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It's Gut Check Time! A Universal Food Immunomarking Technique for Studying Arthropod Feeding Activities

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Abstract

The analysis of arthropod feeding activity is often determined by using species-specific postmortem gut content polymerase chain reaction and enzyme-linked immunosorbent assays (ELISA). Such mono-specific assays require time, resources, and technical expertise to develop for the food item (usually a pest insect species) that is the target of the investigation. A generic predator gut analysis method was described over a quarter of a century ago that does not require the development of a species-specific gut assay. This generic method remained in relative obscurity until about a decade ago. Recently, it has been used to study a wide range of arthropod feeding activities, such as carnivory, herbivory, scavenging, and other feeding interactions. For this review, I have coined this method as the universal food immunomarking technique (UFIT). The UFIT consists of tagging food items (i.e., prey, foliage, carrion, etc.) with a specific protein. In turn, the gut contents of foraging arthropods are examined for the presence of protein-marked food items by a standardized protein-specific sandwich ELISA. In this article, I give examples of the benefits of the UFIT gut assay approach over prey-specific gut assay approaches and tips on conducting a successful UFIT experiment, and provide examples of how it has been adapted to study a wide variety of arthropod feeding behaviors. My goal is to make researchers aware of another valuable tool in the gut analysis toolbox.

Key words: gut analysis, feeding behavior, ELISA, carnivory, herbivory

A novel predator gut content enzyme-linked immunosorbent assay (ELISA) was described a quarter-century ago (Hagler and Durand 1994)—an adaptation of a protein immunomarking technique described 2 yr earlier (Hagler et al. 1992a). The procedure consists of marking potential prey items with a unique protein biomarker. In turn, consumed protein-marked prey items are identified by post-mortem analysis of the predator using a protein-specific ELISA. The novelty of this gut analysis assay is that unlike the conventional prey-specific ELISA and polymerase chain reaction (PCR) gut assays, it does not require the development of a prey-specific probe (i.e., a monoclonal antibody [MAb] for ELISA and DNA primer for PCR). Although the original immunomarking method was described to study predator–prey interactions, it has since been adapted to study arthropod herbivory, nectivory, scavenging, cannibalism, and other interactions. As such, I have coined the procedure as the Universal Food Immunomarking Technique (UFIT). The two protein biomarkers used to date for UFIT research include rabbit immunoglobulin G (IgG) and chicken IgY (often referred to as chicken IgG). The IgG-marked food items are detected by the original and steadfast anti-IgG sandwich ELISAs described over 20 yr ago (Hagler and Durand 1994, Hagler 1997a).

The UFIT has many features that make it an ideal tool for studying many aspects of arthropod feeding activity. Below I review its key attributes and limitations, provide suggestions for conducting a successful UFIT study, and highlight a wide variety of published studies that have used the procedure. My primary objective was to raise awareness among researchers that this technique is well suited for deciphering a wide range of arthropod feeding activities (not just predation), many of which are not possible using prey-specific assay approaches.

Problems Studying Arthropod Food Consumption

Observing arthropod food intake in nature is difficult because of the small size and cryptic behavior of both the consumer (i.e., carnivore, omnivore, or herbivore) and, often, the meal. Moreover, observing arthropod feeding events in nature is usually disruptive to the normal foraging process. Consequently, direct observation of feeding activity (especially for entomophagous arthropods) is

difficult. Additionally, most entomophagous arthropods do not leave any indirect evidence at the scene of a feeding site; most arthropods with chewing mouthparts devour their meal in entirety and those with piercing-sucking mouthparts do not leave species-specific wound sites on their victims. These difficulties have forced researchers to seek indirect methods for assessing arthropod feeding behavior. One of the most common methods is postmortem analysis of arthropod gut contents using food- or prey-specific ELISA and PCR assays (Greenstone and Morgan 1989; Hagler et al. 1992b, 2018a; Greenstone 1996; Harper et al. 2005; Sheppard and Harwood 2005; Gariepy et al. 2007; King et al. 2008; Hagler and Blackmer 2013, 2015). Prey-specific assays have proven very useful for qualitative evaluations of arthropod feeding activity in open-field conditions. However, there are drawbacks to these techniques that limit the quality of data they yield. Many of these drawbacks can be overcome by using the UFIT gut analysis approach.

Limitations of Food- and Prey-Specific Gut Assays

Most arthropod gut analysis research conducted to date has focused on investigating predator–prey interactions (Greenstone 1996, Sheppard and Harwood 2005, King et al. 2008). Specifically, prey-specific assays have been developed to identify biological control agents of major agricultural pests. Of the two most common prey-specific gut assays (ELISA and PCR), PCR has been the predominant method used over the past 15 yr (King et al. 2008). The recent popularity of the PCR assay approach is due, in large part, to the fact that PCR assays are relatively inexpensive and easy to develop when compared to a prey-specific MAb-based ELISA (Greenstone and Shufran 2003, Monzó et al. 2010). However, there are trade-offs when using PCR in place of ELISA. Specifically, mass screening arthropods for prey-specific DNA by PCR remains much more expensive, time-consuming, and labor-intensive than by ELISA (Fournier et al. 2008, Hagler and Blackmer 2013).

Food- or prey-specific assays, whether PCR or ELISA, have inherent limitations in the quality of the data they produce. Food-specific assays are susceptible to yielding various types of false-positive (FP) assay reactions. Three potential kinds of FP reactions are as follows: 1) scavenging events, 2) food-chain errors (secondary feeding), or 3) failed predation attempts.

Arthropods scavenging for food is ubiquitous. Unfortunately, it is well documented that prey-specific assays cannot differentiate between viviphagy and necrophagy (Foltan et al. 2005, Juen and Traugott 2005, Sheppard and Harwood 2005). Any 'predator' that frequently feeds on cadavers of the targeted prey will yield a FP assay error for a predation event. Such FPs will result in gross overestimations of the biological control services rendered by that predator species. The UFIT is an ideal tool for examining arthropod scavenging activity as I highlighted below.

