNA from Fungi, plants, protists and animals for PCR. *BioTechniques* **15**, 138-141.
- Greenstone MH (1999) Spider predation: How and why we study it. *Journal of Arachnology* **27**, 333-342.
- Greenstone MH & Morgan CE (1989) Predation on *Heliothis zea* (Lepidoptera: Noctuidae): An instar-specific ELISA assay for stomach analysis. *Annals of the Entomological Society of America* **82**, 45-49.
- Greenstone MH & Trowell SC (1994) Arthropod predation: A simplified immunodot format for predator gut analysis. *Annals of the Entomological Society of America* **87**, 214-217.
- Grisworld CE, Coddington G, Hormiga G, & Scharff N (1998) Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). *Zoological Journal of the Linnean Society* **123**, 1-99.
- Hagler JR & Naranjo SE (1997) Measuring the sensitivity of an indirect predator gut content ELISA: Detectability of prey remains in relation to predator species, temperature, time, and meal size. *Biological Control* **9**, 112-119.
- Harwood JD, Symondson WO & Sunderland KD (2001a) Monoclonal antibodies to quantify the effects of alternative prey on aphid predation by spiders. *Antenna Bulletin of the royal entomological society* **25(4)**, 257-259.
- Harwood JD, Phillips SW, Sunderland KD & Symondson WOC (2001b) Secondary predation: Quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. *Molecular Ecology* **10**, 2049-2057.
- Harwood JD, Sunderland KD & Symondson WO (2001c) Living where the food is: Web location by linyphiid spiders in relation to prey availability in winter wheat. *Journal of Applied Ecology* **38**, 88-99.
- Humphreys WF (1975) The influence of burrowing and thermoregulatory behaviour on the water relations of *Geolycosa godeffroyi* (Araneae: Lycosidae), an Australian wolf spider. *Oecologia* **21**, 291-311.
- James DG & Price TS (2002) Fecundity in twospotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. *Journal of Economic Entomology* **95(4)**, 729-732.
- Johnson ML (1999) *Comparing Predatory Insects of Helicoverpa spp. in Australian Cotton: Approaches to Measuring Prey Consumption*. Ph.D. thesis, The University of New England, Armidale.
- Jones D (1982) Predators and Parasites of temporary row crop pests: agents of irreplaceable mortality or scavengers acting prior to other mortality factors? *Entomophaga* **27**, 245-266.
- Kidd NA & Jervis MA (1996) Population dynamics. In: *Insect natural enemies. Practical approaches to their study and evaluation* (eds. Kidd NA & Jervis MA), pp. 293-. Chapman and Hall, London.

- Knutson AE & Gilstrap FE (1989) Direct evaluation of natural enemies of the southwestern corn borer (Lepidoptera: Pyrauidae) in Texas corn. *Environmental Entomology* **18**, 732-739.
- Luck RF, Shepard MB, Kenmore PE (1988) Experimental methods for evaluating arthropod natural enemies. *Annual Review of Entomology* **33**, 367-391.
- Mills N (1997) Techniques to evaluate the efficacy of natural enemies. In: *Methods in Ecological and Agricultural Entomology* (eds. Dent DR & Walton MP), pp. 271-291. CAB International, New York.
- Nakamura M & Nakamura K (1977) Population dynamics of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). *Oecologia* **27**, 97-116.
- Nyffeler M (1999) Prey selection of spiders in the field. *Journal of Arachnology* **27**, 317-324.
- Nyffeler M & Benz G (1987) Spider in natural pest control: A review. *Journal of Applied Entomology* **103**, 321-339.
- Nyffeler M, Dean DA & Sterling WL (1989) Prey selection and predatory importance of orb-weaving spiders (Araneae: Araneidae, Uloboridae) in Texas (USA) cotton. *Environmental Entomology* **18**, 373-380.
- Nyffeler M, Breene RG, Dean DA & Sterling WL (1990) Spiders as predators of arthropod eggs. *Journal of Applied Entomology* **109**, 490-501.
- Nyffeler M, Dean DA & Sterling WL (1992) Diets, feeding specialization, and predatory role of two lynx spiders, *Oxyopes salticus* and *Peuceitia viridans* (Araneae: Oxyopidae), in a Texas cotton agroecosystem. *Environmental Entomology* **21**, 1457-1465.
- Nyffeler M, Sterling WL & Dean DA (1994) Insectivorous activities of spiders in United States field crops. *Journal of Applied Entomology* **118**, 113-128.
- Pearce S & Zalucki MP (2002a) Spider ballooning in crops: A web of intrigue. *Australian Grain* **12** (4), vi-vii.
- Pearce S & Zalucki MP (2002b) A wolf with a beneficial bite. *Australian Grain* **12** (5), vi-vii.
