Chapter 10

QUANTIFYING THE IMPACT OF COCCINELLIDS ON THEIR PREY

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10.1 INTRODUCTION

The importance of quantifying the impact of natural enemies in biological control programmes has often been emphasized, but the empirical measurement of impact in the field remains one of the most difficult challenges for ecological entomologists. This is especially true for predatory species such as coccinellids. The impact of coccinellids on their prey is a complex function of their local abundance, voracity and prey fidelity, combined with various indirect effects of their activities on prey survival, some mediated by other natural enemies and some by the host plant. Predation is difficult and time-consuming to observe directly in the field and often leaves no evidence of its occurrence. Since various coccinellid species frequently attack the same prey simultaneously, and may comprise only one fraction of a guild of predators, it can be challenging to partition impact among predator species even when rates of prey removal can be quantified. Despite such difficulties, efforts to quantify coccinellid predation directly are usually considered preferable to more indirect inferences derived from statistical correlations of predator and prey abundance. Too often, the collapse of large populations of **aphids** is attributed to the action of large numbers of coccinellids associated with them when, in actuality, **many biotic and abiotic factors interact to accelerate** such **population declines**. These include, but are not limited to, diminishing host plant quality, induced plant defence responses, development and emigration of winged forms of prey, fungal epizootics, high temperatures, wind and rain, etc. The respective contributions of these factors can be difficult to separate from the impact of predation. The study of *Diuraphis noxia* mortality conducted by Lee et al. (2005) illustrates some of the empirical challenges posed by such confounding factors and the fact that coccinellids, although abundant, may not necessarily suppress aphid populations below economic levels. Notwithstanding the challenges of attributing mortality to particular causes, the **important role of coccinellids** as agents of biological control is **undeniable in many cases**, even if it is difficult to quantify. For example, the mass exodus of spring generation *Hip. convergens* from mature wheat fields is observed with seasonal regularity on the High Plains of the USA and can only result from prodigious aphid consumption in the developing grain, without which more frequent and more extensive pesticide applications would almost certainly be necessary to preserve yields (JPM, pers. observ.). In contrast, Dixon (2005) concludes that, although natural enemies, including coccinellids, may occasionally inflict dramatic mortality on tree-dwelling aphids, it is the interactions between the aphids and their host plants that drive long-term cycles in the abundance of these species.

Coccidophagous coccinellids may match the reproductive rate of their prey, generation for generation, or in the case of *Rodolia cardinalis*, exceed it (Hodek 1973; Chapter 11), resulting in a highly effective numerical response. However, the numerical response of aphidophagous species cannot match the reproductive rate of most aphid species that can achieve multiple generations in the critical period of spring/early summer. Estimation of coccinellid impact on aphids is particularly challenging because of the ephemeral nature of aphid populations, their high reproductive rate (due to parthenogenesis and viviparity), and the sensitivity of biological control outcomes to initial conditions, especially the timing of predator arrival. Smith (1966) characterized five stages of the typical **aphid population cycle:** initiation, exponential growth, peak, collapse and scarcity. In the **initiation phase**, immigrant alatae form nuclear colonies on plants that have recently become suitable hosts; aphids remain at low density for some time because alate reproductive rate is low and many die prior to the maturation and reproduction of their apterous daughters. Although rapid recruitment by coccinellids and other aphid predators at this time can have great impact on the aphid population's growth potential, these predators may not respond in any numbers until aphid density reaches some threshold, and the timing of their arrival may hinge on the distance over which they must travel from other habitats or overwintering sites. It has been pointed out that any estimate of predator impact in established aphid colonies must be accompanied by some estimate of aphid reproduction during the same period in order to be meaningfully interpreted (Latham & Mills 2010). If natural enemy recruitment is either sparse or delayed, the aphid population enters a **phase of exponential growth** that coincides with the onset of reproduction by large numbers of maturing apterae. Soon thereafter, a threshold is surpassed beyond which the aphid replacement rate far exceeds the maximum rate of mortality that can be inflicted by natural enemies, regardless of their numbers, and biological

control can be considered to have failed. Nevertheless, 'success' for coccinellids is not measured by decimation of the aphid population, but rather by achieving reproduction that is properly synchronized with the growth of the aphid colonies in order to ensure the survival of offspring (Kindlmann & Dixon 1993). Thus, females maximize their fitness by laying as many eggs as possible at the beginning of exponential growth phase, the so-called 'oviposition window' (Dixon 2000), so that their larvae can complete development during the peak phase when aphids are maximally abundant. This is followed by the decline or **collapse phase** when the last maturing aphids develop into alatae and disperse, and then finally the **period of aphid scarcity** that persists until a new population cycle is initiated, either on the same or alternative host plants.

From the **perspective of biological control**, three types of outcome are possible for each aphid population cycle. Coccinellids and other aphidophagous predators achieve maximum impact if they **arrive early and in sufficient numbers**, so that most aphid colonies are eliminated prior to the exponential growth phase. This is the anthropocentric objective, but has adverse consequences for the coccinellid population because many larvae starve, and those that survive must do so by resorting to cannibalism and intraguild predation. This scenario may well occur more often than is generally recognized, especially in non-agricultural systems, and thus contribute to a lack of recognition of coccinellid impact, simply because **aphid infestations eliminated** in such **early stages** often go **unnoticed**. At the other extreme, impact will be negligible if coccinellids arrive too late, regardless of their numbers, once a large proportion of aphid colonies are in the exponential growth phase. In many cases, the outcome lies somewhere between these extremes; the peak phase of the aphid population is suppressed to some degree, but perhaps not to the desired level. In these cases, whether impact is considered sufficient with respect to biological control hinges entirely on the economic threshold for aphids on the particular crop plant. Often, the threshold is too low to permit a realistic expectation of control through biological means alone, despite the fact that the potential for coccinellid impact is relatively high. Examples include cases of direct aphid contamination of a consumer product (e.g. the lettuce aphid, *Nasonovia ribisnigri*), and where aphids vector an important plant disease (e.g. *Myzus persicae* vectoring potato leaf roll virus). Thus, from the perspective of pest management, coccinellid impact is relative; it may be substantial in terms of aphid population reduction and still fall short of economic requirements.

10.2 ASSAYS OF CONSUMPTION

There have been many efforts to **quantify the voracity** of coccinellid species as an indication of their potential impact. Bankowska et al. (1978) measured food consumption of coccinellids **in the laboratory** and then multiplied these values by the numbers of beetles observed in alfalfa fields to estimate their impact on aphid populations. Laboratory studies of coccinellid development and reproduction on particular prey species are useful for inferring their potential impact on prey populations (e.g. Michaud 2000, Uygun & Atlhan 2000, Michaud & Olsen 2004, Omkar & James 2004, Mignault et al. 2006) and for predicting functional and numerical responses (Mack & Smilowitz 1982, Atlhan & Ozgokce 2002, Butin et al. 2003). However, coccinellid **foraging efficiency** on a particular prey may vary as function of plant architecture (e.g. Clark & Messina 1998; 5.3.1.1) such that consumption rates measured off plants may yield unrealistic results (e.g. Corlay et al. 2007). Coccinellid **life tables** can be compiled from laboratory feeding data (e.g. Liu et al. 1997, Lopez et al. 2004) but their relevance to nature is low unless survival rates are known for different life stages in the field (e.g. Kirby & Ehler 1977). In general, **mortality** of coccinellid eggs and larval stages is likely much higher in the field than in the laboratory due to factors such as disease, intraguild predation, cannibalism, resource limitation, etc. Furthermore, their impact may be reduced by factors such as emigration, consumption of alternative prey, and climatic factors such as wind and rain that limit foraging activity. Since laboratory consumption assays are usually thought to generate upper limit estimates of impact potential, they complement direct field observations, but cannot substitute for them.