A food chain feeding error is also a well-known source for yielding FP gut assay reaction (Harwood et al. 2001, Sheppard et al. 2005). Food chain errors occur when a high-tiered (secondary) predator yields a positive assay reaction because of a predation event on a lower-tiered predator that had previously eaten the 'targeted prey' item. In this scenario, the actual predator of the targeted prey is reduced in or eliminated from the ecosystem and, in turn, the secondary predator is falsely credited for providing the biological control services. Harwood et al. (2001) examined this phenomenon in an aphid–spider–carabid system (i.e., pest–primary predator–secondary predator) using an aphid-specific indirect ELISA. That study showed that FP errors in this limited food chain scenario were rarely detected under realistic field conditions. However, in an almost identical study using an aphid-specific PCR assay, Sheppard et al. (2005) showed that aphid DNA remains were readily detected in carabid beetles that consumed spiders (the true aphid predator). The conclusion drawn from that study was that FP food chain errors were more prevalent with the aphid-specific PCR assay than the aphid-specific indirect ELISA. The UFIT has also been shown to be vulnerable to FP food chain errors. That study showed that rabbit IgG marker readily passed from the protein-marked prey to the primary predator and then to the secondary predator (Hagler 2016). The detectability of the movement of the IgG through the food chain is likely due to the high sensitivity of the sandwich ELISA format, which is more sensitive than the indirect ELISA gut assay format (see below).

Food-specific assays also cannot pinpoint the amount of food consumed by an individual arthropod. The actual amount of food eaten by an arthropod is not quantifiable due to several uncontrollable biotic (e.g., digestion, meal size, metabolic status, etc.) and abiotic variables (e.g., ambient temperature, humidity, etc.). All of these contributing factors are well documented (Sopp et al. 1992, Greenstone 1996, Hagler and Naranjo 1996, Sunderland 1996, Naranjo and Hagler 1998). In short, mono-specific food detection assays only yield qualitative data. The UFIT, in most cases, also shares this limitation. However, under certain experimental conditions, the UFIT can be adapted to quantify food intake by arthropods (see below).

An unsuccessful predation attempt could also generate an FP gut analysis assay error. A 'failed predation attempt' hypothesis was proposed by Hagler and Naranjo (1996) as a potential source of gut analysis error. It seems plausible that a predator could ingest (or obtain by contact exposure) enough prey protein (for ELISA) or DNA (for PCR) to yield a positive gut assay reaction but fail to kill the targeted prey item. To my knowledge, the vulnerability of prey-specific assays to this type of FP error has not been investigated. However, UFIT has been examined for yielding FP errors due to failed predation attempts (Hagler et al. 2018b). A series of feeding studies conducted with chewing and piercing-sucking type predators exposed to internally or externally IgG-marked prey items showed that the UFIT was effective at detecting IgG remnants in the predators that successfully killed their prey, regardless of the prey marking treatment. However, there were some FP assay errors recorded, primarily for the chewing predator species that had extended contact (i.e., a failed predation attempt) with externally marked prey items. The conclusion drawn was that the FP assay error rate was small, but, when possible, UFIT research should be conducted on internally marked prey items to minimize the chances of obtaining this type of FP assay error. This type of FP error also needs to be examined using the prey-specific gut assay approaches.

An obvious limitation with prey-specific gut assay approaches is that they cannot be used to study intraspecific predation (i.e., cannibalism). Many arthropods are inclined to engage in cannibalism (Polis 1981, Sigsgaard 1996, Wise 2006, Richardson et al. 2010). Like predators that frequently scavenge for food, cannibals can diminish the biological control services provided by any given arthropod community. Another advantage of the UFIT is that it can be adapted to study arthropod cannibalism (see below).

Species-specific PCR assays and most ELISAs cannot differentiate predation events between the various life stages of a targeted prey species. There have been a few species- and egg-specific gut ELISAs developed (Hagler et al. 1991, 1993, 1994; Fournier et al. 2006). These assays can detect predation events on eggs and gravid females, but they are unable to detect predation events on larvae, pupae, and adult males. Again, I will give many examples below of how the UFIT can be adapted to identify life stage-specific predation events.

The Relative Sensitivity of the UFIT and Prey-Specific Assays

There is an implication that prey-specific PCR gut assay is more effective than the prey-specific ELISA approach (Symondson 2002, Sheppard et al. 2005). I believe this is one of two reasons that the prey-specific ELISA procedure was summarily abandoned as a tool for predator gut analysis about 15 yr ago (the other being that some falsely claimed that the PCR was a more cost-effective approach for mass-screening predator specimens). It is noteworthy that most prey-specific gut ELISAs developed during the ELISA gut assay 'revolution' (the 1990s to early 2000s) used the indirect ELISA format (Greenstone and Morgan 1989; Hagler et al. 1991, 1993, 1994; Symondson and Liddell 1993; Symondson et al. 1999). Just before the PCR assay 'revolution' (the early 2000s to present), a study compared the efficacy of various ELISA formats (i.e., indirect, direct, and sandwich ELISA, dot blot and Western blot) at detecting prey remains in predators (Hagler 1998). That study revealed that the sandwich ELISA format was much more effective than the conventional indirect ELISAs. Subsequently, the UFIT ELISA procedure was compared to an anti-*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) indirect ELISA. Mansfield et al. (2008) marked *H. armigera* eggs with rabbit IgG. In turn, beetles that consumed a protein-marked egg were examined by both an *H. armigera*-specific indirect ELISA (Trowell et al. 2000) and the standardized anti-rabbit IgG sandwich ELISA used for UFIT studies (Hagler and Durand 1994). Data revealed that anti-rabbit IgG ELISA had a much higher detection rate for egg predation than the prey-specific indirect ELISA under controlled and open field conditions. Recently, the UFIT was compared to an established PCR assay designed to detect *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Hagler et al. 2015). That study revealed that both tests were effective at identifying *C. carnea* predation for several hours after a meal, but the sandwich ELISA was more reproducible. Specifically, when each predator was examined in triplicate for evidence of a predation event on *C. carnea*, the ELISA subsamples consistently yielded the same outcome. Conversely, the PCR assay was less dependable. Specifically, many of the triplicate subsamples yielded FPs. This outcome was especially surprising because the predator sample preparation was biased for the PCR assay (i.e., the PCR sample preparation protocol was used and the leftover sample buffer was used for the sandwich ELISA), and the prey-marking protocol was not as stringent (i.e., a relatively low concentration of IgG marker was used to externally mark the *C. carnea* larvae) as those employed in previous studies. In short, the standardized sandwich ELISA used for the UFIT outperformed both types of prey-specific assays (Mansfield et al. 2008, Hagler et al. 2015).