- Pyke BA & Brown EH (1996) *The Cotton Pest and Beneficial Guide*. CRDC, CTPM and CRC for Sustainable Cotton Production, Woolloongabba.
- Reischl U & Kochanowski B (1999) Quantitative PCR: A survey of present technology. In: *Quantitative PCR protocols* (eds. Kochanowski B & Reischl U), pp. 3-30. Humana Press, Totowa.
- Room PM (1979) Parasites and predators of *Heliothis* spp. (Lepidoptera: Noctuidae) in cotton in the Namoi valley, New South Wales. *Journal of the Australian Entomological Society* **18**, 223-228.
- Room PM, Titmarsh IJ, Zalucki MP (1991) Life tables. In: *Heliothis: Research methods and prospects* (ed. Zalucki MP), pp. 69-79. Springer-Verlag, New York.
- Saini HS, Sheperd M, Henry RJ (1999) Microwave extraction of total genomic DNA from barley grains for PCR. *Journal of the Institute of Brewing* **105**, 185-190.
- Scott L & Graham G (2001) *Introduction to molecular biology*, p. 89. Centre for Identification and Diagnostics, Brisbane.
- Scholz BC, Cleary AJ, Lloyd RJ & Murray DA (2000) Predation of heliothis eggs in dryland cotton on the Darling Downs. *Proceedings of the Tenth Australian Cotton Conference*, 16-18 August, Brisbane. Australian Cotton Growers Research Association. 113-119.
- Seymour JE & Jones RE (1991) Evaluating natural enemy impact on *Heliothis*. In: *Heliothis Research Methods and Prospects* (ed. Zalucki MP), pp. 80-89. Springer-Verlag, New York.
- Sigsgaard L (1996) Serological analysis of predators of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) eggs in sorghum-pigeonpea intercropping at ICRISAT, India: a preliminary field study. In: *The Ecology of Agricultural pests* (ed. Symondson WOC & Liddell JE). Chapman and Hall, London.
- Sigsgaard L, Greenstone MH & Duffield SJ (2002) Egg cannibalism in *Helicoverpa armigera* on sorghum and pigeonpea. *BioControl Dordrecht* **47**, 151-165.
- Sopp PI & Sunderland KD (1989) Some factors affecting the detection period of aphid remains in predators using ELISA. *Entomologia Experimentalis Et Applicata* **51**, 11-20.
- Sopp PI, Sunderland KD, Fenlon JS & Wratten SD (1992) An improved quantitative method for estimating invertebrate predation in the field using an enzyme-linked immunosorbent assay (ELISA). *Journal of Applied Ecology* **29**, 295-302.
- Stuart MK & Greenstone MH (1990) Beyond ELISA: A rapid, sensitive, specific immunodot assay for identification of predator stomach contents. *Annals of the Entomological Society of America* **83**, 1101-1107.
- Sunderland KD (1988) Quantitative methods for detecting invertebrate predation occurring in the field. *Annals of Applied Biology* **112**, 201-224.
- Sunderland KD, Fraser AM & Dixon AF (1986a) Distribution of linyphiid spiders in relation to capture of prey in cereal fields. *Pedobiologia* **29**, 367-375.
- Sunderland KD, Fraser AM & Dixon AF (1986b) Field and laboratory studies on money spiders (Linyphiidae) as predators of cereal aphids. *Journal Of Applied Ecology* **23**, 433-448.
- Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular Ecology* **11**, 627-641.
- Titmarsh IJ (1992) *Mortality of immature lepidoptera: a case study with Heliothis species (Lepidoptera : Noctuidae) in agricultural crops on the Darling Downs* Ph.D., The University of Queensland.

- Van Den Berg H, Cock MJ (1995) Natural control of *Helicoverpa armigera* in cotton: Assessment of the role of the predation. *Biocontrol Science and Technology* **5**, 453-463.
- Wade M, Zalucki M & Franzmann B (2002) What are damsel bugs doing in cotton? Implications for IPM. *Australian Cotton Grower* **23(7)**, 48-50.
- Wade MR, Zalucki MP & Franzmann BA (2003) Does observer presence influence the behaviour of the damsel bug? *Animal Behaviour* In review.
- Whitcomb WH (1967) Field studies on predators of the second instar bollworm, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Journal of the Georgia entomological society* **2**, 113-118.
- Wolf A (1990) The silken nests of the clubionid spiders *Cheiracanthium pennyi* and *Ceiracanthium punctorium* (Araneae, Clubionidae). *Acta Zoologica Fennica* **190**, 397-404.
- Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J & Symondson WOC (1999) Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? *Molecular Ecology* **8**, 2081-2087.