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Assessing the Trophic Ecology of the Coccinellidae: Their roles as predators and as prey.

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Abstract.

Coccinellidae function in complex food webs as predators, as consumers of non-prey foods, and as prey or hosts of natural enemies. Dietary breadth and its implications remain largely unexplored. Likewise the nature and implications of interactions with other predators in the field are poorly understood. The use of biochemical tools based on nucleic acids, proteins, sugars and other components of coccinellid diets, expands our understanding of their trophic ecology -- but only under field conditions in which coccinellids live, reproduce, forage, and consume prey (including intraguild prey), pollen, fungi, nectars, and other foods. We review the various methods which have been applied to the study of trophic relationships involving the Coccinellidae, their advantages and disadvantages, and some salient innovations and results produced by the range of technologies and their combinations. We advocate employing multiple tools to generate a more complete picture of the trophic ecology of a predator. The false perceptions of the strength and direction of trophic linkages that can result from a methodologically narrow approach is well illustrated by the laboratory and field assessments of coccinellids as intraguild predators, a phenomenon that is discussed in detail here. Assessing intraguild predation, and the breadth of prey and non-prey foods of the Coccinellidae, is essential to the understanding of this group, and for their application as biological control agents.

Key words: biological control, food web, intraguild predation, lady beetle, nutrition, predator, gut analysis, PCR, immunoassay, isotopic analysis, alkaloids

1. Trophic roles of Coccinellidae

Entomophagous coccinellids are major consumers of prey, but are themselves prey for intraguild predators. The processes of finding food and avoiding predation ultimately shape many of the behaviors of lady beetles and the ecological services they provide. Our current knowledge of the dietary breadth of coccinellids is incomplete; it also arises from a variety of approaches and tools used to examine trophic linkages. Likewise, assessments of the strength and outcome of intraguild interactions among coccinellids and other natural enemies are imperfect, and can vary depending on the experimental or observational approaches that are employed.

Coccinellid feeding behavior is much more complex than the stereotype of the aphid-eating lady beetle would suggest. This is not to say that aphidophagous species are unimportant; their conservation and augmentation within cropland can help suppress aphid outbreaks (van Emden and Harrington, 2007; Lundgren, 2009b; Obrycki et al. 2009, this issue). But the family Coccinellidae evolved from coccidophagous ancestors, and much of the extant diversity in the family still specializes on this prey group (Giorgi et al., 2009, this issue; Hodek and Honěk, 2009; this issue). Certain clades have also come to specialize on aleyrodids (Hodek and Honěk, 2009, this issue), mites (Biddinger et al., 2009, this issue), fungi (Sutherland and Parrella, 2009, this issue), plant foliage (Hodek and Honěk, 1996; Giorgi et al., 2009, this issue), and even pollen (Hodek and Honěk, 1996). Alternative foods such as lepidopteran and coleopteran immatures (Evans, 2009, this issue) and non-prey foods (Lundgren, 2009a, this issue) are critical components of optimal diets in most coccinellids, and shape the natural histories of these and other predators (Lundgren 2009b). As a group, coccinellids are extremely polyphagous; and it is increasingly apparent that species and individuals are in many instances quite polyphagous as well. The simple fact is that there isn't a single species for which the entire dietary breadth is known.

The abundance, dispersion, and pest management benefits of coccinellids are influenced by their suite of natural enemies. Parasitoids, parasites (mites) and pathogens (nematodes, viruses, protozoa, bacteria, and fungi) are widespread in many coccinellid populations (Riddick et al., 2009, this issue), and their geographic and host ranges have expanded with the anthropogenic redistribution of coccinellids used in biological control. Perhaps equally important are intraguild predators (including other coccinellids) that regularly consume coccinellid eggs (Harwood et al., 2009) and larvae (Lucas, 2005; Pell et al., 2008), and ants that defend herbivorous prey from coccinellid predation (Majerus et al., 2007). Pressure from intraguild competitors and other natural enemies drives coccinellid spatio-temporal distributions on many scales, as well as their predation capacity, defensive characteristics, and reproductive decisions (Seagraves, 2009, this issue). These intraguild interactions notwithstanding, coccinellids and other natural enemies are now well recognized as operating additively or synergistically in pest suppression (Snyder, 2009, this issue).

Research on coccinellids has advanced mankind's concepts of pest management, the nutritional physiology of insects, and how insects function within complex food webs. However, the complex nature of coccinellid trophic ecology must be appreciated and accommodated for their pest management benefits to be fully realized. Specifically, the dietary breadth of coccinellids can only be fully evaluated using multiple diagnostic methods that account for the polyphagous tendencies of these predators in both space and time. This point is well illustrated by the recent scientific attention devoted to intraguild interactions involving coccinellids, discussed in Section 2. The wide breadth of tools currently applied to assess the diets of predators (and coccinellids in particular) can help to resolve 1) the relative contributions of different foods to the nutritional ecology of coccinellids, and 2) the influence of intraguild predation (IGP) interactions on natural enemy communities comprised in part of coccinellids.

2. Caveats for dietary assessments of predators in the laboratory: A case study involving IGP and coccinellids.

The importance of using multiple techniques to evaluate the strength of trophic interactions by natural enemies is well illustrated by the staggering number of studies recently published on the relative capability of lady beetles as intraguild predators in relation to other natural enemies. These studies have identified that intrinsic characteristics of predator guilds (including size, chemical and physical defenses, mandibular features, dietary breadth, mobility, degree of satiation, etc.) influence which predator will emerge successful from an intraguild encounter. Among natural enemies, coccinellids are comparatively large-bodied, aggressive, and well defended against predation; all of these traits make lady beetles frequent victors in IGP contests. But evidence from larger scale experiments suggest that the consistently strong trophic relationships between coccinellids and IGP competitors measured in the laboratory are unrealistic. Ultimately, this lends credence to our argument that multiple field-based assessment procedures are necessary to define the role of coccinellids in IGP, and the trophic ecology of the group in general.

2.1. IGP contests with non-coccinellid natural enemies. A number of natural enemies suffer asymmetrically from IGP by coccinellids. Within confined conditions, anthocorids (Santi and Maini, 2006) and predaceous Diptera larvae (Lucas et al., 1998; Gardiner and Landis, 2007) usually lose IGP contests with coccinellids. Parasitoid immatures within parasitized hosts are particularly vulnerable to predation (Snyder et al., 2004; Zang and Liu, 2007; Pell et al., 2008). Coccinellids seldom discriminate between parasitized and unparasitized prey (Colfer and Rosenheim, 2001; Bilu and Coll, 2007; Zang and Liu, 2007; Royer et al., 2008), depending on the age of the parasitoid (e.g., parasitoid pupae or mummies are sometimes less preferred than developing endoparasitoids) (Chong and Oetting, 2007; Zang and Liu, 2007; Hodek and Honěk, 2009, this issue). Entomopathogens residing in infected prey are also consumed by coccinellids, and thus these pathogens' ability to suppress a pest population may be reduced by IGP (Pell et al., 2008; Roy et al., 2008). However, even when coccinellids are successful intraguild predators, heterospecific intraguild prey are often poor quality for coccinellids relative to their preferred prey (Phoofolo and Obrycki, 1998; Santi and Maini, 2006; Royer et al., 2008), and IGP is often reduced when alternative prey becomes available (De Clercq et al., 2003; Yasuda et al., 2004; Cottrell, 2005).

Although coccinellids are often successful intraguild predators, they also are victims of IGP. Ants that tend hemipterans are particularly hostile toward foraging coccinellid adults and larvae, although the intensity of these interactions depends on the species involved (Majerus et al., 2007). Adult coccinellids are usually chased away by ants, and larvae are moved away from the prey colony, pushed off of the plant, or killed (Majerus et al., 2007). Pentatomids also overcome coccinellid immatures in intraguild contests in the laboratory (Mallampalli et al., 2002; De Clercq et al., 2003; Pell et al., 2008). Lacewing larvae (chrysopids and hemerobiids) fare well in IGP contests against coccinellids of similar or smaller size (Lucas et al., 1998; Michaud and Grant, 2003; Santi and Maini, 2006; Gardiner and Landis, 2007). Finally, entomopathogens may also harm the intraguild predators that eat infected prey; aphids infected with the entomopathogen *Neozygites fresenii* (Nowakowski) (Entomophthorales: Neozygitaceae) increased mortality, prolonged development, and reduced fitness of *Coccinella septempunctata* L. versus individuals fed healthy prey (Simelane et al. 2008).