Latham and Mills (2009) **compared assays** of plum aphid, *Hyalopterus pruni*, consumption by various predators, both **in the laboratory and in the field**, and found significant discrepancies between the estimates generated. The authors reviewed possible sources of experimental error that could account for

their results, in particular several that could have led to overestimates of consumption in the field. They also pointed out the importance of distinguishing between the **amount of biomass killed versus** the amount **consumed**. The latter value was significantly less than the former when the predator was *Chrysopa nigricornis*, but only slightly less in the case of *Har. axyridis*, leading the authors to infer that consumption assays in laboratory arenas were vulnerable to generating underestimates of predator impact, in contrast to the conventional assumption.

10.3 INDIRECT IMPACTS

Foraging coccinellids may disturb aphid colonies directly by bumping into aphids, and indirectly by stimulating release of the **aphid alarm pheromone** β-farnesene (5.3.1.2), causing adjacent aphids to abandon their feeding sites and incur costs in terms of growth and development (Nelson 2007). Whereas factors such as wind and rain can forcibly dislodge aphids from plants (Cannon 1986, Mann et al. 1995), an **aphid's propensity to drop** voluntarily in response to disturbance varies considerably depending on its species, growth stage, and feeding habits. Some species use quick dropping as an escape response (5.3.2), e.g. *Acyrthosiphum pisum* (Francke et al. 2008) and *Macrosiphum euphorbiae* (J.P.M., unpublished), whereas others may feed with their stylets so deeply imbedded in plant material that quick release of the plant is difficult, e.g. *Aphis fabae* and *Toxoptera citricidus*. Among species that drop readily in response to disturbance, it has been estimated that more aphids may be killed by disturbance than are actually consumed by the foraging beetle (Roitberg et al. 1979, Day et al. 2006). Aphids exposed to alarm pheromone may be reluctant to climb back onto plants (Klingauf 1976) and crop cultivars expressing antibiosis may increase the propensity of aphids to drop (e.g. Gowling & van Emden 1994), thus synergizing the impact of foraging coccinellids. Aphids dislodged from plants may succumb to the action of physical factors such as heat and desiccation on exposed soil (Roitberg & Myers 1979) or be consumed by other ground-dwelling predators before they can recover their host plant (Winder 1990, Losey & Denno 1998a). Thus, **laboratory measurements** of prey consumption rates **may** also **underestimate** various indirect impacts of coccinellids on prey populations in the field (McConnell & Kring 1990).

Historically, **classical biological control** programmes have often failed to adequately quantify the impact of released species following their establishment (Van Driesche & Hoddle 2000). Projects could be deemed successful on the basis of **indirect indicators** of economic benefit such as declining pest populations, reductions in pesticide usage and even diminishing numbers of public complaints about the pest. In actuality, such indirect measures can be driven by various factors other than the impact of specific natural enemies. Ideally, discrete populations of pest and predator should be monitored for several years at a variety of sites, including a series without coccinellid releases (e.g. Van Driesche et al. 1998). In **augmentation** programmes, some assumptions regarding the per capita impact of a coccinellid species are implicit since release rates and intervals must be selected. However, these are most often arrived at by trial and error rather than being interpolated from quantitative data (van Lenteren 2000).

10.4 TRADITIONAL APPROACHES TO THE STUDY OF PREDATION

Luck et al. (1988) outlined six general **techniques for evaluating the impact** of biological control agents in the field, pointing out the limitations of each and the merits of combining different tactics. (See also an earlier review, Hodek et al. 1972.) Of these traditional approaches, the most useful for assessing coccinellid impact have probably been **direct observation** and **exclusion**. Most coccinellid species are sufficiently conspicuous to make direct observation of their activities feasible, although this is not true in all cases. Whereas most aphidophagous species lay brightly coloured eggs in conspicuous clusters in the open, coccidophagous species typically lay eggs singly in concealed locations, making it more difficult to assess their reproductive activity in the field. van Emden (1963) developed a technique for re-visiting marked colonies of *Brevicoryne brassicae* to infer the intensity of mortality inflicted by different aphid natural enemies. A similar approach of re-iterative, non-destructive sampling was used by Michaud (1999) to infer the relative importance of various sources of mortality to *T. citricidus* colonies on citrus terminals.

Frazer and Gilbert (1976) maintained that all practical methods for **counting coccinellids in the field** substantially **underestimated** their **actual**

abundance. Actively foraging individuals are more likely to be observed and satiated individuals more likely to be resting in concealed locations and therefore overlooked (Frazer 1988). The efficacy of direct observation can also be greatly affected by the nature of the plant and the opportunities for concealment it offers, the time of day, prevailing weather conditions and any other factors affecting coccinellid activity. Different **sampling methods** may also give different results for different species and life stages within the same crop and the most efficient methods may vary between crops (Elliott & Michels 1997, Michels et al. 1997). Thus, efforts to sample coccinellids must be carefully tailored to suit a particular situation and take into account the structure of the prey-bearing plant, its stage of development, and differences in detectability among life stages. Since coccinellids are diurnal (5.4.1.1), nocturnal observations are not required to detect them, although they may be needed to determine the full range of predators contributing to prey mortality (Pfannenstiel & Yeargan 2002).

10.4.1 Selective exclusion

Selective exclusion is the method most often used for measuring coccinellid impact under field conditions, likely because many of their prey are comparatively sessile and thus well suited for field cage studies. It is often possible to select a mesh size to permit the selective entry or exclusion of specific natural enemies. Sticky barriers can also be judiciously applied to selectively exclude ants or other non-flying arthropods. Xiao and Fadamiro (2010) used a combination of barriers and exclusion cages to attribute mortality of citrus leafminer, *Phyllocnistis citrella*, in mandarin orange groves proportionally among various parasitoids and predators, including *Har. axyridis*. Exclusion studies can produce convincing data (e.g. Morris 1992), especially when one predatory species is clearly dominant, although data can be more ambiguous when multiple predator species are involved (Chambers et al. 1983, Hopper et al. 1995). Exclusion cages have often been successfully used on aphid colonies to generate estimates of coccinellid impact in situations where they are the dominant predators (Cherry & Dowell 1979, Kring et al. 1985, Liao et al. 1985, Rice & Wilde 1988, Nechols & Harvey 1998, Lee et al. 2005). For example, Wells et al. (1999) used a **combination of partial and total exclusion** treatments to demonstrate the key role of *Scymnus* sp. in suppressing *Aphis gossypii* populations on cotton in the coastal plain of Georgia, USA. The study conducted by Winder (1990) used polyethylene barriers to exclude generalist epigeal predators, primarily carabids and linyphiid spiders, and estimate their contribution to mortality of *S. avenae* on wheat plants, separately from aphid-specific predators. Innovative traps were deployed to assess the rates at which dislodged aphids regained plants and demonstrate that polyphagous ground predators reduced their survival.

Figure 10.1 Exclusion cages mounted in an alfalfa field to determine impact of coccinellid species feeding on pea aphids, *Acyrthosiphon pisum* (photo: Edward Evans). (See colour plate.)

10.4.2 Field cages

Exclusion studies in field crops typically require the installation of large cages anchored to the ground and enclosing multiple plants (Fig. 10.1). In this manner, Costamagna and Landis (2006, 2007) used **direct observations in field cages** to quantify collective predation impact by coccinellid species on *Aphis glycines* relative to other predators in soybean. Selective exclusion of large coccinellid species (primarily *Har. axyridis* and *C. septempunctata* revealed that they exerted a disproportionate amount of pest suppression (Costamagna et al. 2008). On trees or shrubs, it may be sufficient to enclose infested branches or terminals. Michaud (2004) used exclusion cages to monitor the survival of immature Asian citrus psyllid, *Diaphorina citri*, on expanding citrus terminals (Fig. 10.2). This technique was effective in quantifying collective

Figure 10.2 An exclusion cage designed for assessing impact of coccinellids and other natural enemies on aphids and psyllids infesting expanding grapefruit terminals. The cage is constructed from a clear plastic 2-litre soda bottle with mesh sleeves attached to either end with silicone and fastened to the branch with a length of wire secured to the inside of the bottle with packing tape. The sleeve mesh can be selected to permit the passage of particular insects (in this case, parasitoids), while excluding larger ones (coccinellids). The basal sleeve is attached tightly around the branch with a zip tie; the terminal sleeve is sufficiently long to permit unimpeded growth of the shoot for 2–3 weeks and can be untied to permit periodic access and counting of insects (photo: J.P. Michaud). (See colour plate.)