The Three Steps of a UFIT Experiment

A typical UFIT study comprised of three simple steps: 1) administering the protein mark to the food item, 2) collecting and preserving the potentially protein-marked consumer arthropods, and 3) analyzing the consumer arthropods for the presence of the biomarker protein.

Administering Protein Marks to Food Items

External Marking Techniques

Virtually any terrestrial food item (e.g., prey, plant tissue, etc.) can be externally marked by spraying it with a low volume and concentration of IgG protein solution. For large or robust items (e.g., adult beetles, eggs, pupae, plant parts, etc.), the biomarkers can be applied topically with a hand-held spray bottle (Hagler 1997a, Rendon et al. 2018), air paintbrush (Blackmer et al. 2006), or perfume atomizer. Immobile food items (e.g., insect eggs and pupae, plant seeds, etc.) and hardy arthropods (e.g., beetles, large larvae, etc.), can be doused or even submerged briefly into a protein marking solution (Hagler and Miller 2002, Williams et al. 2013, Mansfield and Hagler 2016). For small and delicate food items (e.g., parasitoids, mosquitoes, aphids, whiteflies, small larvae, etc.), I recommend applying the marker with a medical nebulizer (Hagler 1997b, 2006; Hagler and Jackson 1998; Hagler et al. 2002). A nebulizer produces a uniform, fog-like mist that does not appear to have any adverse effects on delicate arthropod species.

Internal Marking Techniques

Some types of prey can be marked internally by feeding them IgG-laced food items. For example, minute parasitoids have been internally marked by eating protein-marked sugar or honey solutions. These parasitoid species retained the IgG marker throughout their adult lifespan (Hagler 1997b, Hagler and Jackson 1998, Hagler et al. 2002) and the IgG marker was subsequently detectable in the guts of predators that consumed the parasitoids (Hagler 2006). Arthropods reared on an artificial diet can be easily marked by incorporating a small amount of IgG into the food. For example, early instar pink bollworm larvae fed a diet containing rabbit IgG retained the biomarker throughout their subsequent instar life stages, prepupae and pupae, though not as adults (Hagler and Miller 2002). I unwittingly learned that IgG protein markers might already be present in some types of artificial diet. For example, raw chicken egg (which contains chicken IgY) is an ingredient in a common *Lygus hesperus* Knight (Hemiptera: Miridae) diet (Debolt 1982). The IgY in the egg product of that diet proved sufficient to mass mark *L. hesperus*. In such cases, adding an IgG marker to the food might not even be necessary. Additionally, researchers should be aware of the potential for FPs generated by an overlooked ingredient in an insect diet.

One important source of false-negative (FN) assay error when using internally marked prey items is the failure of the prey to ingest the marked diet. Unless each prey insect is directly observed feeding on the protein-laced diet (which may be quite labor-intensive in large-scale studies), it is possible that any given prey item may have failed to take on the internal mark. It is, therefore, advisable to retain a subsample of ostensibly marked prey and assay these individual insects directly for the presence of the IgG marker to determine the potential FN rate for a given prey type.

To date, internal marking techniques for UFIT research has been reserved for only marking prey items that will feed on protein marker-laced foodstuffs. However, preliminary studies indicate that a syringe can be used to inject protein markers into specific sites on a plant (J.R.H., in preparation). Studies are underway to help elucidate herbivore foraging preferences on various types of plant tissues (i.e., flowers, fruits, seeds, etc.). This is an area for further research.

Collecting and Handling Protein-Marked Arthropods

A potential source for an FP assay error is by contamination of unmarked specimens during the collection and handling processes. As such, some thought needs to go into developing a reliable sampling protocol for any UFIT study. An adequate sampling scheme should efficiently capture enough of the arthropods of interest without compromising the integrity of the marked and unmarked specimens. A few studies have been conducted to identify reliable methods to sample arthropods for prey-specific gut analysis and protein immunomarking dispersal research (Harwood 2008, Chapman et al. 2010, Greenstone et al. 2011, Hagler and Blackmer 2013). The

information provided in these studies also pertain to UFIT research and should be read for additional information (also see Hagler 2019 in this volume).

Due to the versatility of the UFIT procedure (see the many examples below), it is impossible to provide specific guidelines for sampling arthropods for any given study. The sampling techniques chosen will ultimately depend on the circumstances of the experimental design (e.g., open field, field cage, etc.) and the type of feeding behavior under investigation (i.e., carnivore, herbivore, etc.). However, one constant among UFIT studies is the critical importance of immobilizing arthropod specimens by freezing as soon as possible after they are collected. Immobilization by freezing serves two purposes: 1) it ensures that the arthropods do not come into extended contact with the protein-marked food item (e.g., protein-marked prey or plant material) during the sampling process and 2) it halts their metabolic process. In isolated field situations, this is best accomplished by placing the specimens in an ice chest containing dry ice. To date, many UFIT studies have been conducted on whole plants contained within individual cages in a greenhouse or a field close to my laboratory (Hagler 2006, 2011; Mansfield and Hagler 2016). In such cases, the whole plants can be cut at their base and frozen in entirety in a −20 or −80°C freezer within minutes after harvesting.

Analyzing Arthropods for Protein-Marked Food Remains

Individual frozen arthropods are typically placed into a 1.6-ml microcentrifuge tube, to which 500 to 1,000 µl of Tris-buffered saline or phosphate-buffered saline is then added. Each specimen must be homogenized with a clean tissue grinder to expel the arthropod's stomach contents into the sample buffer. The samples should be thoroughly mixed before analysis to ensure uniform suspension of stomach contents into solution.

Arthropod samples are analyzed to detect the presence of the marked food item by well-established anti-IgG sandwich ELISAs (Hagler et al. 1992a, Hagler 1997a). These same assays have been used faithfully for decades to study a wide variety of arthropod feeding activities (see the many examples given below). These assays have many features that make them an ideal tool for predator gut analysis. First, the ELISAs have been standardized and the ingredients needed to conduct the assays are commercially available at an affordable price. This means that there is no assay development or optimization required. Second, the sandwich ELISAs are easier to learn and perform than the PCR assay. A novice can learn the ELISA in 1–2 d, and the assay does not require a multitude of micro-pipetting steps. Third, the ELISA does not generate hazardous waste. Fourth, the sandwich ELISA is more reliable at detecting food remains in homogenized arthropod samples than the more commonly used prey-specific indirect ELISA (Hagler 1998; Fournier et al. 2006, 2008; Mansfield et al. 2008) and PCR formats (Hagler et al. 2015). Fifth, the sandwich ELISA is much less expensive than the PCR assay (Fournier et al. 2008, Hagler et al. 2015). It is also better suited for mass throughput (Fournier et al. 2006). My laboratory staff can process over 1,000 arthropod samples per day at the cost of approximately US$0.15 per sample (J.R.H., personal observation).