2.2. IGP contests with other coccinellids. Coccinellid species vary greatly in their competitiveness in IGP conflicts. Among coccinellid life stages, eggs are particularly vulnerable to predation, and coccinellids are behaviorally adapted to reduce egg predation from heterospecifics (Seagraves, 2009, this issue). In addition to predator avoidance strategies by ovipositing females (Griffen and Yeargan, 2002; Seagraves and Yeargan, 2006; Seagraves, 2009, this issue), the chemical defenses present in or on coccinellid eggs partially determine their acceptability to heterospecific predators (Sato and Dixon, 2004; Cottrell 2005, 2007; Pell et al., 2008; Ware et al., 2008); perhaps immunity to the chemical defenses of conspecific eggs is why these are such a suitable food for many coccinellids (Burgio et al., 2002; Sato and Dixon, 2004). Larvae are defended from predation by heterospecific coccinellids through their chemistry, behavior and mobility, and their physical characteristics (e.g., exterior spines or waxy secretions). Like heterospecific coccinellid IGP, cannibalism is also a common phenomenon in coccinellids, but differs in important nutritional, selective, and evolutionary implications (Osawa, 2002; Michaud, 2003; Michaud & Grant, 2004; Omkar et al., 2006; Seagraves, 2009, this issue).

2.3. Implications of IGP for biological control. Nearly all the studies in Sections 2.1 and 2.2 assess the relative ability of a coccinellid species to function as an intraguild predator of a conspecific or heterospecific natural enemy within confined experimental conditions (either a Petri dish or a “microcosm”). For example, 73% of the 30 studies on IGP involving coccinellids reviewed by Lucas (2005) were conducted in the laboratory, and 10% were conducted in field cages. These experiments are valuable in assessing the propensity of one species to successfully attack another, all else being equal. But under field conditions, habitat characteristics (e.g., three-dimensional complexity and refugia), availability of alternative food sources, activity cycles of the participants, and avoidance and escape behaviors of potential intraguild prey strongly influence the outcome of these interactions (Lucas, 2005; Majerus et al., 2007; Pell et al., 2008). Also, much of the research to date has focused on interactions in cropland, and the influence of IGP by and on coccinellids in natural systems remains to be substantiated (Pell et al., 2008). Field observations of IGP events (e.g., Colfer and Rosenheim, 2001; Harwood et al., 2009), as well as the defensive characteristics and behaviors of natural enemies, all support the hypothesis that IGP occurs under field conditions and can influence insect communities and biological control. But the results from IGP interactions obtained in the laboratory or confined spaces are of questionable application to field conditions, and should be interpreted with caution.

2.3.1. Effects of IGP by exotics on coccinellid communities. Populations of several coccinellid species endemic to North America and Europe have experienced steep declines in recent years, and exotic coccinellids released for biological control programs are implicated as causal agents based on abundant but circumstantial evidence (Elliott et al., 1996; Michaud, 2002; Brown, 2003; Alyokhin and Sewell, 2004; Evans 2004; Hesler et al., 2004; Snyder and Evans, 2006; Losey et al., 2007; Mizzell, 2007; Hesler and Kieckhefer, 2008; Ware et al., 2009). Within North America, *Adalia bipunctata* (L.), *Coccinella novemnotata* Herbst, and *C. transversoguttata* Faldermann were once the most abundant coccinellids in many habitats. These species are now virtually extinct or extirpated from certain habitats (Losey et al., 2007).

Meanwhile populations of the exotic coccinellids *Coccinella septempunctata* and *Harmonia axyridis* Pallas abound in the habitats where the former species used to be dominant. While it is clear that there has been a recent shift in coccinellid communities in certain systems, analysis does not indisputably support that regional reductions in coccinellid diversity are coupled with the range expansion of invasive species (Harmon et al., 2007). Regardless, the diminishing abundance of some native coccinellids within agroecosystems as exotic species have increased numerically has clear implications for biological control and insect conservation.

2.3.2. IGP and biological control under realistic conditions. The published literature suggests that IGP likely has less pronounced effects on biological control than is indicated by laboratory experiments. The effects of IGP on biological control ultimately depend on the relative contributions that coccinellids and other natural enemies make to the suppression of a target pest. Strong levels of IGP inflicted by coccinellids are not likely to impede biological control in systems where coccinellids are keystone predators, as repeatedly demonstrated under realistic conditions (Mallampalli et al., 2002; Snyder et al., 2004; Rosenheim and Harmon, 2006; Gardiner and Landis, 2007; Zang and Liu, 2007; Costamagna et al., 2008). Another consideration is that predator diversity often favors biological control (Losey and Denno 1998; Cardinale et al. 2003; Aquilino et al. 2005; Snyder, 2009, this issue), but the long-term implications of the introductions of strong IGP competitors that reduce or eliminate other intraguild members for biological control are important to consider. Nevertheless, the example of recent IGP literature clearly indicates the ease with which erroneous conclusions (e.g., the severe consequences sometimes inferred from laboratory IGP contests) can be drawn from a narrow, laboratory approach to assessing the trophic ecology of the coccinellids. A multifaceted, field-based approach that employs observational, microscopic, biochemical, or molecular assessments of coccinellid feeding behavior under field conditions will better define the roles of coccinellids in food webs, both as predators and as prey.

3. Assessing dietary breadth in lady beetles

Several methods have been used to diagnose trophic linkages among insects and natural enemies, as well as the occurrence, frequency, and impact of a predator species on target prey populations. These include direct observation of predation events, controlled manipulation of predator and prey numbers to determine resulting effects, and detection of prey-associated markers in predators having consumed them. Physical dissection and examination of predator guts or feces (e.g., Triltsch, 1999), are valuable, depending on the feeding mode of the predator and the structural integrity of identifiable food components. Prey can be marked with radioactive (McCarty et al., 1980) or stable (Nienstedt and Poehling, 2004) isotopes or external antigenic markers (Hagler and Jackson, 2001); however, this limits studies to the marked subset of a prey population. Researchers using stable isotopic patterns (typically of C and N) not involving enrichment (Hood-Nowotny and Knols, 2007) are challenged by a staggering array of different food combinations and other variables (Daugherty & Briggs, 2007). The self-identifying and unique biochemistries of prey species -- proteins, nucleic acids or other unique organic molecules -- offer versatile opportunities for predation detection and, potentially, predation quantification. These methods have been used to deduce the diets of lady beetles over the past 125 years, but each of these methods carries strengths and weaknesses.

3.1 Observations in field, field cages, and laboratory. Observing coccinellids feeding has many strengths, but also may bias the perceptions of the trophic ecology of coccinellids (Thompson, 1951; Hodek and Honěk, 2009, this issue). Focusing observation efforts on a target prey can identify major predator groups that consume this species, but this approach does not reveal other foods consumed by generalist predators. This same caveat applies to prey-centric studies using biochemical methods described below in Sections 3.5 and 3.6). Moreover, those prey groups or life stages that are sessile or easy to observe over time tend to receive disproportionate attention, and may partially explain why many coccinellids are so often recognized as aphid specialists. Direct observations are extremely valuable (but scarce) in defining the dietary breadth of a predator when they focus on the predators themselves over a range of times and locations rather than a target prey. For instance, direct observations have established that the common species *C. septempunctata* feeds on willow and oak foliage (Brassler, 1930) in addition to non-aphid prey (Kanervo, 1940).

3.1.1. Use of sentinel prey, and nocturnal sampling. Placing sentinel prey in the field can be very useful in assessing the intensity of predation and the species responsible for biological control. It may be especially useful where pest density is insufficient to permit observation of adequate numbers of predators. Kidd and Jervis (1996) and Mills (1997) describe the caveats in deploying sentinel prey, including positioning, quality, and density considerations. Manipulation of prey density may also lead to important insights. For example, Evans and Toler (2007) used prey density manipulation in open alfalfa fields to demonstrate the aggregation of native coccinellids to high aphid density, but not to high alfalfa weevil larval densities; *C. septempunctata* responded high densities to both prey. Andow (1990, 1992) assessed predation of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) sentinel egg masses in different corn ecosystems, including that by the major coccinellid predator, *Coleomegilla maculata* DeGeer.

Pfannenstiel and Yeargan (2002) and Pfannenstiel (2005) observed predation on sentinel Lepidoptera eggs throughout the diel cycle, determining that larval and adult *C. maculata* had distinct periods of activity for consuming foliar prey. In spite of the widespread preconception that lady beetles are diurnal, these studies and others (Vickermann and Sunderland, 1975; Weber et al., 2008) have discovered significant nocturnal predation. Meyhöfer (2001) used unattended 24-hr video recording of parasitized and unparasitized *Aphis fabae* Scopoli (Hemiptera: Aphididae) to identify and characterize behaviors of individual predators eating parasitized aphids, showing that six major groups, including coccinellids, nocturnally consumed immature parasitoids.