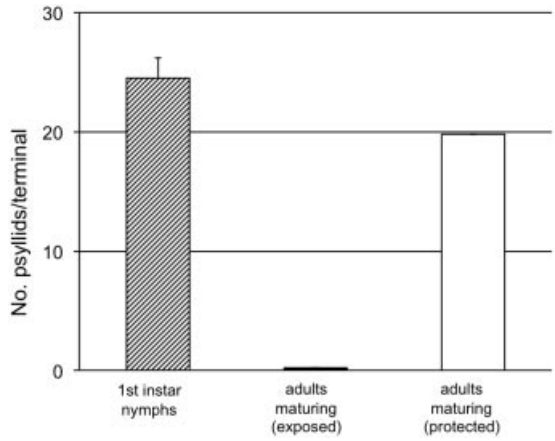

Figure 10.3 Mean numbers of first instar psyllid nymphs per grapefruit terminal and the numbers maturing to adulthood with and without exclusion of four coccinellid species (summarized from Michaud 2004).

predator impact caused primarily by four coccinellid species separately from that of the introduced parasitoid *Tamarixia radiata*. Although the various sources of juvenile psyllid mortality could not be fully partitioned, the comparative importance of ladybird species could be inferred from their relative abundance and the fact that their exclusion improved psyllid survival by a factor of 140 (Fig. 10.3). In addition, it was determined that parasitoid immatures suffered >95% mortality within their psyllid hosts as a consequence of intraguild predation by the coccinellids. Subsequently, Qureshi and Stansly (2009) applied a similar approach in south Florida and obtained similar results.

All forms of **enclosure modify** the **physical environment**, typically increasing temperature, reducing illumination and air flow, and providing some degree of unnatural protection from physical stresses such as wind and rain. This is sometimes addressed by including a **partial exclusion** treatment to reproduce the environmental effects of the cage while permitting the entry of natural enemies. However, physical enclosure, whether partial or complete, often results in significant changes in plant growth form, creating the potential for indirect effects on the prey population. The use of exclusion cages on aphids may also result in the **trapping of alatae** and thus lead to artificially elevated populations through forced re-infestation of the same plants (Lee et al. 2005). Studies that exclude coccinellids from plants naturally infested with their prey are always preferable to those where infestations are artificially established, although this is sometimes a necessity. **Artificial infestations** entail two significant risks; the plants infested manually may vary in quality in ways not evident to the researcher, and laboratory-raised insects may be unduly stressed when they are moved from protected rearing environments to the field. Exclusion of coccinellids can also be accomplished with insecticide treatments (e.g. Annecke et al. 1969) but these are often difficult to implement without affecting pest populations.

10.4.3 Cage inclusion

Cage inclusion, as opposed to exclusion, where predators are added to cages, has also been used to quantify coccinellid impact in the field. For example, Cudjoe

et al. (1992) used selective cage inclusion and exclusion to quantify the relative impacts of the indigenous predator *Parexochomus troberti* and the exotic parasitoid *Anagyrus lopezi* on colonies of the cassava mealybug, *Phenacoccus manihoti*, in Ghana. Butin et al. (2003) demonstrated the proportionally greater impact on *Adelges tsugae* of *Scymnus ningshanensis* compared to *Sasajiscymnus (= Pseudoscymnus) tsugae* by caging colonies of the adelgid in the field with and without each of the two predators. Such studies generate inferences on potential coccinellid impact derived from relative comparisons of species-specific **functional and numerical responses** in a closed system, but they have few advantages over laboratory studies because they ignore searching efficiency and exclude important factors such as emigration and intraguild predation. However, they may be useful for assessing impact potential when the provision of prey is difficult to achieve in the laboratory, or when particular natural enemy interactions are of interest. For example, Straub and Snyder (2006) infested potato plants with *M. persicae* in field cages and then inoculated them with various predator combinations.

10.4.4 Manual removal

One alternative to exclusion cages is the manual removal of immature coccinellid life stages from a fraction of infested host plants to compare the subsequent survival and growth of prey colonies with and without predator removal. This technique was used effectively by Berthiaume et al. (2000) to demonstrate the impact of *Anatis mali* on *Mindarus abietinus* infesting balsam fir (*Abies balsamea*) in Christmas tree plantations. The feasibility of this approach may depend to some degree on host plant architecture and the ease of discovery of egg masses, but it has the advantage of high selectivity and not interfering with secondary sources of prey mortality. However, manual removal of immature stages will underestimate impact whenever predation by transient adults is significant. Rates of coccinellid recruitment to various host plant-prey combinations can also be assessed using **sentinel prey** on potted plants in a trap-line approach. A useful method for sampling potential parasites and predators is placing aphid colonies in the field and periodically collecting all natural enemies that arrive to attack them. Natural enemies of *Aphis fabae* were thus sampled in Germany (Müller 1966) and of *Schizaphis graminum* in the Czech Republic (Starý & Gonzalez 1992). Although impact is not quantified with this technique, it is effective for assessing functional responses of coccinellid species and measuring the **speed of prey patch discovery**. Michaud and Belliure (2000) infested young citrus trees with various numbers of *T. citricidus* and revisited them daily for a period of weeks to tally the growth trajectories of aphid colonies and rates of natural enemy recruitment. Similarly, Noma et al. (2005) placed pots of wheat infested with *D. noxia* in a series of field locations for 2–7 day periods and then collected them to assess predation and parasitism. Although this approach was more effective for assessing parasitism, predatory larvae were also recovered. This approach could be used to estimate rates of prey discovery by coccinellids, but would more require frequent observations to detect transient visitation, or the use of trap plants with sufficiently large numbers of prey to stimulate oviposition by adult females.

10.5 STATISTICAL APPROACHES

Chambers and Aikman (1988) discussed the advantages and limitations of four different **analytical procedures** for estimating the extent to which predation was responsible for observed changes in aphid densities. Various regression techniques are often used to analyze patterns of coccinellid abundance in relation to that of their prey, both spatially and temporally (e.g. Rautapaa 1972, Elliott et al. 1999, Kriz et al. 2006). **Regression models** that incorporate plant growth stage and counts of predators and prey can be used to forecast whether the pest's economic injury threshold will be surpassed based on a current data sample from the field. This approach can be of practical value for guiding management decisions and providing indications of the collective impact of coccinellid assemblages on their prey at particular stages of crop growth or season. The limitation of such correlational analyses is that causal relationships are not necessarily implied and no information is generated with respect to underlying mechanisms.

It is possible to construct **life tables** for herbivores that partition mortality by life stage and then use key factor analysis to quantify the proportion of variance in abundance that is attributable to various submortalities, including coccinellid predation (Manly 1990, Dent 1997). This approach has not been used extensively to assess coccinellid impact, possibly because

actual rates of prey removal can be difficult to assess and attribute to particular species, and without reliable estimates in this regard, the approach has little advantage over other regression techniques. Podoler et al. (1979) constructed **partial life tables** for black olive scale, *Saissetia oleae*, that revealed *Chil. bipustulatus* to be a key mortality factor for immature stages. Similarly, Hamilton et al. (1987) used a life table approach to infer significant impact of coccinellid predation on numbers of *S. graminum* on grain sorghum in central Missouri, USA. Life tables constructed for cereal leaf beetle, *Oulema melanopus*, (Shade et al. 1970) and citrus black fly, *Aleurocanthus woglumi*, (Cherry & Dowell 1979) also implicated coccinellid predation as key mortality factors for these pests.