The sandwich ELISA consists of six simple steps. The steps involved in the ELISA along with the reagents used in each step are given in Table 1. A detailed description of each step of the sandwich ELISA and a simple flow diagram of the procedure is given in this volume (Hagler 2019).

Scoring Specimens for the Presence of a Marked Food Item

Another advantage of ELISA is that the outcome of each assay can be quantitatively measured. The measurement is a chromogenic reading that is proportional to the amount of the targeted protein mark sandwiched between the antibodies used in the ELISA. This avoids the nonsubjective scoring of the specimens as is typical with the PCR assay approach. Moreover, it provides a better means to depict the data in the tabular or graphical form. That is, both the ELISA optical density values and percentages of the population scoring positive for presence of the mark can be reported. Again, a review of the various methods used to score arthropod specimens by ELISA for the presence or absence of targeted proteins is given in this volume (Hagler 2019).

Examples of UFIT Studies

The impetus for developing the UFIT was to create a generic and easy-to-use tool for predator gut analysis that did not require the development of a prey-specific assay (Hagler and Durand 1994). Below are some examples of how the UFIT has been employed to examine a broad range of arthropod feeding activities. It is important to note that every study listed below used the same standardized ELISA(s) described by Hagler (1997a).

Simple Trophic Level Studies

The UFIT was used to investigate the efficacy of an augmentative biological control agent on hornworm caterpillars, *Manduca sexta* L. (Lepidoptera: Sphingidae). Kelly et al. (2014) examined the open-field predatory activity of a stink bug, *Podisus maculiventris* Say (Hemiptera: Pentatomidae), on IgG-marked hornworm caterpillars. The field site contained strategically placed stink bug pheromone lures that served to retain mass released stink bugs and herbivore-induced volatile plant lures (methyl salicylate [MeSA]) to retain the hornworms. Data revealed that 4% of the recaptured stink bugs tested positive for the protein used to mark hornworms, thus showing that hornworm predation occurred, but at a low frequency. However, the study also revealed that protein-marked caterpillars were depleted by predators at a higher rate in stink bug-augmented tomato plots that occurred near the MeSA and pheromone lures. Additionally, wolf spider predation was recently examined on rabbit IgG-marked *Helicoverpa* spp. (Lepidoptera: Noctuidae) larvae that were strategically placed in a cotton field (Rendon et al. 2018). Their study showed that 2.1% of field-collected spiders examined tested positive for the presence of the IgG marker.

Some types of prey items can be marked directly in their habitat by adding IgG to a baited feeding station (a self-marking technique). For example, trophic interactions between woodland ants, *Aphaenogaster rudis* (Emery) (Hymenoptera: Formicidae), and subterranean termites, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae), was determined by feeding termites (prey) paper impregnated with rabbit IgG in order to internally mark them. Subsequently, the intercolony distribution (movement of the consumed prey item) of the marked termite prey items was monitored within the ant colony (Buczkowski and Bennett 2007).

Multiple Trophic Level Studies

The UFIT has been used to decipher complex trophic level interactions in agroecosystems. Such studies are often conducted in concert with field cage methodologies. For example, the UFIT and field cage methods were used in combination to investigate the interguild and intraguild predator activity among members of the cotton arthropod assemblage (Hagler 2006). The cage treatments contained

Table 1. Step-by-step instructions for conducting the standardized anti-rabbit IgG and anti-chicken IgG sandwich ELISA used for UFIT research[1]

Assay	Step	Description	Dilution	Volume	Duration	Vendor[2]	Catalog #
Anti-rabbit IgG	**1**	Coat each ELISA sample well with IgG antibody (primary antibody)	1:500 in TBS	50 µl per well	≥60 min	Sigma Chemical Co.	R2004
	→	Discard the IgG antibody from each well					
	2	Coat each well with blocking solution	1% nonfat milk mixed in dH_2O	300 µl per well	≥30 min	Local supermarket	
	→	Discard the blocking solution					
	3	Add arthropod samples to individual sample wells[3]	See footnote 3	100 µl per well	≥60 min		
	→	Discard the samples from each well					
	→	Wash each well 3× with TBS	Undiluted	300 µl per well	Briefly		
	4	Add IgG antibody containing the HRP conjugate (secondary antibody)	1:2,000 in TBS	50 µl per well	≥60 min	Sigma Chemical Co.	A6154
	→	Wash each well 3× with TBS	Undiluted	300 µl per well	Briefly		
	5	Add TMB substrate solution	Undiluted	50 µl per well	10 min	BioFx Laboratory, Inc.	TMBW-1000–01
	6	Read ELISA reactions with microplate reader set at 650 nm					
Anti-chicken IgG	**1**	Coat each ELISA sample well with IgY antibody (primary antibody)	1:500 in TBS	50 µl per well	≥60 min	Sigma Chemical Co.	C6409
	→	Discard the IgY antibody from each well					
	2	Coat each well with blocking solution	1% nonfat milk mixed in dH_2O	300 µl per well	≥30 min	Local supermarket	
	→	Discard the blocking solution					
	3	Add arthropod samples to individual sample wells	See footnote 3	100 µl per well	≥60 min		
	→	Discard the samples from each well					
	→	Wash each well 3× with TBS	Undiluted	300 µl per well	Briefly		
	4	Add IgY antibody containing the HRP conjugate (secondary antibody)	1:10,000 in TBS	50 µl per well	≥60 min	Sigma Chemical Co.	A9046
	→	Wash each well 3× with TBS	Undiluted	300 µl per well	Briefly		
	5	Add TMB substrate solution	Undiluted	50 µl /well	10 min	BioFx Laboratory, Inc.	TMBW-1000–01
	6	Read ELISA reactions with microplate reader set at 650 nm					

[1]The anti-rabbit and anti-chicken assays are designed to detect rabbit IgG (Sigma Chemical Co., #I5006) and chicken IgY (US Biological, I1903-15), respectively.