3.1.2. Manipulation of predator density. Manipulation of predator density, and testing for subsequent changes in pest (prey) numbers and/or crop damage, is “the most convincing test of predator impact” (Symondson et al., 2002). The very large number of studies employing predator augmentation, field cages, or exclusion by physical or sometimes by chemical means (Luck et al., 1988; Mills, 1997; Obrycki et al., 2009, this issue), are beyond the scope of this review. In laboratory feedings and microcosms, as in field cages with simplified food webs, treatments must be based on realistic densities and species assemblages if these results are to be relevant to the open field. Many coccinellid studies, including IGP studies reviewed above, fail to compare tested arenas and conditions with what might be expected in a field ecosystem. Thus, while prey augmentation can be a powerful tool for assessing the pest suppression capabilities of a predator, the caveats associated with this method need to be recognized.

3.2 Gut dissections. Examining the gut contents of coccinellids microscopically is an affordable, low-technology method that can give a very good overview of the full dietary breadth of a predator species. This method only functions when solid food is ingested, and so cannot be applied to fluid-feeding life stages (e.g. neonate coccinellid larvae). Even in those insects which ingest solid food, it is not suited to distinguishing soft, amorphous prey and plant parts, or liquids such as honeydew and floral and extrafloral nectars, all of which may be important components of coccinellid diets (Lundgren 2009a, this issue; 2009b). As Crowson (1981, p. 161) points out, microscopic analysis of gut contents (in common with the use of laboratory feedings) requires "acquaintance with the natural habitat and with the sort of potential foods which are present in it."

3.2.1. Forbes and Triltsch: The first and the most comprehensive gut analyses. A number of researchers have dissected the guts from coccinellids to determine their range of food consumption (Table 1). One of the first of these analyses was conducted by Stephen Forbes (1883), who examined the gut contents of several common coccinellids and carabids of Illinois (USA). In virtually all coccinellid species, fungal spores and pollen together made up approximately half of the estimated volume of gut contents. Approximately half of the *C. maculata* adult guts contained aphids with a few mites. About 54% of gut contents contained pollen and/or fungal spores. Around 40% of *Hippodamia convergens* Guérin-Méneville and *H. glacialis* (Fabricius) adults contained arthropods (including a millipede, caterpillar, aphids, and chinch-bugs). In both genera, the non-prey gut contents included pollen of various plants, especially composites and grasses, and fungal spores (particularly *Helminthosporium* and *Cladosporium*). Nearly two-thirds of *Coccinella novemnotata* and *C. transversoguttata* (n = 3 each) consumed aphids; fungi and small amounts of pollen were also found in their guts. Although Forbes only examined a few individuals of each species, his work was instrumental in establishing that coccinellids consume much more than just their preferred foods such as aphids.

Only a few studies have undertaken broad dietary assessments of coccinellids using gut analysis (Table 1); of these, Triltsch (1997, 1999) provides the best exploration of dietary spectrum for a single polyphagous insect predator species, *Coccinella septempunctata* in Germany. Nearly 2,000 adults and larvae from three locations near Berlin were examined over a 2-yr period. Aphids and fungal spores were the most frequently observed foods, found in 44% and 42% of adults respectively. More than one food type was found in 68% of non-empty adult guts (calculated from Triltsch, 1999, Table 2). Non-aphid arthropod prey (found in 13% of adults) included thrips, Collembola, mites, Hymenoptera, Diptera larvae, and coccinellid larvae. Pollen was found in a maximum of 23% of adults in May and September. In addition to the comprehensive catalogue of foods consumed by *C. septempunctata*, Triltsch analyzed the sex-specific, stage-specific, seasonal, physiological, and geographic effects on the diet of *C. septempunctata*, and clearly illustrated that alternative foods are common components of this aphidophagous species' diet, even when aphids were extremely abundant.

3.2.2. Temporal patterns in food consumption. In addition to the diversity of foods that most coccinellids consume, one of the strongest conclusions that can be drawn from published gut content analyses is the seasonal shifts in diet experienced by most coccinellids. In part, the dietary breadth is reflective of the local food abundance available to the foraging coccinellid (Putman, 1964; Ricci et al., 1983; Ricci, 1986a, b; Hemptinne et al., 1988). For instance, in Australia *Scymnodes lividigaster* (Mulsant) and *Ileis* (=*Leptothea*) *galbula* (Mulsant) consumed different foods on different host plants (Anderson, 1982). In Israeli citrus orchards, *Chilocorus bipustulatus* (L.) switches from diaspidid scales in spring to coccid scales later in the year, based on the relative abundances of these two food sources (Mendel et al., 1985). Aphid consumption by *Rhyzobius litura* (Fabricius) peaked during April and October (Ricci, 1986a). The central pattern in these studies is one of large and consistent seasonal variation in food consumption, which exceeds year-to-year and location-to-location effects (Ricci 1986a, b; Triltsch 1997, 1999).

3.2.3. Diet and physiological status. The physiological status of the coccinellid is also likely to dictate which foods are consumed and when. Gut dissections of field-collected coccinellids have revealed that adults tend to consume the most food during the pre-reproductive and reproductive phases (Anderson, 1982; Triltsch, 1999). Recently eclosed *C. septempunctata* adults ate more fungi, more non-aphid arthropods, and fewer aphids, than did overwintered adults (Triltsch, 1999). Also, females are likely to consume more food than males, although qualitative differences in their diets have not been documented (Triltsch, 1999; Lundgren et al., 2005).

The developmental stage of the coccinellid sometimes affects their diet. Larvae and adult coccinellids do not necessarily differ in their diets (Ricci et al., 1983; Ricci, 1986a, b). These examples notwithstanding, it is often the case that larvae consume different foods than the adults, reflecting their unique predatory abilities and nutritional needs. Lundgren et al. (2004) found similar proportions of *C. maculata* larvae and adults consuming prey and pollen in maize fields. However, in the same study, larvae of *Harmonia axyridis* were much more likely to consume pollen than were adults of this species. In *C. septempunctata*, although larval and adult diets were similar, the larvae ate less pollen and more conspecifics than did adults (Triltsch, 1999).

3.2.4. Gut dissections and the overemphasis on prey specialization. Gut dissections often reveal the importance of alternative foods to the trophic ecology of coccinellids, even in the presence of essential prey (sensu Hodek and Honěk, 1996). Even when essential prey is widely available, it may constitute only a fraction of a coccinellid's diet (Anderson, 1982; Ricci et al., 1983; Ricci, 1986a, b; Ekbom, 1994; Triltsch,1999; Lundgren et al., 2004; Ricci and Ponti, 2005; Ricci et al., 2005). Gut dissections may identify previously unknown essential foods, such as pollen and fungi for the aphidophagous *Rhizobius litura* (Ricci, 1986a, Ricci et al., 1988). Also important, gut dissections reveal that coccinellids often simultaneously consume numerous species of prey (sometimes as many as five or six prey species), thereby seriously calling into question any degree of specialization in these often polyphagous predators (Putman, 1964; Anderson 1982; Ricci et al., 1983; Ricci, 1986a, b; Triltsch, 1999; Ricci and Ponti, 2005). Finally, non-prey foods, including plant trichomes, pollen, fungal spores and inorganic debris, are frequently consumed concurrently with prey, and even more intensively when prey becomes scarce (Forbes, 1883; Putman, 1964; Anderson, 1982; Ricci et al., 1983; Hemptinne and Desprets, 1986; Ricci, 1986a, b; Hemptinne et al., 1988; Triltsch, 1999; Ricci and Ponti, 2005; Ricci et al. 2005; Lundgren, 2009b, this issue).

3.2.5. Strengths and weaknesses of gut dissections. Gut dissection remains a straightforward and productive method for rapid low-cost dietary assessment, which often identifies unexpected contents. Triltsch (1999) points out that the gut dissection technique fails to detect insect egg consumption, which may be significant for coccinellids. Prey are not equally easy to identify or to count. Small prey such as thrips and aphids are often easily identified in gut contents, but the necessary fragmentation of large prey such as *Oulema* (Coleoptera: Chrysomelidae) and *Coccinella* larvae present more of a challenge. Another important point is that not all gut contents are intentionally consumed (Putman, 1964; Triltsch, 1999). For example fungal spores are often consumed incidentally with honeydew meals. Studies of specific foraging behaviors may shed light on intent, and analysis of nutritional qualities of different diets may shed light on value (see Lundgren, 2009b, this issue). There is no assurance that unintentionally ingested materials lack value, nor that intentionally ingested foods are valuable. Gut dissections simply reveal that the current knowledge of coccinellid diet is incomplete, at best.