10.6 RESOLVING COCCINELLID IMPACT WITHIN COMPLEX COMMUNITIES

Since coccinellids may serve as either **intraguild predators** or intraguild prey (Chapter 7), interactions between coccinellid species, and between coccinellids and other natural enemy taxa, may serve to either enhance or diminish herbivore suppression. For example, coccinellids often consume aphid parasitoids in their larval form (5.2.7) and this has been construed to impede, rather than assist, aphid control in systems where parasitism is inferred to be a key source of mortality (Colfer & Rosenheim 1995, Ferguson & Stiling 1996). In some cases, intraguild predation may have little or no effect on herbivore suppression (i.e. Snyder & Ives 2003) whereas in others, the role of coccinellids as key predators can more than counter their negative impact as intraguild predators of parasitoids (Michaud 2004, Gardiner & Landis 2007).

Tamaki and Weeks (1972) showed that the addition of *C. transversoguttata* improved control of *M. persicae* by predatory Hemiptera (*Geocoris bullatus* and *Nabis americoferus*) on caged sugar beet plants in a greenhouse, one of the first illustrations of how coccinellid predation can complement other biotic sources of mortality (Fig. 10.4). It has been pointed out that coccinellids are mobile predators that respond well to prey aggregations, whereas more **sedentary, resident predators** such as earwigs (*Forficula* spp.) are more likely to respond to aphid colonies early in the establishment phase when the proportional impact of predation is much higher (Piñol et al. 2009). The latter authors used simulation modelling to explore the interactions of significant parameters affecting aphid population dynamics in a citrus grove that could give rise to the patterns of abundance observed.

10.6.1 Multi-species combinations

Many recent studies have sought to determine whether other multi-species combinations including one or more coccinellids either facilitate or impede the suppression of prey populations, and have yielded a wide range of results. Negative effects can arise via intraguild predation or foraging interference, whereas positive effects can be generated by spatial or temporal **niche partitioning** among predatory species. These include the differential use of prey life stages, preferences for foraging on different plant parts, or variation in diurnal or seasonal activity. Losey and Denno (1998b) used a combination of laboratory and field studies to show a synergistic impact of *C. septempunctata* and the carabid *Harpalus pennsylvanicus* on pea aphids that was double the sum of their individual predation impacts. Similarly, Cardinale et al. (2003) demonstrated a 'super-additive' effect of multiple natural enemies (*Har. axyridis*, *Nabis* sp. and *Aphidius ervi*) on populations of *A. pisum* caged on alfalfa. In contrast, Cardinale et al. (2006) inferred interference interactions among adult *Har. axyridis*, *C. septempunctata* and *Col. maculata* based on a lower level of aphid suppression when all three were present together than would be expected based on the sum of their effects when foraging alone. Straub and Snyder (2008) manipulated natural enemy diversity (*Aphidius matricariae*, *C. septempunctata*, *Hip. convergens* and *Nabis* sp.) in field cages of potatoes and collards infested with *M. persicae*. Their findings suggested that the identity of species within predator communities was more important for aphid control than species richness, per se, and that the coccinellid component (*C. septempunctata* and *C. transversoguttata*) had the greatest impact on aphid numbers. The authors concluded that increased natural enemy diversity **strengthened aphid suppression** because intraspecific competition was stronger than interspecific competition among predator species due to resource partitioning. Snyder (2009) reviewed predator diversity studies that included one or more coccinellid species and concluded that positive effects of increas-

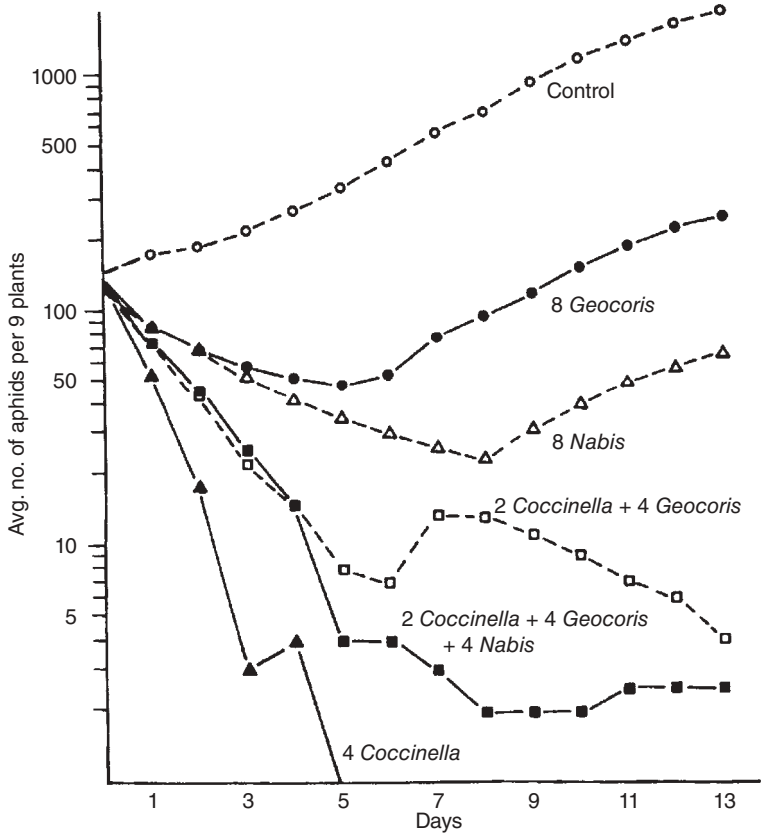

Figure 10.4 Effectiveness of three predators, used alone or in combination, against populations of the green peach aphid (after Tamaki and Weeks 1972).

ing predator diversity were generally more common than negative ones. Thus, coccinellids are emerging as potentially key predators that often act within a larger guild of natural enemies, the full complement of which may be required to provide acceptable levels of pest suppression.

10.6.2 Demographic-based estimates

In an effort to resolve the issue of aphid reproduction confounding the rate of removal by predators, Latham and Mills (2010) compared what they termed 'demographic-based' versus 'observation-based' estimates of **mealy plum aphid predation** in California. The latter represents a truly novel approach to assessing predator impact, albeit one requiring a large investment in baseline observations. Periodic counts of aphids and predators throughout the season were used to estimate aphid abundance and predator densities, by species and life stage, that were then expressed in terms of leaf area on particular dates. Consumption rates were estimated for larval and adult predators of each species based on direct observation. Since the predators were diurnal (with the exception of adult *Chrysopa nigricornis*), their period of activity was assumed to correspond to actual day length. The proportion of time spent feeding was estimated from a series of point observations in the field, and the time required to consume an aphid estimated from laboratory observations. Collectively, this information was used to generate an estimate of aphid predation per unit of leaf area

on all sampling dates that could be compared to the demographic estimate of aphid population dynamics. The latter was obtained from a simple model of aphid population growth using estimates of intrinsic rate of increase obtained from field cages from which predators were excluded. Despite the numerous sources of error inherent in this approach (thoroughly enumerated by the authors), the study confirmed an intuitive inference; the guild of predators (including *Har. axyridis*) were neither numerous enough nor voracious enough to effect directional changes in aphid population dynamics, nor suppress the aphids below economically damaging levels, although they were able to influence the rate of population growth or decline at some points in the cycle.