[2]The various products listed are only suggestions; similar products can be purchased from other vendors.

[3]Each arthropod sample should be homogenized in 500 to 1,000 µl of TBS and shaken for ≥1 h on an orbital shaker prior to adding to the ELISA sample well.

Abbreviations: IgG, immunoglobulin G; TBS, Tris-buffered saline; HRP, horseradish peroxidase; TMB = 3,3′,5,5′-Tetramethylbenzidine

a manipulated arthropod assemblage of about a dozen predator and three pest species: rabbit IgG-marked *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) larvae, chicken IgG-marked *L. hesperus* nymphs, and sentinel-placed *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechilidae), egg masses. An inclusion cage treatment was designed to allow foraging fire ants, *Solenopis xyloni* McCook (Hymenoptera: Formicidae), to freely enter the cages. Conversely, an exclusion cage treatment prevented ants from entry in to the caged arenas. The population dynamics data yielded from the field cage portion of the study revealed that substantial interguild and intraguild predation was occurring on most of the arthropod species in the assemblage, especially in the ant inclusion cages. The UFIT was then used to pinpoint which predators fed on the marked pests by conducting three postmortem gut content analyses on each predator. Specifically, *P. gossypiella* egg predation events were detected using a species and egg-specific sandwich ELISA (Hagler 1998), and *T. ni* and *L. hesperus* predation events were detected using respective UFIT ELISAs. The gut analyses revealed that each prey species tended to attract a particular group of predator species.

Quantifying Predation Rates

The UFIT, when used in tandem with field cage methods, was used to pinpoint the number of predation events on IgG-marked *L. hesperus* by members of the cotton predator assemblage (Hagler 2011). For that study, sets of two *L. hesperus* nymphs, one marked with rabbit IgG and the other with chicken IgG, were released into 360 individual field cages containing a single cotton plant and the natural (unmanipulated) predator population (note that two *L. hesperus* per cotton plant is a density often found in nature; Naranjo et al. 2004). Seven hours after release, the cages were removed from the field, the indigenous predator population was counted, and every predator was examined for the presence of rabbit and chicken IgG. Data revealed that 74 of the 556 native predators trapped within the 360 field cages contained IgG-marked *L. hesperus* in their guts. This study served as a proof-of-concept example that, under certain manipulative experimental conditions, the UFIT can be used to quantify predation rates.

Herbivory/Granivory

For the most part, gut content analysis methods are usually used to study predator–prey interactions. However, the UFIT has been used to study arthropod herbivory. Lundgren et al. (2013) marked dandelion seeds with rabbit IgG and strategically placed them in research plots. Subsequently, ground-dwelling arthropods were collected in pitfall traps and examined for the presence of the protein marker in their guts. The data generated showed that about a quarter of the field-collected arthropod herbivores tested positive of the presence of the mark. Blubaugh et al. (2016) used the UFIT to simultaneously examine predation and herbivory of omnivorous ground beetles in open field research plots containing manipulated densities of vegetation (i.e., vegetation vs bare ground), weed, and prey. For that study, they marked weed seeds with rabbit IgG and pupal prey with chicken IgG. The study showed that omnivorous carabids tracked the rabbit IgG-marked weed seeds, but not chicken IgG-marked prey, and that strict carabid predators were not found to consume either type of protein-marked food resource.

Nectivory

The UFIT has enormous potential for studying pollination and nutritional ecology. Nectar and pollen provide dietary resources for a wide variety of arthropods (e.g., bees, butterflies, parasitoids, predators, pests) (Wackers et al. 2007, Lee and Heimpel 2008). The UFIT can be easily adapted to examine these multitrophic level interactions by marking the potential nectar or pollen resources. For example, the flow of nectar resources into bee colonies was determined by feeding foraging honey bees a sugar solution supplemented with rabbit IgG (DeGrandi-Hoffman and Hagler 2000). The protein-marked sugar reward was quickly delivered to the hive by the returning foragers and traced to food storage areas and brood combs within the colonies.

The foraging behavior and food allocation was examined by feeding odorous house ants, *Taponia sessile* (Say) (Hymernoptera: Formicidae), a rabbit IgG-laced sugar solution (Buczkowski and Bennett 2009). The UFIT helped to identify resource partitioning of foraging ants to nestmates. Data showed that foragers distributed the IgG-labeled sugar reward to the majority of workers in less than a day and that the workers retained more of the marker than queens or larvae. Further results showed that the odorous house ants exhibit high foraging site fidelity. That is, they travel along well-established paths and forage on a local scale.

Scavenging. The UFIT is an ideal tool for studying arthropod scavenging behavior. In a proof-of-concept study, Zilnik and Hagler (2013) used the UFIT to differentiate necrophagy from viviphagy. Whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), and green lacewing, *Chrysoperla rufilabris* (Bermeister) (Neuroptera: Chrysopidae), cadavers were marked with rabbit IgG and live individuals with chicken IgG. The marked prey items were fed to two predaceous beetle species. Data revealed that marker proteins from both the corpse and live prey items were detectable in the majority of the beetles for 12 h after prey consumption. Subsequently, the UFIT was used to measure the frequency of predation and scavenging on *L. hesperus* by *Collops vittatus* (Say) (Coleoptera: Melyridae), *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), and *C. carnea*. Again, the cadaver and live prey were tagged with the rabbit IgG and chicken IgG markers, respectively (Mansfield and Hagler 2016). The study contained about 100 cotton plants, each enclosed within an individual cage (sample unit). The gut analyses showed that scavenging was much more prevalent than predation. The data yielded from this study should alert researchers that previous work using prey-specific gut assays might have overestimated the biological control services, if the targeted predators were frequently engaging in carrion feeding activity. If scavenging is prevalent in ecosystems, the data yielded by the prey-specific gut assay methods will grossly overestimate the biological control services rendered by any given predator species.

Cannibalism. The UFIT is also adaptable for studying intraspecific predation events (i.e., cannibalism). Field cage studies were conducted to detect the frequency of cannibalism and intraguild predation occurring in a cotton predator assemblage. In that study, early instar *C. carnea* larvae were marked with rabbit IgG, and late instars with chicken IgG. The two larval life stages (which are known to be cannibalistic) were then introduced into field cages containing other generalist predator species. The UFIT data revealed a very low frequency of cannibalism and a relatively high frequency of intraguild predation (i.e., the other generalist predators fed on the protein-marked *C. carnea* larvae), respectively (J.R.H., in preparation).