3.3 Frass analysis. In spite of its widespread use in other studies on animal feeding ecology (Litvaitis, 2000), only four researchers have analyzed the frass of coccinellids to yield insights on their diet (Table 1). Conrad (1959) stationed sticky surfaces beneath sentinel egg masses of European corn borer *Ostrinia nubilalis*, to capture frass of *Coleomegilla maculata*. On average 16% of egg masses were partially consumed, and predation frequency on *O. nubilalis* eggs decreased as aphids and corn pollen increased in the corn field. This is the only published example that used frass identification to investigate predation by coccinellids under field conditions. Putman (1964) and Ricci et al. (2005) make non-specific reference to the diet determination of coccinellids using frass examination, but the intensity of their efforts is unclear.

Honěk (1986) used frass production as an estimate of prey consumption and predator satiation. Although this study did not distinguish dietary components, measurements of frass production in field-collected *C. septempunctata* led to the conclusion that most predators are far from satiated over the course of a growing season, an ingenious answer to an oft-posed ecological question.

Frass analysis is unlikely to yield markers for specific prey, and is not associated easily with specific predators in the field. However, association of predator- and prey- specific markers, as with mammalian studies (e.g., Deagle et al., 2006), has not been attempted. Quantification and analysis of frass is likely to be useful in laboratory and other controlled experiments concerning digestive dynamics and energetics of predator nutrition and physiology.

3.4 Isotopic methods. Radioactive labeling, stable isotopic or elemental labeling, and stable isotope analysis of natural patterns in the field are the three main applications of isotopic analysis in diagnosing trophic linkages between coccinellids and target prey.

3.4.1. Radiolabeled prey. Herbivores, or the plants on which they feed, can be labeled with radioisotopes (e.g., ^{32}P, ^{3}H, or ^{14}C). ^{32}P injected into thistle plants bioaccumulated into three coccinellid species, presumably via the herbivore *Anuraphis* sp. (Pendleton and Grundmann, 1954). Independent calibrations are necessary to quantify the consumption of the marker by each predator species, since each retains the markers for different amounts of time (Garg and Gautam, 1994). Room (1979) and Thead et al. (1987b) used radiolabeled heliothine moth eggs and larvae to identify predators, including coccinellids, and Thead et al. (1987b) quantified predation in field cages, correcting for the rate of marker retention in respective predators (Thead et al., 1987a). Radiolabeling is hazardous to the environment and to researchers, and its persistence within a food web can lead to IGP and scavenging being misdiagnosed as predation. Its application is restricted to specialized trophic and metabolic studies in the laboratory, some of which may also be addressed through stable isotopic enrichment techniques. Nevertheless, laboratory studies of food and water dynamics have successfully used radiolabeling to address a number of trophic relationships involving coccinellids (Ferran et al., 1981; Taylor, 1985; Houck and Cohen, 1995; Holte et al., 2001).

3.4.2. Stable isotopic and elemental enrichment. Enrichment of suspected prey or other food items such as nectar or pollen with stable isotopes such as ^{15}N and ^{18}O (Hood-Nowotny and Knols, 2007), or rare elements such as Rb (Akey et al., 1991), has been used to identify and investigate predation by coccinellids. Nienstedt and Poehling (2004) used open-topped field enclosures in wheat with laboratory-raised ^{15}N-enriched aphids to determine predation by carabids, staphylinids, spiders, and coccinellids. *Coccinella septempunctata* and *Propylea quatuordecimpunctata* (L.) contained the isotopes, but this signature could have originated from other prey species since the barriers did not restrict the movement of these predators. Steffan et al. (2001) found that *Hippodamia convergens* acquired ^{15}N enrichment when they consumed nectar of Chinese cabbage which had been fertilized with enriched KNO_3 fertilizer. Rb marking (see Akey et al., 1991) has been used to mark the phytophagous coccinellid, *Epilachna varivestis* Mulsant (Shepard and Waddill, 1976), and various predators including *H. convergens* and *Scymnus loewii* Mulsant in a cotton-sorghum system (Prasifka et al., 2001). Of the isotopoic methods, stable isotopic enrichment and elemental enrichment may prove the most useful for specific questions, where technology is available for atomic absorption spectrometry, and the residence time for the enrichment component is appropriate to the coccinellids under study.

3.4.3 Diagnosing trophic relationships using naturally occurring stable isotopes. Based on distribution of ^{13}C and ^{15}N in plants and their respective herbivores, field and laboratory studies have established that isotopic proportions in predaceous coccinellids are responsive to dietary changes and thus are potentially useful in studying trophic relationships (Scrimgeour et al., 1995; Ostrom et al., 1997; Prasifka et al., 2004; Gratton and Forbes, 2006; Park and Lee, 2006). Gratton and Forbes (2006) established that different tissues within *Harmonia axyridis* and *C. septempunctata* registered $\delta^{13}C$ in response to changes in their diets from aphids on soy (C3 plant) to aphids on corn (C4 plant). In theory, this raises the prospect for more intricate tracking of trophic dynamics. In practice, stable isotope ratios may be produced by a large range of different food combinations, as well as species- and stage-specific physiological effects in prey and predators; therefore, application of this method appears to involve too much complexity to yield clearcut conclusions in trophic studies (Daugherty and Briggs, 2007).

3.5 Immunoassay methods. Methods to assess predation that are based on mammalian immune reactions or cell lines have been in use for about 60 years, and possess a wide range in specificity and sensitivity, from early precipitin tests to highly specific and sensitive monoclonal antibody-based ELISA methodology (Greenstone, 1996; Harwood and Obrycki, 2005). Early predation studies focused on fluid-feeding predators such as predatory Heteroptera and spiders, or prey not amenable to gut dissection, such as Lepidoptera eggs and larvae (see Table 11.1, Greenstone, 1996). Because of this taxonomic selectivity in application of immunoassays, or possibly because coccinellids were uncommon in the systems investigated, they are less represented in early predation studies. For instance, Vickermann and Sunderland (1975) examined over 600 predators of 24 species for aphid consumption, using microscopic gut analysis for coccinellid larvae and adults, carabids, and adult staphylinids, but using precipitin testing for all others.

About 20 published studies (Table 2) have used immunoassays to examine coccinellid predation. Many of these (e.g., Ashby, 1974; Whalon and Parker, 1978; Hagley and Allen, 1990) tested a wide range of predators to identify important consumers of a focal pest. Some of the most extensive immunoassay-based predator analyses involving coccinellids were conducted by Hagler and Naranjo (1994, 1996, 1997), who studied predation of whiteflies and pink bollworm eggs by *Hippodamia convergens* in Arizona using prey-specific monoclonal antibodies. Based on frequency of detection, coccinellids were determined to be unimportant predators in some cases (e.g., Whalon and Parker, 1978) and very important predators in others (e.g., Hagley and Allen, 1990; Huang et al., 1992). Early workers (Dempster, 1960; Rothschild, 1966) already recognized the difficulties with translating detection frequency into a quantitative measure of predation, a conundrum which continues to challenge researchers (Hagler and Naranjo, 1996; Sunderland, 1996). However, quantitative ELISA (Symondson et al., 2000; Harwood et al., 2004) provides more information for each sampled predator (as with qPCR versus conventional PCR, discussed below), information which can be related to quantity of prey consumed.

Marking of predators with common antigens (Hagler and Jackson, 2001) can be combined with prey-specific immunoassays (Hagler and Naranjo, 2004) to provide insights into movement and prey consumption of both endemic and released predators. Marking prey with inexpensive, user-friendly antigens can be applied to efficiently detect prey consumption by numerous predators (100s or 1,000s), but is unreliable for piercing-sucking species (Hagler and Durand, 1994). Recently, Mansfield et al. (2008) compared prey-specific indirect ELISA with an anti-rabbit IgG prey marker using sandwich ELISA, for predation detection in a coccinellid and a melyrid predatory beetle in Australia cotton, and judged the detection of the marker to be more specific and sensitive. But sensitivity, especially in larger predators such as many coccinellids, depends on the specifics of the ELISA format used (Hagler, 1998). Marking of prey is an extra step which is useful only for certain research applications (Hagler and Jackson, 2001). Horton et al. (2009) have measured movement of generalist predators -- coccinellids, chrysopids, and Heteroptera, and spiders -- from different cover crops to pear orchard canopy, using inexpensive egg albumin immunomarker and ELISA (see Jones et al., 2006). The coccinellid *Hyperaspis lateralis* Mulsant showed the greatest proportion of cover-crop markers among canopy-captured predators, suggesting unexpected feeding on marked prey in the cover crops in addition to known predation on mealybug and scale insect prey on pear trees.