Our knowledge of coccinellid nutritional ecology has been greatly advanced by laboratory feeding studies that have tested the suitability of prey species for development and reproduction (5.2). However, our understanding of why coccinellids are and are not effective in suppressing prey populations would benefit from more complete knowledge of the full range of foods that species actually utilize under natural conditions (5.2.5, 5.2.9). Direct observation yields only fragmentary information in this regard, and one alternative is to dissect specimens and examine gut contents (Ricci et al. 1983, Mendel et al. 1985, Triltsch 1997; 5.2.1) or attempt to identify prey fragments in frass (Davidson & Evans 2010). Fortunately, newly developed molecular techniques have considerably enhanced our ability to identify prey types within gut contents beyond simple visual recognition of body parts. Since these techniques tend to yield only qualitative information, their value in aiding determinations of coccinellid impact on herbivores lies in complementing other techniques that yield more quantitative measures.

10.7 POST-MORTEM ANALYSIS OF PREDATION

Techniques for elucidating predator–prey interactions in the field have received considerable attention in recent years (reviewed by Symondson 2002, Sheppard & Harwood 2005, Harwood & Greenstone 2008, Weber & Lundgren 2009). These reviews have, to some extent, superseded earlier assessments of post-mortem techniques (e.g. Kiritani & Dempster 1973, Boreham & Ohiagu 1978, Washino & Tempelis 1983, Sunderland 1988, 1996, Greenstone 1996) due to the rapidly advancing nature of molecular biology and biochemistry. However, the over-riding consensus has been that post-mortem analyses offer valuable insights, and sometimes advantages, for studying the ecology and impact of predator communities (including coccinellids) in complex environments. Despite a proliferation in the use of molecular approaches in ecology, the extent to which predation can be 'quantified' by such techniques remains controversial.

10.7.1 Antibody-based analysis of predation

Vertebrate antibodies have been used since the 1940s to qualify the presence (or absence) of target prey in predator guts (Brooke & Proske 1946). Brooke and Proske (1946) developed a **polyclonal antibody** in rabbits to identify trophic relationships between *Anopheles quadrimaculatus* and its natural enemies using a **precipitin test**. Although the specificity of the assay was too general to enable species-level elucidation of trophic relationships (the antiserum was reported to be 'order-specific'), this study provided the groundwork for the subsequent adoption of serology as the tool of choice for predator–prey research throughout the latter half of the twentieth century. Subsequent studies documented predation upon a diverse spectrum of prey with specificity reported at the level of order, family, genus and species (see Greenstone 1996 for a review). However, despite the plethora of studies diagnosing predation in the field using polyclonal antisera, it is surprising how few have focused on food relations of the Coccinellidae. In contrast, considerable attention has been directed towards examining ecological interactions between the Carabidae, Araneae and Hemiptera and their prey (see Boreham & Ohiagu 1978, Sunderland 1988, 1996, Greenstone 1996). **Enzyme-linked immunosorbent assay (ELISA)** technology (Fig. 10.5) relies of the binding of prey-specific antibodies to target antigen and subsequent visualization of the reaction using a spectrophotometer to signify the presence (or absence) of prey material within each ELISA plate well. The ELISA format offers a rapid, cost-effective and widely adopted approach for food web studies.

Early serological laboratory experiments illustrated their utility for evaluating coccinellid predation (Pettersson 1972), and Whalon and Parker (1978) demonstrated that a **polyclonal antiserum** developed in

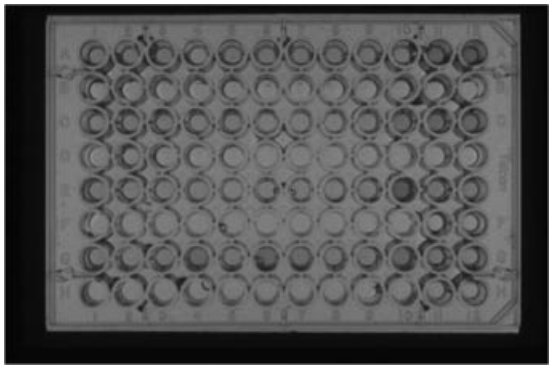

Figure 10.5 A 96-well microtitre plate following enzyme-linked immunosorbent assay. The amount of antigen–antibody binding is signified by the intensity of the reaction; absorbance is typically monitored by spectrophotometer to infer qualitative (and occasionally semi-quantitative) assessments of predation (photo: James Harwood). (See colour plate.)

rabbits could detect *Lygus lineolaris* proteins in *Col. maculata* for 42 hours after consumption. However, it was the study of Vickerman and Sunderland (1975) that provided the first insight into coccinellid feeding behaviour in the field. Traditionally, the adoption of gut dissection relied on the successful identification of indigestible remains of prey in coccinellid guts (Forbes 1883, Eastop & Pope 1969, Ricci 1986, Ricci & Ponti 2005, Ricci et al. 2005) or faecal samples (Honěk 1986), but Vickerman and Sunderland (1975) screened over 200 species of predators, including four coccinellids, collected by sweep netting and vacuum sampling in spring barley fields in the United Kingdom. High levels of predation upon *Metopolophium festucae* and/or *Sitobion avenae* were documented using a precipitin test (Fig. 10.6). The use of antisera to confirm consumption of specific prey types is thus a potentially useful tool for complementing direct observations of predation in studies that aim to assess coccinellid

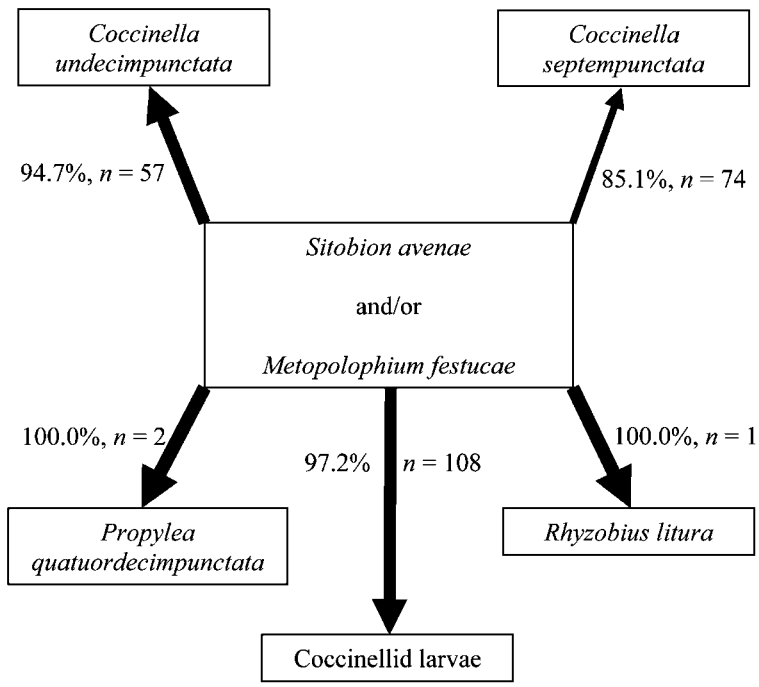

Figure 10.6 Adult coccinellid predation on the cereal aphids *Sitobion avenae* and *Metopolophium festucae* (summarized from Vickerman & Sunderland 1975). Size of arrow corresponds to strength of trophic pathway and the numeric value represents the percentage of predators screening positive for target prey.

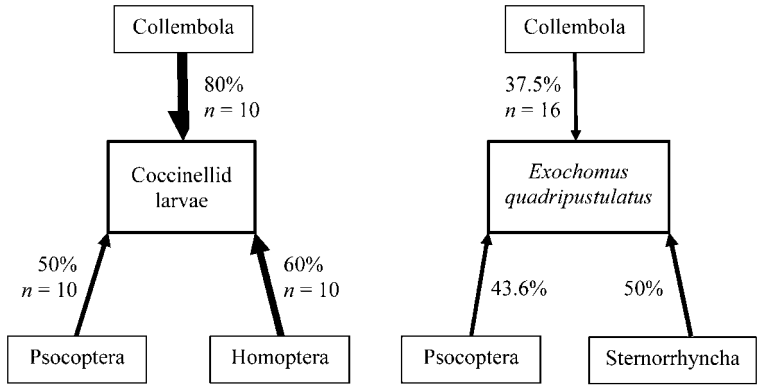

Figure 10.7 Adult and larval coccinellid predation on Homoptera, Collembola and Psocoptera (summarized from Turner 1984). Size of arrow corresponds to strength of trophic pathway and the numeric value represents the percentage of predators screening positive for target prey.

impact relative to other generalist predators, especially when these may be species that are nocturnal, cryptic or otherwise difficult to observe directly. For example, where estimates of predator abundance are used in models of herbivore suppression, these could be adjusted by an amount proportional to the percentage of each species testing positive for the focal prey.