Other food UFIT studies

I have highlighted just a few of the examples of how the UFIT has been used to study various aspects of arthropod food intake. Table 2

Table 2. Various types of arthropod feeding interactions that have been examined using one or both of the anti-IgG UFIT ELISAs

Broad classification	Targeted food item	Research topic(s)	Reference
Carnivory	Insects	Identifying predators of pests, quantifying predation rates, studying trophic level interactions, pinpointing life stage feeding activity	Hagler and Durand (1994) Hagler (2006, 2011) Mansfield et al. (2008) Blubaugh et al. (2016) Rendon et al. (2018) Kelly et al. (2012, 2014) Buczkowski and Bennett (2007)
	Carrion	Scavenging	Zilnik and Hagler (2013) Mansfield and Hagler (2016)
	Blood meals	Bed bugs	Sivakoff et al. (2016)
	Intraspecific prey	Cannibalism	J.R.H., In preparation
	Spiders		
	Mollusks		
Herbivory	Foliage	Weed biocontrol agents	Williams et al. (2011, 2013)
	Seeds	Weed biocontrol agents	Blubaugh et al. (2016), Lundgren et al. (2013)
	Nectar	Ants, termites, honey bees, trophallaxis	DeGrandi-Hoffman and Hagler (2000) Buczkowski and Bennett (2007) Buczkowski et al. (2007)
	Flowers		
	Fruit		
	Grass		
	Pollen		
Omnivory	Plant and animal	Carnivory versus granivory	Blubaugh et al. (2016)
Fungivory	Fungus		
Detritivory	Decomposing matter		
Bacterivory	Bacteria		
Coprophagy	Feces	Flies emerging from cow pats	Peck et al. (2014)
Trophallaxis	Regurgitated food	Honey bees, ants, termites	DeGrandi-Hoffmann and Hagler (2000) Buczkowski et al. (2007) Baker et al. (2010) Song et al. (2015)
Feeding on bait	Uptake of protein-marked bait	Pesticide laced bait, foraging behavior, dispersal	DeGrandi-Hoffmann and Hagler (2000), Baker et al. (2010), Song et al. (2015), Hogg et al. (2018)

lists all the studies conducted to date. I also provide some suggestions for other types of feeding activities than could be studied using the UFIT. In short, there are many research opportunities for conducting creative research on arthropod feeding activity.

Recent Developments in UFIT Research

Recently, it was discovered that the purified IgG biomarkers can be replaced with whole rabbit and chicken sera (J.R.H., in preparation). Preliminary predator feeding trials indicate that the anti-IgG sandwich ELISAs are more responsive to the whole sera biomarkers than to their IgG counterparts. Whole sera are available for bulk purchase at a fraction of the cost of the highly purified IgGs, representing significant per-volume cost savings without any loss of assay reactivity. As such, greater volumes and concentrations of the biomarkers can be used in future studies to label food items for UFIT studies.

To date, only two biomarker detection assays (i.e., anti-rabbit IgG and chicken IgG ELISAs) have been described for UFIT research. A third UFIT sandwich ELISA is under development that detects rat IgG and, in turn, can be used to assay whole rat serum marker (J.R.H., in preparation). The anti-rat sandwich ELISA is being used simultaneously with the other two IgG-specific assays. I do not doubt that more protein-specific ELISAs will serve as an invaluable tool for deciphering multi-trophic feeding interactions.

Conclusions

There are a variety of techniques available for analyzing arthropod gut contents for the presence of specific food types. Most of these techniques require technical expertise, time, and money to develop and apply to field research. The UFIT procedure, as shown above, can be used to investigate a wide variety of arthropod feeding activities. The assay procedure has been standardized. As such, there is very little technical expertise, time, or money required to conduct meaningful research (e.g., studies with large sample sizes and strong experimental designs). Moreover, the technique can be used to investigate arthropod feeding behaviors that are not possible with the prey-specific assay approaches (e.g., scavenging, cannibalism, stage-specific feeding events, etc.). Given all these attributes, the UFIT procedure should be a tool in the toolbox of every field entomologist.

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References Cited

Baker, P. B., J. R. Hagler, R. Marchosky, S. A. Machtley, J. M. Brown, M. A. Riehle, and D. E. Bellamy. 2010. Utilizing rabbit immunoglobulin G protein for mark-capture studies on the desert subterranean termite, *Heterotermes aureus* (Snyder). Insect Soc. 57: 147–155.

Blackmer, J. L., J. R. Hagler, G. S. Simmons, and T. J. Henneberry. 2006. Dispersal of *Homalodisca vitripennis* (Homoptera: Cicadellidae) from a point release site in citrus. Environ. Entomol. 35: 1617–1625.

Blubaugh, C. K., J. R. Hagler, S. A. Machtley, and I. Kaplan. 2016. Cover crops increase foraging activity of omnivorous predators in seed patches and facilitate weed biological control. Agric. Eco. Environ. 231: 264–270.

Buczkowski, G., and G. Bennett. 2007. Protein marking reveals predation on termites by the woodland ant, *Aphaenogaster rudis*. Insect. Soc. 54: 219–224.

Buczkowski, G., and G. Bennett. 2009. The influence of forager number and colony size on food distribution in the odorous house ant, *Tapinoma sessile*. Insect. Soc. 56: 185–192.

Buczkowski, G., C. Wang, and G. Bennett. 2007. Immunomarking reveals food flow and feeding relationships in the eastern subterranean termite, *Reticulitermes flavipes (Kollar)*. Environ. Entomol. 36: 173–182.

Chapman, E. G., S. A. Romero, and J. D. Harwood. 2010. Maximizing collection and minimizing risk: does vacuum suction sampling increase the likelihood for misinterpretation of food web connections? Mol. Ecol. Resour. 10: 1023–1033.

Debolt, J. W., 1982. Meridic diet for rearing successive generations of *Lygus hesperus*. Ann. Entomol. Soc. Amer. 75: 119–122.

DeGrandi-Hoffman, G., and J. R. Hagler. 2000. The flow of incoming nectar through a honey bee colony as revealed by a protein marker. Insect. Soc. 47: 1–5.

Foltan, P., S. Sheppard, M. Konvicka, and W. O. C. Symondson. 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Mol. Ecol. 14: 4147–4158.

