ABSTRACT.

Little effort has been made to characterize the diel pattern of predation on insect pests in the field, particularly predatory events that occur nocturnally. Round-the-clock observations in systems such as potatoes, cotton, soybean, maize, and ley crops under varying cultural practices, and woodlands, with additional published studies, highlight several important considerations when working on predator communities:

- Predator communities differ greatly between day and night. Depending on the system, cursorial spiders, carabid and other predatory beetles, cockroaches, ants, and earwigs, are important after dark.

- Predators have distinct diel patterns of behavior, which do not conform simply to a nocturnal/diurnal dichotomy; circular statistics are important in diel analysis.

- Diurnal appraisals are at best biased; predators causing significant mortality to target pests may be completely overlooked.

- Sentinel (emplaced) prey are uniquely useful, provided they represent target pests and are stationed realistically. Predator taxa and diversity vary greatly with prey type, habitat, and position.

- Combining direct observations with other methods (including DNA and protein-based molecular tools) strengthens predation assessments

- Disturbed habitats tend to host lower predator diversity and only during limited diel windows; cultural techniques which enhance complexity in agricultural habitats may expand the diel period for higher intensity and diversity of predation on pests.

We conclude that nocturnal predation must be addressed explicitly to accurately characterize predator-prey systems, and that round-the-clock observation of sentinel prey is a key technique for assessing pest suppression as an ecosystem service.
INTRODUCTION.

Predation in agricultural systems is at least as common after dark, as during the day. Yet research which investigates predation throughout the diel cycle is rare indeed. Over 30 years later we can still echo the sentiment of Vickerman & Sunderland (1975) that “the nocturnal activity of terrestrial invertebrates seems to have been largely overlooked in crop ecosystems.

Both natural and managed ecosystems show diel rhythms in predator activity, including predation events. The diel patterns vary by species, season, and habitat; furthermore, community patterns vary over these same temporal and ecological dimensions. To identify and characterize important ecological processes including predation, and to build effective management methods for sustainable agricultural systems, it is essential to have full knowledge of the multiple dimensions of diel activity.

It is important not just to think or design studies in terms of “nocturnal” versus “diurnal”, nor to classify species or species groups into such categories. Conditions and behavior vary over the entire 24 hours; for instance, temperature and dew are very different from beginning to end of scotophase, with great influence on predator behavior.

Early ecological workers considering mainly the Carabidae (e.g. Williams 1959; Thiele & Weber 1968) showed a variety of patterns of activity, as measured typically by pitfall capture or actographs of recently-caught beetles in captivity. Dennison & Hodkinson (1983) related woodland carabid and staphylinid diel activity patterns to species, body size, and season, with a variety of nocturnal, diurnal, crepuscular, and intermediate or combined activity patterns. Dennison & Hodkinson (1984) used presence-absence diel activity data for 13 carabids, 9 staphylinids and one leiodid, to cluster the species in a “diurnal activity dendrogram” according to average linkage clustering. This resulted in 3 loose clusters as well as two unique species. The pattern was far from a simple behavioral dichotomy of nocturnal versus diurnal. In agricultural systems, Luff (1978) and Kiritani et al. (1972) observed similarly diverse diel patterns, respectively, in carabids of an English vegetable system, and in spider predators of the green rice leafhopper Nephotettix cincticeps Uhler (Homoptera: Cicadellidae), in Japanese rice paddies.

That the diel pattern of occurrence, activity or predation might vary according to habitat was proposed by Park (1941), who viewed diel patterns from the perspective of niche-filling, with more symmetric diel activity accompanying advanced community succession. However, only a very few studies have taken place in both natural and agricultural systems to examine these trends in detail, especially in terms of predation risk to herbivores.