Using a **modified Ouchterlony plate approach** (after Pickavance 1970), Turner (1984) was able to distinguish the consumption of insect prey from three orders (Psocoptera, Sternorrhyncha and Collembola) from the consumption of predatory arthropods from eight orders, including three coccinellid species (Fig. 10.7). In the same year, Leathwick and Winterbourn (1984) used the Ouchterlony approach to develop an antiserum targeting *Acyrthosiphon kondoi* and *A. pisum* from alfalfa in New Zealand, and reported that 70% of *C. undecimpunctata* tested positive for these aphids (n = 73). Although no cross reactivity was observed with *Sidnia kinbergi*, *Calocoris norvegicus* or *Philaenus spumarius*, there was a weak reaction to another hemipteran, *Nysius huttoni*. The characterization process of polyclonal and monoclonal antibodies typically requires extensive screening of the developed antibody against non-target organisms due to the possibility for cross-reactivity (whereby the 'pest-specific' antibody binds to the antigens of other invertebrates). Thus, the possibility of overestimating predation with this technique exists, especially when few non-target species are screened during characterization.

Hagley and Allen (1990) adopted an **immunoelectro-osmophoresis** approach on cellulose acetate membranes to examine the food relations of generalist predators of the green apple aphid, *Aphis pomi*, in apple orchards in Ontario. Although no cross-reactivity testing was reported and replication of field-collected specimens was low (n = 168), evidence was obtained of the importance of coccinellids as predators of the green apple aphid relative to other specialist and generalist aphid predators (Fig. 10.8). Such data can complement more conventional field-based studies designed to assess coccinellid effectiveness in pest suppression.

The principal limitation of polyclonal antisera has been an inherent lack of specificity that derives from their mode of production (simple immunization of a vertebrate animal, typically a rabbit, to produce polyclonal antisera targeting determinants common to many organisms). Therefore, potential 'false-positive' reactions generate interpretative errors that can lead to an overestimation of interaction pathway strength. Occasionally, cross-reacting material can be reduced through purification. For example, affinity chromatography can employ cross-reacting antibodies to bind the target antigen so that non-reacting material can be eluted from the column (Cuatrecasas 1970, Schoof et al. 1986). However, levels of sensitivity are sometimes compromised. Although species-specific (Dempster 1971, Pettersson 1972, Sunderland & Sutton 1980, Nemoto et al. 1985) and even stage-specific (Ragsdale

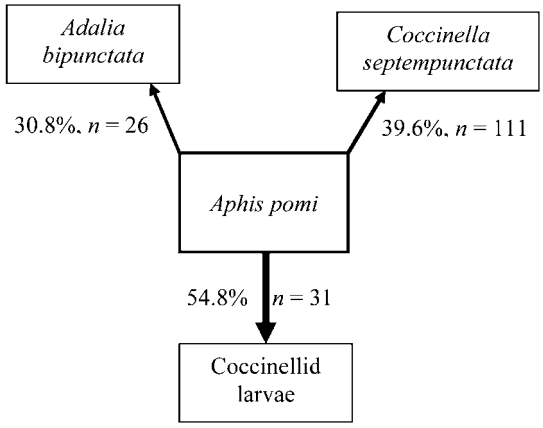

Figure 10.8 Adult (*Adalia bipunctata* and *Coccinella septempunctata*) and larval coccinellid predation on *Aphis pomi* in Ontario apple orchards (summarized from Hagley & Allen 1990). Size of arrow corresponds to strength of trophic pathway and the numeric value represents the percentage of predators screening positive for target prey.

et al. 1981) diagnostic systems have been developed using a polyclonal approach, the problems with specificity led to the proliferation of monoclonal antibody technology in food web biology during the 1990s. This approach relies on the immunization of a vertebrate animal, typically a mouse, with target material, and the subsequent extraction of lymphocytes that produce a hybridoma following fusion with myeloma cells. The hybridomas are cloned and single cells can be cultured *in vitro* to produce monoclonal antibodies of desired specificity (Köhler & Milstein 1975). Monoclonal antibodies have been used to target pests such as *Helicoverpa zea*, *Lygus hesperus*, *Bemisia tabaci*, *Pectinophora gossypiella*, *Heliothis virescens*, the slugs *Deroceras reticulatum*, *Arion hortensis* and *Tandonia budapestensis*, and aphids (reviewed by Greenstone 1996, Symondson 2002, Sheppard & Harwood 2005, Weber & Lundgren 2009). However, high initial development costs (Fournier et al. 2008, Harwood & Greenstone 2008) have driven monoclonal antibody-based research in the direction of pest management assessment and the development of pest-specific monoclonals. Many predators, including most coccinellids, have the ability to subsist on alternative prey types prior to colonizing pest-infested plants, with potentially important consequences for their physiological condition and the timing of their immigration into crop habitats. Thus, the ability to recognize such consumption through the use of specific monoclonal antibodies could supplement our understanding of coccinellid effectiveness, or lack thereof, in particular contexts.

Large-scale field trials have been implemented to identify patterns of food web processes and have, in some cases, purported to quantify the role of natural enemies in biological control. In the largest study of its kind, Hagler and Naranjo (2005) utilized a whitefly-specific ELISA to assay 32,262 field-collected predators and assess the effect of insecticide regimes on the predator complex associated with *Bemisia tabaci*. It is perhaps surprising that such studies have yet to address role of coccinellids in agroecosystems, given the prior development of an aphid-specific monoclonal antibody.

Early-season predation has long been considered important in pest regulation by generalist predators (Settle et al. 1996, Landis & van der Werf 1997). Using an aphid-specific monoclonal antibody, Harwood et al. (2004) revealed that spider communities can impact aphids before their populations increase. By correlating the falling rates of aphids (and alternative prey) into webs with their consumption by the spiders, disproportionately high rates of feeding on *Sitobion avenae* were observed precisely when numbers were at their lowest. Despite the obvious power of monoclonal antibody technology, and its ability to yield somewhat quantitative inferences with respect to prey consumption, it has not yet been employed to any significant extent in studies addressing the impact of coccinellids on their prey. Hagler and Naranjo (1994) used monoclonal antibodies for *B. tabaci* and *P. gossypiella* to identify these species in the guts of 613 *Hip. convergens* adults, the only study to date that has used monoclonals to study coccinellid prey consumption. Whereas only 1.0% of *Hip. convergens* screened positive for eggs of pink bollworm, 38.0% contained whitefly, albeit with significant variation evident throughout the season, probably due to temporal variation in the abundance of whitefly and other (potentially preferred) prey.

10.7.2 Protein marking for predation analysis

Immunolabelling has been used for many years as a tool for marking insects and tracking dispersal (Hagler & Jackson 2001), but has recently been used to analyze predation (e.g. Hagler & Naranjo 2004, Hagler 2006).

This approach employs immunoglobulin labels that are applied either internally or externally to prey prior to their consumption, and then detected and visualized within the gut of a predator. A recent field assessment comparing the efficacy of protein labelling and monoclonal antibody-based ELISA (Mansfield et al. 2008) suggested superior levels of detection in the former. An extended period of detection was attributed to a longer persistence of rabbit protein markers in the predators (*Dicranolaius bellulus*, *Hip. variegata*, *C. leonina transversalis* and *Cheiracanthium* spp.) compared to antigen from *Helicoverpa armigera*. Post-consumption detection periods are critical in gut content analyses and can vary from a few hours to more than a week. Thus, immunolabelling incurs a compromise between the increased likelihood of detection over an extended period and the possibility of overestimating feeding events when prey remain detectable in guts for longer periods.