Immunoassays specific for Bt Cry proteins produced by transgenic crops can be used to track tritrophic interactions within transgenic cropland. For instance, Harwood et al. (2005, 2007b) showed that coccinellids, particularly *Coleomegilla maculata*, acquire the Cry toxin from Bt field corn before pollen shed, and peak detection was well after anthesis. This led to the conclusion that the predators must have ingested Bt-containing prey or plant parts other than pollen (see Moser et al., 2008).

3.6 DNA-based methods. Polymerase chain reaction (PCR) has within the past decade been applied to detect DNA of target prey within the guts of coccinellids (Table 2). Only a few of these studies have applied PCR to answer trophic questions in the field, whereas several carabid and spider predation studies have involved far more field sampling (e.g., see Harwood and Greenstone, 2008; Lundgren et al., in press). The goal of most PCR-based analyses has been to demonstrate the viability of a specific detection system in the laboratory, sometimes including a few field samples. From this work it is clear that the detection of prey DNA may depend on a large number of factors. These include the choice of marker sequence and particularly its length;

time since feeding; temperature; species, physiological state and mass of predator; ingestion of target or other food material before, during, and after predation on the prey of interest; quantity of prey; number of DNA sequences in the prey (depending in turn on life stage and cell number, number of nuclear or mitochondrial (or other) copies of sequence present per cell); and preservation of the sample (Sheppard and Harwood, 2005; Weber and Lundgren, 2009).

Prey DNA may be detected as a result of scavenging or secondary predation, which are considered false positives or erroneous detections when predation of live prey is of interest (Sheppard et al., 2005; Juen and Traugott, 2005). These quantitation issues, as well as potential sources of false positives, are shared with immunoassay methods (Hagler and Naranjo, 1996; Harwood et al., 2001; Calder et al., 2005). Since predators may differ radically in their digestion rates, species- and stage-specific determination of marker disappearance is necessary for each species when ranking their relative contributions to the suppression of a target prey (Greenstone et al., 2007). Hoogendoorn and Heimpel (2001) employed markers of four different lengths to improve determination of time since prey consumption, based on the more rapid disappearance of longer markers, which is in accord with disintegration of DNA markers expected by random ligation (Deagle et al., 2006).

Quantitative PCR (qPCR, also known as real-time PCR) has several traits that suggest it may eventually supplant conventional PCR, in part because of its ability to reduce both analysis time and the subjectivity of the results: it relies on flourometric quanitation rather than visual band detection on an agarose gel, and allows the verification of the precise target DNA sequence based on its melting temperature. Used widely in medicine and forensics, qPCR has been applied to predation investigations involving several non-coccinellid systems (Deagle et al., 2006; Troedsson et al., 2007; Nejstgaard et al., 2008; Lundgren et al., in press). With respect to coccinellids, Zhang et al. (2007b) quantified the amount of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) DNA consumed by *Propylea japonica* (Thunberg) using qPCR, and related it to initial meal size and time since consumption in the laboratory. Weber and Lundgren (2009) demonstrated the value of qPCR for quantification of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) eggs by *C. maculata*, with quantitation of number of eggs consumed, and effect of subsequent meals on the retention of the DNA marker, for which the quantitative half-life ranged from 16 to 59 minutes. Additionally, marker DNA quantity and frequency of detection allowed the ranking of commonly-used sample preservation protocols such as freezing and placing samples in ethanol, demonstrating their critical importance to PCR-based gut analyses. Quantitative PCR adds additional information when measuring predation compared to conventional PCR, but as with conventional PCR, preliminary laboratory studies need to be performed on a study system before clear interpretations of field measures of prey consumption are possible.

Detection of arthropod prey has been the focus of gut analysis studies for coccinellids and other predators, but PCR methods may also be used to detect plant tissues consumed by insect herbivores (Matheson et al., 2008; Jurado-Rivera et al., 2009). PCR detects fungi and pollen consumed by coccinellids (Lundgren and Weber, unpublished data). Plant and fungal foods have been largely neglected in arthropod studies using biochemical techniques, in spite of widespread success with detecting fungi (Atkins and Clark, 2004), pollen (Zhou et al., 2007) and other plant tissues (Ferri et al, 2008) in environmental samples. PCR methods also have a variety of other applications to studies of coccinellids, their food, and natural enemies. PCR is seeing wide use in diagnosis and identification of parasites (e.g., male-killing bacteria in Coccinellidae; Majerus, 2006) and also for parasitoids (although not so far in the Coccinellidae)(Harwood and

Greenstone, 2008). Other molecular methods such as temperature gradient gel electrophoresis (Harper et al., 2006) may come into use in predation studies as the field continues its meteoric development.

3.7 Gas chromatography–Mass spectrometry of coccinellid-specific alkaloids.

Coccinellids produce species-specific alkaloids (Glisan King and Meinwald, 1996) which are quantifiable by GC-MS, and may be useful in identifying key intraguild predators of coccinellids (Hautier et al., 2008; Sloggett et al., 2009). The alkaloids produced by *Adalia bipunctata* and *Coccinella septempunctata* were detectable in *Harmonia axyridis* that consumed these intraguild prey in the laboratory (Hautier et al., 2008). Moreover, these intraguild prey-based alkaloids are persistent within the predator (Sloggett et al., 2009); adaline was detectable through pupation in *H. axyridis* fed *A. bipunctata* (Hautier et al., 2008). Sloggett et al. (2009) demonstrated they could distinguish six common species in Kentucky using a combination of nine alkaloids present in one or more species. Hautier et al. (2008) detected exogenous coccinellid alkaloids from three different species in nine of 28 field-collected *H. axyridis*. This method, if applied to field research, has the potential advantage of at least somewhat quantitative measurement of multiple prey markers in a single predator (Sloggett et al., 2009) for analysis of intraguild or higher-level (vertebrate) predation of coccinellids. Longer persistence of some coccinellid alkaloids (Hautier et al., 2008) could increase the potential for false positives by IGP of an intraguild predator.

3.8 Other techniques for trophic analysis of Coccinellidae. Electrophoretic detection of prey (Solomon et al., 1996) has been used in predation studies, but not with the Coccinellidae, and its use has been supplanted by other biochemical techniques. Specific biochemicals present in the prey may affect coccinellids preying upon them (Hodek and Honěk, 2009, this issue), including alkaloids of legumes, quantified in aphids for their effect on three coccinellids eating them (Emrich, 1992).

Magnetic resonance microscropy (MRM, an attunement of MRI) has been used for detecting endoparasitoids and for visualizing the effects of diet on internal organs of *C. septempunctata* (Geoghegan et al., 2000). Although Greenstone (2006) judged MRM of little potential use in distinguishing meals ingested, nor for identifying parasites or parasitoids, there may be applications in distinguishing parasitized and nonparasitized insects for biological control introductions and for examining endoparasitic development.

Sugar is another important food source for coccinellids as evidenced by the number of coccinellids known to consume sugar sources under field conditions and the importance of sugars in supporting various life processes in coccinellids (Lundgren, 2009a, this issue). Glucophagy under field conditions has only been recorded from direct observations. However, the methodology developed for examining sugar feeding in adult mosquitoes and hymenopteran parasitoids is easily transferable to study in coccinellids. These methods include the application of the colorimetric anthrone reagent (which allows the detection and quantification of fructose and sucrose within insect stomachs) (Olson et al., 2000; Heimpel et al., 2004) or the use of TLC, GC, or HPLC to detect specific mono-, di-, and oligo-saccharides in the stomachs of an insect (Heimpel et al., 2004).

3.9. Challenges and trade-offs in application of methods to coccinellid trophic relationships. Methods for gut analysis have evolved as biochemical methods have become available (Figure 1). Gut dissections, immunoassays, and PCR, along with several other methods mentioned above, are all useful in assessing the trophic ecology of coccinellids. Careful observations and manipulations, coupled with gut dissections and more recently with biochemical methods to measure food consumption, have yielded a trophic tapestry for lady beetles, which even for so-called specialists often includes a wide array of arthropod, fungal, and plant-derived foods. The two leading biochemical methods for prey detection are antibody-based analysis of prey proteins, and polymerase chain reaction (PCR) -based analysis of unique prey DNA sequences. In concert with gut dissection to identify the spectrum including previously unknown dietary components, PCR will probably develop as the leading method for trophic quantification, but not supplanting immunological methods, which have some advantages as well as economy of scale. Each of these techniques has advantages and disadvantages. In general, immunoassays are more expensive to develop, but much less expensive per sample to use once developed (a 15-fold difference, Fournier et al., 2008; or 24- to 32-fold, Harwood and Greenstone, 2008), and are able to distinguish amongst different life stages of the same prey based on respective proteins present (e.g., Greenstone and Trowell, 1994; Sigsgaard, 1996). Studies with immunoassays can be based on larger field samples (over 10,000 in two cases, Hagler and Naranjo, 1996, 2005), with the more power to provide meaningful ecological answers. PCR-based methods offer more rapid and inexpensive development, and transferability based only on the information contained in the marker nucleic acid sequence. So far, PCR application to studies of the Coccinellidae has generally involved too few samples in the field, perhaps a consequence of their much higher per-sample marginal expense. Only a very few studies using biochemical methods have sought to answer questions of relevance to coccinellid biological control. Careful and realistic manipulations in the field, along with greater sample size and replication, will allow both more precise trophic determinations, whatever predation detection methods are used, and potential evaluations of the value of habitat modifications and food supplementation in the effective management of Coccinellidae for biological control.