Activity of predators is important, but with biological control, and particularly in agroecosystems, the focus is on the prey, usually pest species, of concern to crop protection. Making the prey the focus of interest makes predation events themselves, rather than predator activity (movement) or location within the ecosystem, and allows the best understanding of ecosystem services in terms of desired, practical biological control.
The focus on particular prey, in particular pests in agricultural systems, is likely to yield more useful results about biological control, especially predation risk, than is general sampling or observation. Molecular methods may focus on prey-specific molecules with valuable results, but the ecological context of these markers must be established in order to confirm how (predation, scavenging, secondary predation), where (within the crop system) and when (during the diel cycle) the prey is obtained by different predators. This will allow proper application of molecular tools in determining the importance of the predator in suppressing the pest of interest.

Use of sentinel prey, either the pest of interest or a factitious (surrogate) species which has been demonstrated to be equivalent as a prey item, is extremely valuable in assessing the intensity of predation and the species responsible for biological control. It may be especially useful where pest density is insufficient to observe adequate numbers (sometimes as a result of very effective biological control!). Kidd & Jervis (1996) describe the caveats in deploying sentinel prey, including positioning, quality, and density considerations.

Here, we describe the deployment of eggmasses of Colorado potato beetle (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) in potato crops, monitored day and night to determine predator occurrence and predation on prey of interest. In addition, we discuss additional studies undertaken by the authors, using sentinel eggs of Lepidoptera in field crops such as cotton, soybeans, and corn, and sentinel larvae in vegetable and field crops under organic transition.

**MATERIALS AND METHODS.**

The field site was a 1-ha potato field in Beltsville, Maryland, USA. Once in July 2006, and at 3 times during 2007, during continuous 48-hour periods we observed 240 field-collected sentinel egg masses of Colorado potato beetle for damage and presence of arthropods on or nearby, every 3 h, at 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 hours.

Eggs were numbered and photographed prior to attachment to leaves in the field using staples. Previous work had shown that attachment to the tops of leaves resulted in similar predation to attachment underneath, in spite of the fact that the large majority of eggs are laid by females underneath leaves, perhaps to prevent desiccation. Plots of 20 plants each were placed in 3 different cultural treatments of potatoes (conventional tilled, killed rye cover crop, & killed vetch cover crop), with 2 replicates in each of two fields, planted in April with cultivar 'Kennebec' and minimal pesticide treatment. The results shown are for the sample period 26-28 July 2006, and do not show cultural effects.

Observations were made with a minimum of disturbance, using red LED headlamps when needed after dark. All egg masses with ≥50% estimated damage were replaced immediately. Damaged egg masses were enumerated to quantify depletion (before minus after).

To analyze the diel patterns, we used circular statistics (Batschelet 1981; Fisher 1993; Zar JH. 1999) with Oriana software (Kovach 2004). Circular statistics and polar plots are essential to analysis and visualization of cyclical temporal data.
RESULTS.

Of the 255 predation events observed in July 2006, 56% occurred after dark, with the fewest observed during the afternoon hours. The natural enemy complex is dominated by beetles and true bugs (Table 1). The most frequently observed predator in 2006, larval *Chauliognathus* sp. (Coleoptera: Cantharidae), has not been reported previously as a CPB egg predator (Ferro 1994; Heimpel & Hough-Goldstein 1992; Hilbeck et al. 1997).

The diel pattern of occurrence of predators on sentinel eggmasses is unique by species, with the exception that Pentatomidae and *Geocoris* sp. (Hemiptera: Geocoridae) have very similar patterns of occurrence (Table 1). *Chauliognathus* sp. has a late-nocturnal peak of abundance, corresponding to the period of heavy dew on plants (Fig. 1). In contrast, *Lebia grandis* Hentz adults (Coleoptera: Carabidae) are primarily early night-active, ceasing activity after midnight but becoming active again typically soon after dawn. *Lebia grandis* egg consumption (not shown) exceeded that of all other predators. Bugs were almost exclusively diurnally predatory on CPB eggs. Among pentatomids, *Perillus bioculatus* (F.) is more specialized on CPB than is *Podisus maculiventris* (Say), and was the dominant species, as nymphs, in our study. Big-eyed bugs (*Geocoris* sp.) was strongly diurnal based on predation events; adults and nymphs were equally represented on CPB eggmasses. However, their primary prey was probably potato leafhopper (*Empoasca fabae* (Harris)) (Hemiptera: Cicadellidae) nymphs, which were abundant. *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is an extreme generalist feeder abundant in some potato fields, particularly following a corn rotation crop. Adults are diurnal and crepuscular feeders, with activity most broadly distributed of all predators.