The frequency with which adult *Hip. convergens* fed on *L. hesperus*, *P. gossypiella* and *Trichoplusia ni* eggs was elucidated under varying light regimes in a field-cage system where the three pests were released after immunolabelling with rabbit immunoglobulin G (*T. ni*) or chicken immunoglobulin G (*L. hesperus*) (Hagler 2006). Consumption of *P. gossypiella* was detected with a *P. gossypiella* egg-specific monoclonal antibody. Predation on *T. ni* in the field occurred with the greatest frequency, with the peak proportion testing positive (~16%) between 0700–1300 hours, and lower frequencies of predation during the period before and after dusk (~11%) (3 hours light followed by 3 hours dark) and during the night (~11%), with little evidence of predation during the period before and immediately after dawn (3 hours dark followed by 3 hours light). Very low predation on *L. hesperus* and *P. gossypiella* was documented (<2% positive for prey proteins). Therefore, this technique can be used to discern not only **quantitative patterns** of coccinellid prey selection when multiple species are present, but also **diurnal cycles** of prey consumption. Although the low cost and ease of application of protein labels is appealing, the requirement for prey labelling and subsequent release into field plots for consumption by predators limits its utility and renders it impractical for assessing the consumption of prey that are naturally distributed in open-field conditions.

10.7.3 Detection of prey-specific DNA

Buoyed by increased access to molecular laboratories and equipment, ecologists have increasingly utilized **DNA-based gut content analysis** to examine the feeding relationships of terrestrial arthropods. This approach relies on the ability to visualize unique fragments of DNA in predator guts, typically the mitochondrial cytochrome oxidase I (COI) gene (Fig. 10.9),

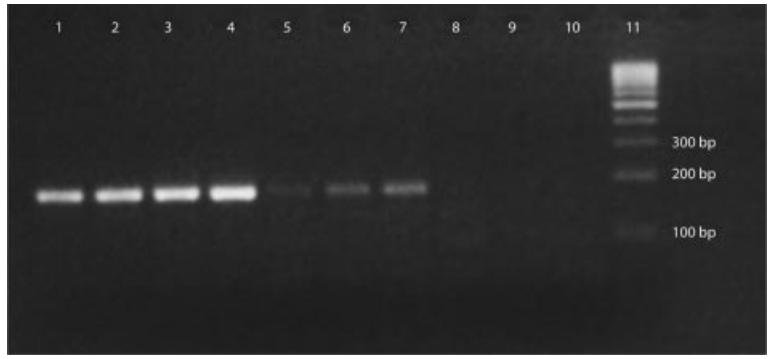

Figure 10.9 Agarose gel electrophoresis of PCR-amplified DNA. The presence of bands signifies the amplification of 'prey' DNA in predator samples, thus indicating the presence of prey in predator guts. This data can be used to identify the presence (or absence) of prey material and therefore infer the frequency of predation events (and thus impact of predators) in the field. Lanes 1–2, positive controls (target prey DNA) to ensure visualization of DNA on gel; lanes 3–7 field-collected predators screening positive for prey DNA; lanes 8–9, starved predators screening negative for prey DNA; lane 10, negative control; lane 11, 100 bp ladder to separate fragment sizes. Photograph courtesy of Eric G. Chapman (University of Kentucky).

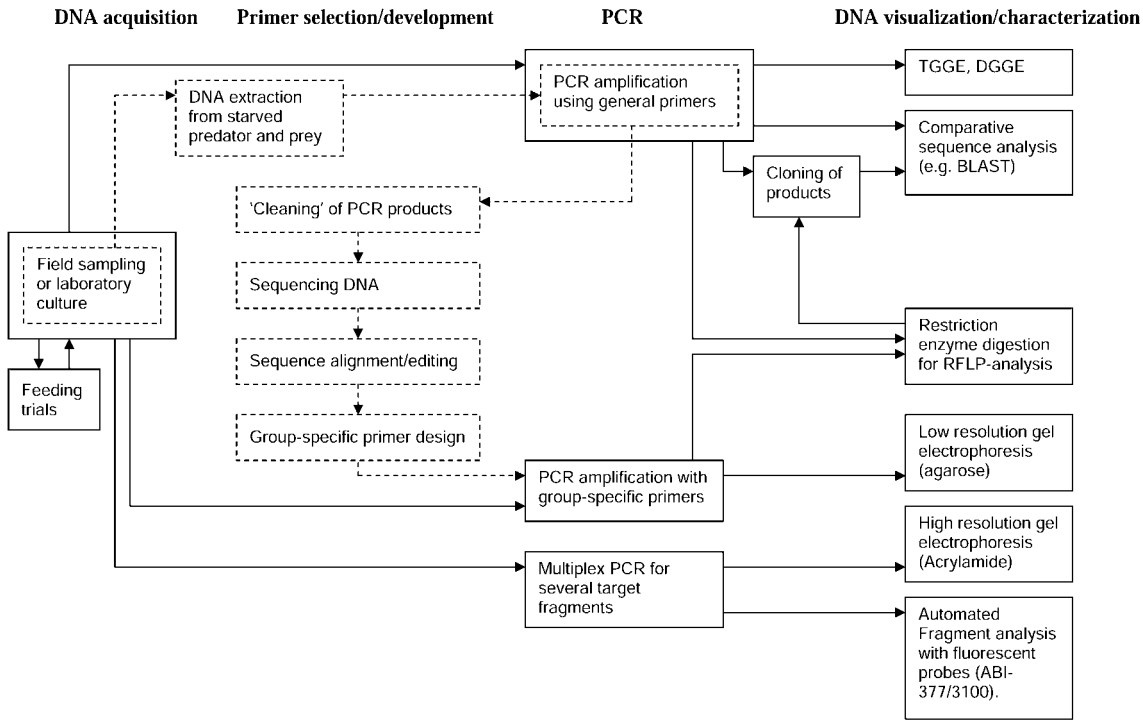

Figure 10.10 Decision flow diagram for the development of a gut-content analysis approach for detection of predation by PCR (from Sheppard & Harwood (2005), courtesy of Wiley-Blackwell).

although other targets have also been used. Traditionally, the approach was limited by an inability to detect DNA beyond a **short period after ingestion** (Asahida et al. 1997), thus reducing the likelihood of detecting foraging events under natural conditions. However, a reduction in target fragment size has enabled extended detection in both gut contents (Zaidi et al. 1999, Chen et al. 2000) and faecal material (Kohn & Wayne 1997, Farrell et al. 2000). For example, Zaidi and colleagues (1999) demonstrated that *Culex quinquefasciatus* DNA could be reliably detected in guts of *Pterostichus melanarius* up to 28 hours after digestion. Although the period of delectability is shorter than that of some monoclonal antibody-based assays, shorter detection periods may be advantageous when only the most recent foraging behaviour is of interest (Sheppard & Harwood 2005).