4. Coccinellidae: A complex trophic ecology.

The Cocccinellidae are a ubiquitous and highly diversified beetle group (Giorgi et al., 2009, this issue). In spite of the volume of research into their evolution, behavior, and physiology, the breadth and diversity of trophic ecology within the group as a whole – and also within tribe, genus, species, populations, and for individuals -- remains to be fully substantiated and as a result is underappreciated. In answer to the question, “are we studying too few taxa?” (Sloggett, 2005), the answer is yes. But also, we apply too few techniques and ignore the biases inherent in each technique, a fact well illustrated by the demonstrated implications of laboratory based assessments of IGP contests involving coccinellids. Application of a combination of careful experimental designs, manipulations and observations with increasingly accessible technology, including biochemical methods, will enhance understanding of this group, and the corresponding application of biological control as a lynchpin of sustainable pest management.

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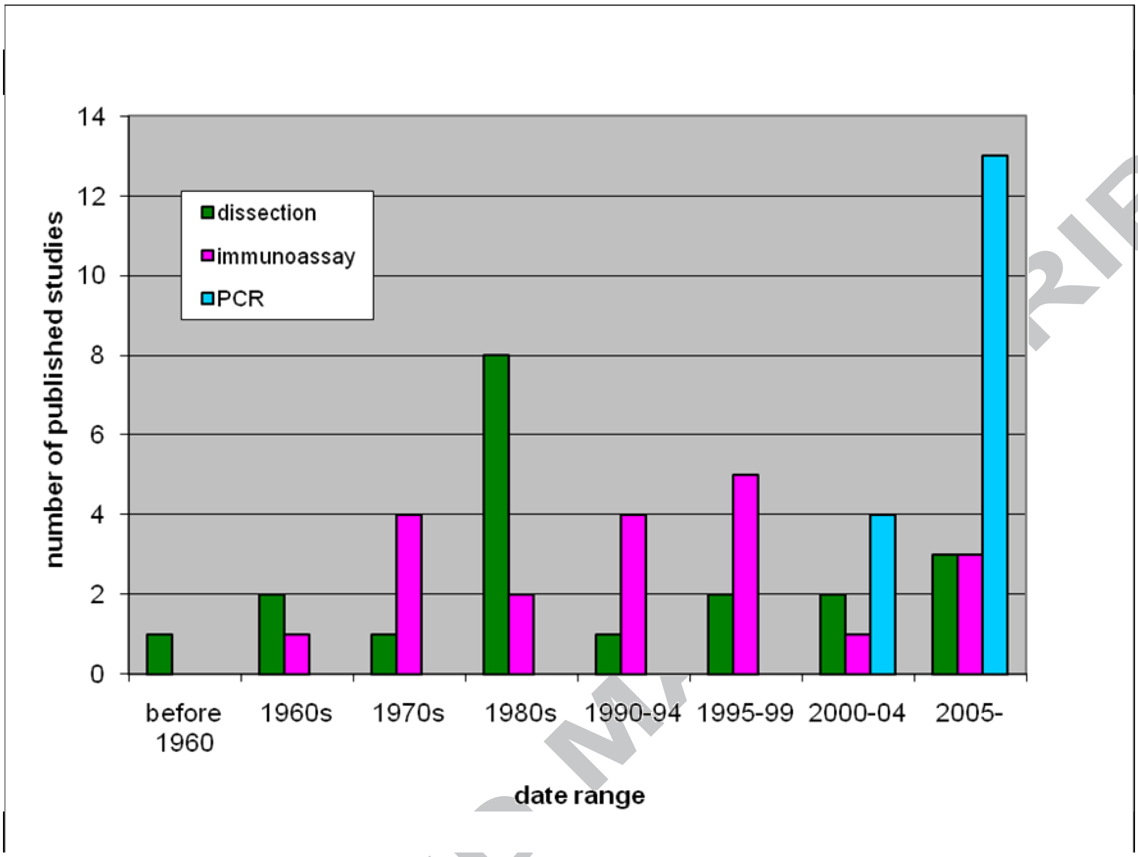
Figure 1. Coccinellid gut content studies, by method, versus year of publication.

Table1. Predation detection studies involving the Coccinellidae: gut dissection and frass analysis.

[attached as Excel spreadsheet]

Table 2: Predation detection studies involving the Coccinellidae: biochemical methods.

[attached as Excel spreadsheet]


14
12
10
8
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number of published studies
dissection
immunoassay
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1960s
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1183

1184

<u>Predator species</u> (coccinellid adults unless noted, with number of individuals dissected)	**<u>habitat</u>**	**<u>location</u>**	**<u>objective(s)</u>**	**<u>techniques</u>**	**<u>reference</u>**
Coleomegilla maculata (De Geer) (14) *Hippodamia convergens* Guérin-Méneville (9) *Hippodamia glacialis* (F.) (4) adults of 4 other species (total 10) and *H. convergens* larvae (2)	various habitats, mostly not where aphids were abundant	USA: Illinois	Determine food of common coccinellids of Illinois in a variety of habitats, especially away from aphids	unspecified collection with subsequent gut dissection	Forbes (1883)
Coleomegilla maculata	corn fields	USA: Delaware	Determine importance of *C. maculata* adults as predators of European corn borer eggs	field deposition of frass under sentinel European corn borer eggmasses as an indicator of predation by *C. maculata*	Conrad (1959)
Adalia bipunctata (L.) (216) *Coccinella trifasciata* L. (73) *Coleomegilla maculata* (79) *Coccinella transversoguttata* Faldermann (66) adults of 5 other species (total 73) and *Adalia bipunctata* larvae (28)	peach orchard	Canada: Ontario	Determine diets of coccinellids in peach orchards, and their importance as biological controls of peach pests	limb-jarring with subsequent dissection or frass examination	Putman (1964)
Rhizobius litura (F.) (adults, number unspecified)	composites and grasses	UK: England	Determine habits of coccinellids in various seasons	unspecified collection with subsequent dissection	Eastop and Pope (1969)
Coccinella septempunctata (74) *Coccinella undecimpunctata* L. (57) 2 other species (4) *Coccinella* sp. larvae (108)	small grains	UK: England	Examine diel pattern of abundance of aphid predators in canopy and ground level in cereal crops; determine by gut dissection or immunoassay frequency of predation for all predators	sweep-netting, vacuuming and hand collection at 3h intervals day and night; Coccinellidae adults and larvae, Carabidae, and adult Staphylidae determined by gut analysis; all others by precipitin tests	Vickerman and Sunderland (1975)
Scymnodes lividigaster (Mulsant) (3,836) *Illeis galbula* (Mulsant) (1,096)	6-ha grassy area with shrubs and trees	Australia: region of Sydney	Determine diets and use of different plants over 2 years in relation to cycles of dormancy and reproduction.	unspecified weekly collections from particular host plants, with subsequent gut dissection	Anderson (1982)
Micraspis lineata (Thunberg) (195 adults and an unspecified number of larvae)	6-ha grassy area with shrubs and trees	Australia: region of Sydney	Determine gut contents for common aphidophagous species through 3 years in relation to dormancy and reproduction	unspecified weekly field collections, with subsequent gut dissection	Anderson and Hales (1983)
Chilocorus bipustulatus (L.)	citrus orchard	Israel	Determine food of adults over 10-month period, compared to field occurence of prey; measure residence time of prey in gut	unspecified collection every 3 weeks; comparison with feeding of known prey in lab	Mendel et al. (1985)