Figure 2 shows the abundance and species composition of predators on eggmasses by sample period. Predation was least prevalent in the mid-afternoon, and most prevalent late in the scotophase. Sampling during one period of the 24-hour diel cycle could yield very different conclusions: communities were as little as 10% similar between 5:00 and 17:00 (percent similarity, Renkonen 1938, sum of the minimum of proportion by species). In particular, the predator responsible for the most egg consumption, *L. grandis*, was completely absent on egg masses at mid-day during this sample period, in spite of being abundant at night (Fig. 2). Certain periods showed rapid temporal community change in the three hours between samplings: from 5:00 to 8:00 and from 8:00 to 11:00 there are 42.0% and 48.0% respective changes in community composition (1 minus the percent similarity), and again from 17:00 to 20:00 and from 20:00 to 23:00 there are changes of 27.5% and 49.5% respectively. Other successive time periods were very similar, in particular from 23:00 to 2:00 the difference was only 7.1%.
Table 1. Diel pattern of occurrence all predators on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Mean time vector in 24-hour time, with p-value, according to Rayleigh’s test, the null hypothesis of which is uniformity of occurrence by time of day.

<table>
<thead>
<tr>
<th>Species</th>
<th>occurrences</th>
<th>mean time vector</th>
<th>p-value</th>
<th>Watson’s F-test for separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chauliognathus sp.</td>
<td>121</td>
<td>3:22</td>
<td>&lt;0.005</td>
<td>a</td>
</tr>
<tr>
<td>Lebia grandis</td>
<td>18</td>
<td>0:17</td>
<td>&lt;0.005</td>
<td>b</td>
</tr>
<tr>
<td>Coleomegilla maculata</td>
<td>43</td>
<td>14:20</td>
<td>0.24</td>
<td>abc</td>
</tr>
<tr>
<td>Geocoris sp.</td>
<td>36</td>
<td>13:40</td>
<td>&lt;0.005</td>
<td>c</td>
</tr>
<tr>
<td>Pentatomidae</td>
<td>24</td>
<td>13:04</td>
<td>0.02</td>
<td>c</td>
</tr>
</tbody>
</table>

Fig. 1. Diel pattern of occurrence of cantharid larvae, *Chauliognathus* sp., on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Mean time vectors and 95% confidence interval shown with 00:00 as midnight.
Fig. 2. Occurrence of predators on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Areas of pies are proportional to number of occurrences during the sample period (every 3 hours for 48 hours), with a maximum of 40 occurrences at 2:00 and a minimum of 16 at 14:00.

DISCUSSION.

Our results in potato are consistent with those of Pfannenstiel (2005) and Pfannenstiel & Yeargan (2002) in field crops, in that nocturnal predation was equal to, or exceeded, diurnal predation. However, compared to the dominance of cursorial spiders and ants feeding on sentinel lepidoptera eggs at night in Texas cotton, corn, and soybeans, our plant canopies hosted few of these predators and they were very rarely found on eggmasses. Colorado potato beetle eggs as well as larvae represent a distinct prey item from lepidoptera eggs, which may not attract
spider or ant predation. The predator complex of Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) for Kentucky soybean and sweet corn is more similar to our observed fauna in Maryland, with the exception that in Maryland, hemipteran predator species were different, and phalangids were never important. In 2006 sampling, we found little difference between cultural treatments (data not shown), perhaps because canopy closure and relatively small plot size resulted in a relatively uniform distribution of predators among plots.