Although the details are beyond the scope of this chapter, DNA-based techniques utilize a series of protocols that enable the acquisition of DNA, the design of molecular markers, the choice of a mechanism for amplifying target DNA, and the final visualization of results (Fig. 10.10). Although considerable care is required during the development and optimization of molecular detection systems, the relative ease of sequencing DNA and designing prey-specific primers has allowed cost-effective identification of a broad spectrum of food resources exploited by generalist predators. This advantage has been further enhanced by the **adoption of multiplex PCR** (Fig. 10.11) which amplifies prey DNA in a single reaction (e.g., Harper et al. 2005). The identification of diverse alternative prey is usually beyond the scope of monoclonal antibody-based analyses due to prohibitively high development costs, although these can be greatly offset in large-scale studies (e.g. Hagler & Naranjo 2005) due to the low per-sample screening cost of ELISA. Since PCR costs 15 to 20 times more than ELISA to screen each predator (Fournier et al. 2008, Harwood & Greenstone 2008), the trade-off between development cost

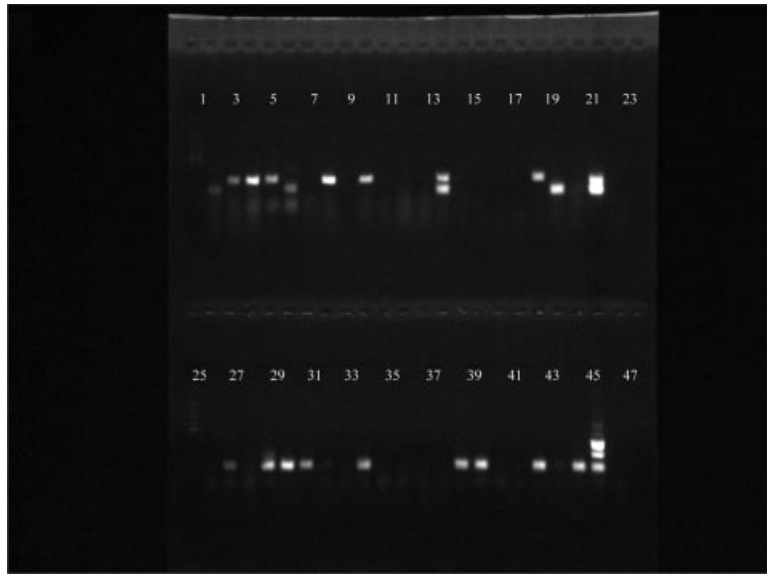

Figure 10.11 Agarose gel electrophoresis of multiplex-PCR-amplified DNA. Fluorescently labelled PCR primers allow the simultaneous amplification of prey DNA from within predator guts based upon variation in target fragment (e.g. lanes 5 and 6). Such technology even allows for the simultaneous amplification of multiple prey within a single sample (e.g. lane 14). Photograph courtesy of R. Andrew King (Cardiff University).

and screening cost influences the relative suitability of these two techniques for particular applications.

In the most comprehensive molecular gut-content study to date, Fournier et al. (2008) screened over 1200 predators (including *Hip. convergens* and *Har. axyridis*) by PCR against species-specific primers of the glassy-winged sharpshooter *Homalodisca vitripennis*. Although laboratory feeding trials revealed 100% successful detection of sharpshooter DNA for 8 hours after consumption, no field-collected coccinellids tested positive for the target prey, in contrast to spiders (18%), hemipterans (14%), mantids (13%), ants (12%) and neuropterans (10%). Similarly, very low levels of coccinellid predation on *Ostrinia nubilalis* were reported by Hoogendoorn and Heimpel (2002). Targeting the ITS1/18S region of nuclear DNA, the decline in detectability of the DNA of prey in predator guts followed a negative quadratic function over 12 hours and just 1% of field-collected *Col. maculata* and *Har. axyridis* were positive for these prey in a corn agroecosystem (Fig. 10.12).

Recently, Chacón et al. (2008) examined the food relations of four coccinellids along with *Chrysoperla carnea* and *Orius insidiosus* that were associated with the soybean aphid, *Aphis glycines*. Molecular gut-content analyses have providing valuable insights into the role of particular natural enemies in controlling this pest (Harwood et al. 2007, 2009, Chacón et al. 2008). For example, greater than 90% of *O. insidiosus*, a species previously reported to be an important predator of this aphid (Harwood et al. 2007, 2009), screened positive for soybean aphids, in contrast with previous reports that had used different molecular markers. Similarly, Zhang et al. (2007a) qualified *Bemisia tabaci* predation rates by a complex of predators, including Coccinellidae, in Chinese cotton agroecosystems (Fig. 10.12). We expect that molecular gut-content analyses will play an increasingly important role in evaluating food web dynamics and will serve to complement quantitative population monitoring and traditional field experiments in various ways to significantly advance our understanding of the relative

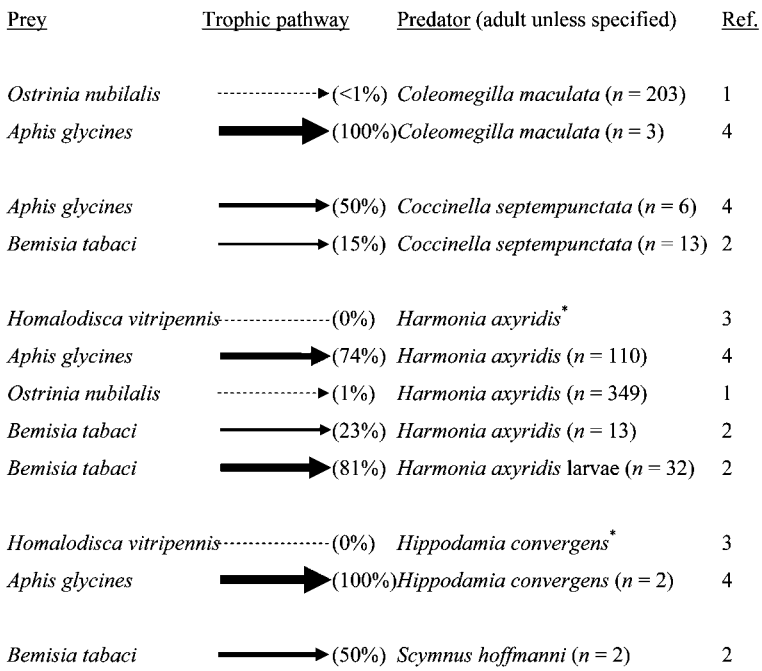

Figure 10.12 Frequency of coccinellid predation in the field, revealed by molecular gut content analysis summarized from: 1, Hoogendoorn and Heimpel (2002); 2, Zhang et al. (2007a); 3, Fournier et al. (2008); 4, Chacón et al. (2008). Size of arrow corresponds to strength of trophic pathway and the numeric value represents the percentage of predators screening positive for target prey. *, exact sample size unspecified.

effectiveness of coccinellids as biological control agents in various ecological contexts.

10.8 CONCLUSIONS

Laboratory feeding studies remain a useful approach for determining prey acceptability and suitability for coccinellids and consumption assays produce estimates of potential impact, but the strength of laboratory studies usually lies in providing a base for interpreting field observations. Traditional methods of assessing the impact of coccinellids on focal prey species continue to be invaluable and the best insights are usually obtained through a combination of approaches selected for their suitability for particular prey–plant systems, as each has inherent limitations. Although molecular analyses have advanced our understanding of the trophic relations of predatory arthropods, including the Coccinellidae, they have yet to yield data that accurately **quantify predation** in the field. The better studies have linked population surveys with molecular gut-content analysis to strengthen inferences of trophic relationships and mechanisms of prey preference, as opposed to simply generating binary data on the presence/absence of target prey in predator guts. Semi-quantitative estimates of predation have been made using gut-content analysis by estimating the concentration of recognizable prey proteins in gut samples by ELISA (e.g. Symondson & Liddell 1993, Symondson et al. 2000, Harwood et al. 2004, Winder et al. 2005) or of prey DNA by quantitative PCR (Zhang et al. 2007b, Lundgren et al., 2009). Such studies provide valuable insights into the relative amount of recognizable material in the predator at a given time, but do not offer 'quantitative' estimates of predation because the number (and/or biomass) of prey consumed and the

time elapsed since consumption cannot be inferred. Thus, although molecular gut-content analyses offer particular benefits for elucidating trophic interactions, they remain fraught with interpretive sources of error. Nevertheless, when used in combination with quantitative data from field experiments, they can generate unique insights that cannot be obtained with other approaches.

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