Coccinella septempunctata *Coccinella quinquepunctata* L. 5 other species	trees and herbaceous habitats	Czech Republic	Determine the usefulness of frass production as a measure of aphid or other prey consumption, and of predator satiation	sweep-netting and other collection with subsequent confinement in laboratory with measurement of frass production	Honěk (1986)
Rhizobius litura *(adults and larvae, number unspecified)*	small grains	Italy	Determine diet over season in relation to habitat and management	D-vac with subsequent dissection	Ricci (1986a)
Tytthaspis sedecimpunctata (L.) *(adults and larvae, number unspecified)*	meadows, small grains, sunflower, safflower, fallow fields	Italy	Determine diet over season in relation to habitat and management	D-vac with subsequent dissection	Ricci et al. (1983); Ricci (1986b)
Adalia bipunctata (156 adults)	fruit orchards	Belgium	Determine importance of pollens in spring diet and ovarian maturation	limb-jarring with subsequent dissection	Hemptinne and Desprets (1986)
Propylea quatuordecimpunctata (L.) (number unspecified)	forests, fields, wheat	Belgium	Determine amount and types of pollen in spring	limb-jarring and sweep-netting with subsequent dissection	Hemptinne et al. (1988)
Coccinella septempunctata (number unspecified)	alfalfa, clover, peas	Sweden: region of Uppsala	Determine the importance of C-7 and various generalists as predators of pea aphid, relative to season and numbers of prey	pitfall trapping with subsequent dissection	Ekbom (1994)
Coccinella septempunctata (1803 adults, 175 larvae)	small grains; also fallow, maize, and hibernating locations	Germany: region of Berlin	Document diet of C-7 in relation to season, life-stage, reproduction, and dormancy, habitat and location	Sweep-netting with subsequent dissection	Triltsch (1997, 1999)
Hippodamia convergens	lab, on dogwood (*Cornus florida* L.) (Cornales: Cornaceae)	USA: Tennessee	Determine if *H. convergens* can spread the dogwood anthracnose fungus in its frass, and if chaser diet has an effect	Examination of frass for viable spore counts of *Discula destructiva Redlin* (Fungi imperfecti) conidia	Hed et al. (1999)
Coleomegilla maculata (31 adults, 26 larvae) *Harmonia axyridis* (Pallas) (28 adults, 190 larvae)	corn field before and during pollen-shed	USA: Illinois	Investigate pollen consumption relative to predator for two common coccinellids (adults and larvae) in cornfields	Hand collection before and during pollen-shed, with subsequent dissection to determine proportion of gut contents which was corn pollen	Lundgren et al. (2004)
Coleomegilla maculata (40 adults, 45 2nd, 36 3rd, and 90 4th instar larvae)	corn field during pollen-shed	USA: Illinois	Quantify pollen consumption by *C. maculata* larval instars and adults, under lab and field conditions	Hand collection of larvae and adults, with subsequent dissection and quantification of pollen in adult and larval guts, compared to lab feeding	Lundgren et al. (2005)
Ceratomegilla notata (Laicharting) (180 adults and 120 larvae)	subalpine and alpine pastures and meadows, 800-1700m	Italy: Alps	Study abundance, diet, and foraging behavior	D-vac with subsequent dissection	Ricci and Ponti (2005)

Coccinella septempunctata (240 adults)	8 different habitats, 200-2000m	Italy: Tiber Valley and Alps	Determine *Coccinella septempunctata* prediapause diet	D-vac with subsequent dissection of gut contents and (?) frass	Ricci et al. (2005)

Table 2. Predation detection studies involving the Coccinellidae: biochemical methods.

Predator species	**prey**	**habitat**	**location, or source of lab cultures**	**objective(s)**	**techniques**	**reference**
IMMUNOLOGICAL STUDIES						
Coccinella septempunctata *Propylea quatuordecimpunctata* *Adalia bipunctata* and many (>80) other potential predators	*Conomelus anceps* (Germar) (Hemiptera: Delphacidae)	wetlands dominated by *Juncus* (rushes)	UK: England	Examine population dynamics of a major herbivore, including predation patterns, with aid of immunoassays	precipitin	Rothschild (1966)
Coccinella septempunctata *Adalia bipunctata* and several other predators	*Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)	lab, field (habitat undescribed)	Sweden	develop immunoassay for *R. padi* which is species-specific and detectable in predators	precipitin	Pettersson (1972)
Coccinella undecimpunctata nabids, phalangids, carabids, syrphids	*Pieris rapae* L. (Lepidoptera: Pieridae)	lab, cabbage	New Zealand	Develop immunoassay for prey; determine detectability time-course; sample predators in field for 2 years	precipitin	Ashby (1974)
Coccinella septempunctata	*Acrythosiphon pisum* (Harris) (Hemiptera: Aphididae)	lab, field (habitat undescribed)	UK	Establish immunoassay for pea aphid	precipitin	Chiagu and Boreham (1978)
Coleomegilla maculata 4 species of predatory bugs	*Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae)	lab; apple orchards	USA: Vermont	Develop antibodies for prey; determine detectability time-course; sample predators in field	precipitin	Whalon and Parker (1978)
Coccinella undecimpunctata nabids, hemeriobiids, phalangids	*Acrythosiphon pisum* *Acrythosiphon kondoi* (Shinji) (Hemiptera: Aphididae)	alfalfa	New Zealand	Assess predators of alfalfa aphids using an immunoassay with sweep-netting during day and night time	precipitin	Leathwick and Winterbourn (1984)
Coccinella septempunctata *Propylea quatuordecimpunctata* *Exochomus quadripustulatus* (L.) and coccinellid larvae	Psocoptera, Psyllidae, Collembola	larch (*Larix decidua*)	UK: England	Develop immunoassays and determine predators of insects feeding on epiphytes of larch bark.	precipitin	Turner (1984)
Coccinella septempunctata *Adalia bipunctata* coccinellid larvae and several other predators	*Aphis pomi* DeGeer (Hemiptera: Aphididae)	apple orchard	Canada: Ontario	Develop immunoassay for green apple aphid and determine importance of predators	polyclonal Ab with immunoelectrophoresis	Hagley and Allen (1990)
Coccinella septempunctata	*Mythimna separata* (Walker) (Lepidoptera: Noctuidae)	lab; wheat	China: Henan and Jiangsu	Develop ELISA assay for oriental armyworm; determine detectability time-course for *Pardosa*; determine main predators	ELISA, unspecified	Huang et al. (1992)

Hippodamia convergens *Collops, Geocoris*, *Orius*	*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)	lab	USA: Arizona	Mark prey with rabbit IgG and determine usefulness as marker to detect predation by four species	ELISA (sandwich) following marking of prey with rabbit IgG	Hagler and Durand (1994)
Hippodamia convergens *Collops vittatus* (Say)	*Bemisia tabaci* *Pectinophora gossypiella*	lab; cotton fields	USA: Arizona	Use double diagnostic to determine predation in 2 cotton fields by 2 beetles predators over growing season	ELISA (indirect) with 2 monoclonal Abs	Hagler and Naranjo (1994)
Hippodamia convergens *Collops, Geocoris*, *Orius* and others	*Bemisia tabaci* *Pectinophora gossypiella*	cotton fields	USA: Arizona	Use double diagnostic to determine predation in 2 cotton fields by 9 predators over growing season	ELISA (indirect) with 2 monoclonal Abs	Hagler and Naranjo (1996)
Menochilus sexmaculatus F. 3 other predators and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae	*Helicoverpa armigera*eggs	pigeonpea, sorghum	India: Andhra Pradesh	Apply egg-specific heliothine assay of Greenstone and Trowell (1994) to determine importance of predators in damaged crops	ELISA (indirect) with monoclonal Ab	Sigsgaard (1996
Hippodamia convergens, Geocoris, *Orius*	*Pectinophora gossypiella* eggs	lab	USA: Arizona	Test effects of temperature, time, and meal size on detection in 3 predators	ELISA (indirect) with monoclonal Ab	Hagler and Naranjo (1997)
Hippodamia convergens	*Pectinophora gossypiella* eggs	lab	USA: Arizona	Test effects of temperature, time, and meal size on detection in 3 predators	ELISA (indirect) and dot blot with monoclonal Ab	Hagler et al. (1997)
Hippodamia convergens	*Pectinophora gossypiella* eggs	lab	USA: Arizona	Test effects of 5 different immunoassays on detection of prey in predator	ELISA (indirect, direct, sandwich), dot blot and Western blot with monoclonal Ab	Hagler (1998)
Hippodamia convergens	*Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae)	cotton, cantalope	USA: Arizona	Track movement and whitefly feeding of released and native *H. convergens*	ELISA (sandwich) with chicken and rabbit IgGs; ELISA (indirect) with whitefly monoclonal Ab	Hagler and Naranjo (2004)
Coccinella septempunctata *Cycloneda munda* *Harmonia axyridis* *Coleomegilla maculata*	Cry1Ab-endotoxins from transgenic corn	corn field	USA: Kentucky	Test herbivore and predators for movement of BT toxins in food-web	ELISA (sandwich)	Harwood et al. (2005)
Harmonia axyridis *Chrysoperla carnea*	*Homalodisca coagulata* (Say) *Homalodisca liturata* Ball (Hemiptera: Cicadellidae)	lab; shrubs and trees	USA: California	Develop immunoassay specific to prey: sharpshooter eggs	ELISA (indirect and sandwich; sandwich superior) with monoclonal Ab	Fournier et al. (2006)
Coccinella septempunctata *Cycloneda munda* *Harmonia axyridis* *Coleomegilla maculata*	Cry1Ab-endotoxins from transgenic corn	corn field	USA: Kentucky	Test coccinellids for internal Bt toxins before and during pollen-shed	ELISA (sandwich)	Harwood et al. (2007b)
Coccinella variegata (Goeze) *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae)	*Helicoverpa armigera*	lab; cotton	Australia: Narrabri, NSW	Compare value and sensitivity of specific immunoassay versus immunomarker applied to H. armigera eggs in lab and field	ELISA (indirect) for prey eggs; ELISA (sandwich) for anti-rabbit IgG label	Mansfield et al. (2008)