Fournier, V., J. R. Hagler, K. Daane, J. de Leon, R. Groves, H. Costa, and T. J. Henneberry. 2006. Development and application of a glassy-winged and smoke-tree sharpshooter egg-specific predator gut content ELISA. Biol. Control. 37: 108–118.

Fournier, V., J. Hagler, K. Daane, J. de León, and R. Groves. 2008. Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. Oecologia. 157: 629–640.

Gariepy, T. D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. J. Appl. Entomol. 131: 225–240.

Greenstone, M. H. 1996. Serological analysis of predation: past, present and future, pp. 265–300. *In* W. O. C. Symondon and J. E. Liddell (eds.), The ecology of agricultural pests—biochemical approaches. Chapman & Hall, London, UK.

Greenstone, M. H., and C. E. Morgan. 1989. Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA assay for stomach analysis. Ann. Entomol. Soc. Amer. 82: 45–49.

Greenstone, M. H., and K. A. Shufran. 2003. Spider predation: species-specific identification of gut contents by polymerase chain reaction. J. Arachnol. 31: 131–134.

Greenstone, M. H., D. C. Weber, T. C. Coudron, and M. E. Payton. 2011. Unnecessary roughness? Testing the hypothesis that predators destined for molecular gut-content analysis must be hand-collected to avoid cross-contamination. Mol. Ecol. Resour. 11: 286–293.

Hagler, J. R. 1997a. Field retention of a novel mark-release-recapture method. Environ. Entomol. 26: 1079–1086.

Hagler, J. R. 1997b. Protein marking insects for mark-release-recapture studies. Current Trends in Entomol. 1: 105–115.

Hagler, J. R. 1998. Variation in the efficacy of several predator gut content immunoassays. Biol. Control. 12: 25–32.

Hagler, J. R. 2006. Development of an immunological technique for identifying multiple predator-prey interactions in a complex arthropod assemblage. Ann. Appl. Biol. 149: 153–165.

Hagler, J. R. 2011. An immunological approach to quantify consumption of protein-tagged *Lygus hesperus* by the entire cotton predator assemblage. Biol. Control. 58: 337–345.

Hagler, J. R. 2016. A false positive food chain error associated with a generic predator gut content ELISA. Entomol. Exp. Appl. 161: 187–192.

Hagler, J. R. 2019. Super mark it! a review of the protein immunomarking technique. Ann. Entomol. Soc. Amer. doi:10:1093/aesa/say039.

Hagler, J. R., and F. Blackmer. 2013. Identifying inter- and intraguild feeding activity on an arthropod predator assemblage. Ecol. Entomol. 38: 258–271.

Hagler, J. R., and F. Blackmer. 2015. Evidence of intraguild predation on a key member of the cotton predator complex. Food Webs. 4: 8–13.

Hagler, J. R., and C. M. Durand. 1994. A new method for immunologically marking prey and its use in predation studies. Entomophaga. 39: 257–265.

Hagler, J. R., and C. G. Jackson. 1998. An immunomarking technique for labeling minute parasitoids. Environ. Entomol. 27: 1010–1016.

Hagler, J. R., and E. Miller. 2002. An alternative to conventional insect marking procedures: detection of a protein mark on pink bollworm by ELISA. Entomologia Experimentails et Applicata. 103: 1–9.

Hagler, J. R., and S. E. Naranjo. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study, pp. 383–399. *In* W. O. C. Symondon and J. E. Liddell (eds.), The ecology of agricultural pests–biochemical approaches. Chapman & Hall, London, UK.

Hagler, J. R., A. C. Cohen, F. J. Enriquez, and D. Bradley-Dunlop. 1991. An egg-specific monoclonal antibody to *Lygus hesperus*. Biol. Control. 1: 75–80.

Hagler, J. R., A. C. Cohen, D. Bradley-Dunlop, and F. J. Enriquez. 1992a. A new approach to mark insects for feeding and dispersal studies. Environ. Entomol. 21: 20–25.

Hagler, J. R., A. C. Cohen, D. Bradley-Dunlop, and F. J. Enriquez. 1992b. Field evaluation of predation on *Lygus hesperus* using a species- and stage-specific monoclonal antibody. Environ. Entomol. 21: 896–900.

Hagler, J. R., A. G. Brower, Z. Tu, D. N. Byrne, D. Bradley-Dunlop, and F. Javier Enriquez. 1993. Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci*. Entomol. Exp. Appl. 68: 231–236.

Hagler, J. R., S. E. Naranjo, D. Bradley-Dunlop, F. J. Enriquez, and T. J. Henneberry. 1994. A monoclonal antibody to pink bollworm egg antigen: a tool for predator gut analysis. Ann. Entomol. Soc. Am. 87: 85–90.

Hagler, J. R., C. G. Jackson, T. J. Henneberry, and J. Gould. 2002. Parasitoid mark-release-recapture techniques: II. Development and application of a protein marking technique for *Eretmocerus* spp., parasitoids of *Bemisia argentifolii*. Biocontrol Sci. Technol. 12: 661–675.

Hagler, J. R., F. Blackmer, and D. W. Spurgeon. 2015. Accuracy of a prey-specific DNA assay and a generic prey-immunomarking assay for detecting predation. Methods Ecol. Evol. 6: 1426–1434.

Hagler, J. R., D. J. Nieto, S. A. Machtley, D. W. Spurgeon, B. N. Hogg, and S. L. Swezey. 2018a. Dynamics of predation on *Lygus hesperus* (Hemiptera: Miridae) in alfalfa trap-cropped organic strawberry. J. Insect Sci. 18: 12.

Hagler, J. R., S. R. Casey, and S. Mansfield. 2018b. Standardization of prey immunomarking: does a positive test always indicate predation? BioControl. doi:10.1007/s10526-018-9905-5.

Harper, G. L., R. A. King, C. S. Dodd, J. D. Harwood, D. M. Glen, M. W. Bruford, and W. O. Symondson. 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Mol. Ecol. 14: 819–827.

Harwood, J. D. 2008. Are sweep net sampling and pitfall trapping compatible with molecular analysis of predation? Environ. Entomol. 37: 990–995.

Harwood, J. D., S. W. Phillips, K. D. Sunderland, and W. O. Symondson. 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Mol. Ecol. 10: 2049–2057.

Hogg, B. N., E. H. Nelson, J. R. Hagler, and K. M. Daane. 2018. Foraging distance of the Argentine ant in California vineyards. J. Econ. Entomol. 111: 672–679.