DNA PCR STUDIES						
Hippodamia convergens *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae)	*Rhopalosiphum maidis* *Rhopalosiphum padi* and 4 other grain aphids	lab	USA: Oklahoma	Distinguish 6 common aphids in 2 predators by PCR; determine time course and sensitivity of detection method for *R. maidis* markers	conv. PCR (mito. CO-II, 3 markers: 198, 246and 339 bp) after -20C dry freezing	Chen et al. (2000)
Coleomegilla maculata	*Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)	lab	USA: Minnesota	For common European corn borer predator, determine detectability time-course for 4 marker sequences versus time, meal size, predator weight, sex, or life stage (4th instar vs. adult)	conv. PCR (4 markers in nuclear ITS-1: 150, 256, 369, and 492 bp) after -20C dry freezing then -20C in 70% EtOH	Hoogendoorn and Heimpel (2001)
Harmonia axyridis *Coleomegilla maculata*	*Ostrinia nubilalis*	lab, corn field	USA: Minnesota	For *Harmonia*, determine detectability time-course and if different from *Coleomegilla*, and sample field populations provided ECB eggs in plots	same as Hoogendoorn and Heimpel (2001)	Hoogendoorn and Heimpel (2003)
Curinus coeruleus Mulsant	*Scotorythra rara* Butler (Lepidoptera: Geometridae) *Eupithecia monticolans* Butler (Lepidoptera: Geometridae)	lab	USA: Hawaii	Develop specific marker for later testing of exotic predators of prey of conservation concern	conv. PCR (mito. CO-I of 140, 151, and 170 bp) after killing by immersion in 100% EtOH or crushing between filter paper and air-drying	Sheppard et al. (2004)
Coccinella septempunctata *Propylea quatuordecimpunctata* *Harmonia axyridis* *Coleomegilla maculata*	*Coccinella septempunctata* *Propylea quatuordecimpunctata* *Harmonia axyridis* *Coleomegilla maculata*	lab	Canada: Québec	Determine feasibility of detection of IGP by 4 coccinellid species by PCR, testing egg consumption by last instar larvae	conv. PCR (nuclear ITS-1 of 105, 115, and 120 bp resp.; CO-I, 137 bp, for *C. maculata*) after -80C dry freezing	Gagnon et al. (2005)
Coleomegilla maculata *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae)	*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)	lab	USA: Maryland	Develop specific prey marker and determine detectability time-course in two important predators	conv. PCR (mito. CO-I, 214 bp) after -20C dry freezing	Greenstone et al. (2007)
Orius insidiosus (Say) (Hemiptera: Anthocoridiae)	*Harmonia axyridis* *Neohydatothrips variabilis* (Beach) (Thysanoptera: Thripidae) *Aphis glycines* Matsumura (Homoptera: Aphididae)	lab and soy fields	USA: Indiana	Determine predation patterns for *Orius*, including intra-guild predation of *Harmonia* eggs and larvae	conv. PCR (mito. CO-I, 261 bp for *Harmonia*; 160 to 255 bp for others) after -20C dry freezing, then placement in 95% EtOH (lab) or on ice until -80C dry freezing (field collections)	Harwood et al. (2007a)
Adalia bipunctata	*Rhopalosiphum padi*	lab	Sweden	Determine effect of time and temperature on probability of prey detection by PCR	conv. PCR (mito. CO-II, 331 bp) after -70C dry freezing	McMillan et al. (2007)
Propylea japonica (Thunberg) (lab, field) *Coccinella septempunctata* (field) *Harmonia axyridis* (field) *Scymnus hoffmanni* Weise (field) and additional predators (field)	*Bemisia tabaci*	lab, cotton field	China: Beijing area	Develop specific prey marker; determine detectability time-course in *P. japonica*; survey predators in field for marker	conv. PCR (SCAR, 240 bp) after -70C dry freezing (lab) or on ice until -70C dry freezing (field collections)	Zhang et al. (2007a)

Propylea japonica (lab, field) *Harmonia axyridis* (field) *Scymnus hoffmanni* (field) and additional predators (field)	*Bemisia tabaci* Biotype B	lab, cotton field	China: Beijing area	Develop marker specific to Biotype B; quantify meal size and decay curves as well as survey predators in field	quantitative PCR (SCAR, 93 bp) after -70C dry freezing (lab) or on ice until -70C dry freezing (field collections)	Zhang et al. (2007b)
Harmonia axyridis *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) *Zelus renardii* (Kolenati) (Hemiptera: Reduviidae)	*Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae)	lab	USA: California	Develop marker specific to prey, glassy-winged sharpshooter; determine detectability time-course; compare with ELISA of Fournier et al. (2006)	conv. PCR (mito CO-I, 197 bp) after -80C dry freezing, as well as ELISA as in Fournier et al. (2006)	Fournier et al. (2008)
Hippodamia variegata (Goeze) *Nabis kinbergii* (Reuter) (Heteroptera: Nabidae) *Venator spenceri* Hogg (Araneae: Lycosidae)	*Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae)	lab	Australia	Determine effects of time, temperature, chaser diet, sex and weight on probability of prey detection by PCR.	conv. PCR (mito. CO-I, 293 bp) after -80C dry freezing	Hosseini et al. (2008)
Serangium sp. Syrphid larvae	*Bemisia tabaci*	cassava	Uganda	Determine important predators on whitefly vector of cassava mosaic virus	conv. PCR (mito. CO-I, 814 bp) with room-temp. 80% EtOH	Rowley et al. (2008)
Harmonia axyridis *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae)	*Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae)	lab and corn fields	China	Develop *R. maidis* marker, determine detectability time-course, sample field for presence in predators	conv. PCR (mito CO-II, 339 bp) after -20C dry freezing (within 1h for field collections)	Song and Cong (2008)
Harmonia axyridis *Propylea japonica* *Chrysopa pallens*	*Aphis glycines*	lab and soy fields	China	Develop *A. glycines* marker, determine detectability time-course, sample field for presence in predators	conv. PCR (two markers of mito CO-I, 197 and 253 bp) after -20C dry freezing (within 1h for field collections)	Song et al. (2008
Orius insidiosus	*Harmonia axyridis* *Neohydatothrips variabilis* *Aphis glycines*	lab and soy fields	USA: Indiana	Determine predation patterns for *Orius*, including intra-guild predation of adults and nymphs on *Harmonia*	conv. PCR (markers as in Harwood et al., 2007) after placement in 95% EtOH, then -20C freezing	Harwood et al. (2009)
Coleomegilla maculata	*Leptinotarsa decemlineata*	lab	USA: Maryland	Determine quantitative disappearance of marker by qPCR based on time,	quantitative PCR (mito. CO-I, 214 bp) with various preservation tests	Weber and Lundgren (2009)
CHROMATOGRAPHY STUDIES						
Coccinella septempunctata *Coccinella quinquepunctata* *Propylea quatuordecimpunctata*	*Macrosiphum albifrons* Essig (Hemiptera: Aphididae)	lab	Belgium	Determine effect of alkaloids of *Lupinus* spp. host plants (4 bitter, 3 non-bitter, plus pea control) on larval development of coccinellids	GC analysis of lupine alkaloids in host plant and in aphids, combined with laboratory feedings of coccinellid larvae	Emrich (1992)
Harmonia axyridis	*Adalia bipunctata* *Coccinella septempunctata*	lab, potato fields	Belgium	Determine method and residence time for 2 alkaloids in Harmonia, with a small field sample	GC-MS of coccinellid prey alkaloids	Hautier et al. (2008)

Harmonia axyridis *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae)	*Hippodamia convergens*	lab	USA: Kentucky	Determine method and residence time for hippodamine in Harmonia and Chrysoperla, demonstrate quantification, determine alkaloids for 6 common spp.	GC-MS of coccinellid prey alkaloids	Sloggett et al. (2008)