Juen, A., and M. Traugott. 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator-prey system. Oecologia. 142: 344–352.

Kelly, J. L., J. R. Hagler, and I. Kaplan. 2012. Employing immunomarkers to track dispersal and trophic relationships of a piercing-sucking predator, *Podisus maculiventris* (Hemiptera: Pentatomidae). Environ. Entomol. 41: 1527–1533.

Kelly, J. L., J. R. Hagler, and Ian Kaplan. 2014. Semiochemical lures reduce emigration and enhance pest control services in open-field augmentation. Biol. Control. 71: 70–77.

King, R. A., D. S. Read, M. Traugott, and W. O. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17: 947–963.

Lee, J. C., and G. E. Heimpel. 2008. Floral resources impact longevity and oviposition rate of a parasitoid in the field. J. Anim. Ecol. 77: 565–572.

Lundgren, J. G., P. Saska, and A. Honěk. 2013. Molecular approach to describing a seed-based food web: the post-dispersal granivore community of an invasive plant. Ecol. and Evol. 3: 1641–1652.

Mansfield, S., and J. R. Hagler. 2016. Wanted dead or alive: scavenging versus predation by three insect predators. Food Webs. 9: 12–17.

Mansfield, S., J. R. Hagler, and M. Whitehouse. 2008. A comparative study of the efficiency of a pest-specific and prey-marking ELISA for detection of predation. Entomol. Exp. Appl. 127: 199–206.

Monzó, C., B. Sabater-Muñoz, A. Urbaneja, and P. Castañera. 2010. Tracking medfly predation by the wolf spider, *Pardosa cribata* Simon, in citrus orchards using PCR-based gut-content analysis. Bull. Entomol. Res. 100: 145–152.

Naranjo, S. E., and J. R. Hagler. 1998. Characterizing and estimating the effect of heteropteran predation, pp. 171–197. *In* M. Coll and J. Ruberson (eds.), Predatory heteroptera in agroecosystems: their ecology and use in biological control. Thomas Say Publ. in Entomology. Entomological Society of America, Lanham, MD.

Naranjo, S. E., P. C. Ellsworth, and J. R. Hagler. 2004. Conservation of natural enemies in cotton: role of insect growth regulators in management of *Bemisia tabaci*. Biol. Control. 30: 52–72.

Peck, G. W., H. J. Ferguson, V. P. Jones, S. D. O'Neal, and D. B. Walsh. 2014. Use of a highly sensitive immunomarking system to characterize face fly (Diptera: Muscidae) dispersal from cow pats. Environ. Entomol. 43: 116–122.

Polis, G. A. 1981. The evolution and dynamics of intraspecific predation. Annu. Rev. Ecol. Syst. 51: 225–251.

Rendon, D., J. R. Hagler, P. W. Taylor, and M. E. A. Whitehouse. 2018. Molecular, ecological and behavioral approaches for assessing *Helicoverpa* spp. larva consumption by wolf spiders in a Bt-cotton field. Biol. Control. 122: 51–59.

Richardson, M. L., R. F. Mitchell, P. F. Reagel, and L. M. Hanks. 2010. Causes and consequences of cannibalism in noncarnivorous insects. Annu. Rev. Entomol. 55: 39–53.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through complex predator–prey food webs. Functional Ecology. 19: 751–762.

Sheppard, S. K., J. Bell, K. D. Sunderland, J. Fenlon, D. Skervin, and W. O. Symondson. 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol. Ecol. 14: 4461–4468.

Sigsgaard, L. 1996. Serological analysis of predators of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) egg in sorghum-pigeonpea intercropping at ICRISAT, India: a preliminary field study, pp. 367–381. *In* W. O. C. Symondon and J. E. Liddell (eds.), The ecology of agricultural pests – biochemical approaches. Chapman & Hall, London, UK.

Sivakoff, F., S. C. Jones, S. A. Machtley, and J. R. Hagler. 2016. Bed bug (*Cimex lectularius*) protein marking for mark-capture dispersal research: proof of concept experiments. J. Med. Entomol. 53: 1370–1377.

Song, J., E. P. Benson, P. A. Zungoli, P. Gerard, and S. W. Scott. 2015. Using the DAS-ELISA test to establish an effective distance between bait stations for control of *Linepithema humile* (Hymenoptera: Formicidae) in natural areas. J. Econ. Entomol. 108: 1961–1971.

Sopp, P. I., K. D. Sunderland, J. S. Fenlon, and S. D. Wratten. 1992. An improved quantitative method for estimating invertebrate predation in the field using an enzyme-linked immunosorbent assay (ELISA). J. Appl. Ecol. 79: 295–302.

Sunderland, K. D. 1996. Progress in quantifying predation using antibody techniques, pp. 419–455. *In* W. O. C. Symondon and J. E. Liddell (eds.), The ecology of agricultural pests – biochemical approaches. Chapman & Hall, London, UK.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Mole. Ecol. 11: 627–641.

Symondson, W. O. C., and J. E. Liddell. 1993. A monoclonal antibody for the detection of arionid slug remains in carabid predators. Biol. Control. 3: 207–214.

Symondson, W. O. C., T. Gasull, and J. E. Liddell. 1999. Rapid identification of adult whiteflies in plant consignments using monoclonal antibodies. Ann. Appl. Biol. 134: 271–276.

Trowell, S. C., N. W. Forrester, K. A. Garsia, G. A. Lang, L. J. Bird, A. S. Hill, J. H. Skerritt, and J. C. Daly. 2000. Rapid antibody-based field test to distinguish between *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 878–891.

Wackers, F. L., J. Romeis, and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Annu. Rev. Entomol. 52: 301–323.

Williams, L., J. R. Hagler, and K. Tonkel. 2011. Retention of Immunolabels by *Diorhabda carinulata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar. Entomol. Exp. Appl. 141: 154–162.

Williams, L., J. R. Hagler, and K. Tonkel. 2013. Does dimethyl sulfoxide increase protein immunomarking efficiency for dispersal and predation studies? Entomol. Exp. Appl. 148: 275–286.

Wise, D. H. 2006. Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. Annu. Rev. Entomol. 51: 441–465.

Zilnik, G. and J. R. Hagler. 2013. An immunological approach to distinguish arthropod viviphagy from necrophagy. BioControl. 58: 807–814.