In contrast, Lundgren et al. (2006) aimed to created strong cultural differences among lower-input pasture system and higher-input tilled vegetable and cash grain systems. The low-input system, transitioning to organic practices, had little diel variation in soil-surface predation rates of tethered factitious prey caterpillars (greater waxmoth, Galleria mellonella L. [Lepidoptera: Pyralidae]). In contrast, an intensively-managed vegetable system showed significantly higher predation during a 3.5-hour night-time sampling period, than either morning or afternoon 3.5-hour observation windows. Predation was not related to pitfall trap captures of potential predators. However, predation was very significantly correlated with overall abundance of insectivorous predators, as measured by quadrat sampling in the different systems. This correlation was in spite of the fact that observed predation involved only a fraction of the predators sampled in the quadrats, and none of the most abundant group, spiders.

Frank & Shrewsbury (2004) tested the effect of nearby conservation strips for predation on tethered black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae) larvae and pupae, and showed a significant effect on larval predation at 6m and 10m distance but not at two closer distances nor at any distance on pupal predation. Again in this case the predation risk showed no relationship to pitfall trap catches, reinforcing the importance of prey-centered measurement of predation, rather than interception of moving epigeal predators.

Clearly the reluctance of entomologists to undertake time-consuming night-time observations has limited the valuable data otherwise available. One technological fix for this is the technique of unattended video recording of sentinel prey carried out in Germany by Meyhöfer (2001). Using 16 monochromatic surveillance cameras illuminated at night by infrared LEDs, he observed colonies of parasitized and unparasitized aphids, Aphis fabae Scopoli (Homoptera: Aphididae) in German sugar beets. These continuous 24-hour video observations allowed detailed study of parasitoid survival with predator presence and time spent in prey patches, and to identify individual predators eating parasitized aphids. The video recordings also allowed detailed determination of diel patterns of predation by species groups.

The video recording technique has also been employed in New Zealand by Merfield et al. (2004) and Frank et al. (2007). Time-lapse video recorders were allowed to run for 12 hours during the scotophase only. Merfield et al. (2004) used blowfly, Calliphora stygia F. (Diptera: Calliphoridae), live eggs as factitious prey and thereby demonstrated differences in predation patterns based on field borders as well as a previously undiscovered commensal interaction between predatory mites and the harvestman Phalangium opilio L. Frank et al. (2007) demonstrated differences in predator complex, but not overall predation pressure, between vineyard floor and canopy, where the prey were tethered larval light brown apple moths, Epiphyas postvittana (Walker)(Lepidoptera: Tortricidae).
Other technologies can be coupled with diel pattern studies to yield insights into predator dynamics. Vickerman & Sunderland (1975), working in English small grains, examined nocturnal activity, demonstrating changing distribution along a vertical axis using manual sweep-netting (for the plant canopy) and D-vac sampling (principally for the soil surface). They also examined predator guts for recent aphid predation, using physical examination and antigen testing. Polyphagous predators, including staphylinids, carabids, earwigs, and spiders, were found at night, particularly in the plant canopy, and these were more likely to have fed recently on aphids at night than during the day. More recently, Lundgren et al. (in press) used a timed pitfall trap in concert with augmentation of western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) and rootworm-specific quantitative PCR to elucidate diel and seasonal patterns as well as to discover several new predators of this key pest in South Dakota corn.

Remedying the serious nocturnal deficiency in agroecology will take more than video cameras and rotating pitfall traps! We ignore the scotophase at our peril. As the observations in Maryland potatoes show, novel predators may be present and undetected unless nocturnal observations are undertaken. Similarly, the discovery of an Asian cockroach, *Blattella asahinai* (Dictyoptera: Blattellidae) as an important predator in soybean and possibly other crops in southern USA (Pfannenstiel et al. 2008) was only by virtue of ongoing nocturnal ecological research. This exotic species had probably been present for over 20 years in the USA (Pfannenstiel et al. 2008).

More generally, we as ecologists need to take the entire diel cycle into account, when contemplating “ecological engineering for pest management” (Gurr et al. 2004). Nectaries, for instance, are not just to the benefit of syrphids and parasitic hymenoptera; in the scotophase spiders which can be important agricultural predators (Pfannenstiel 2008) make use of floral and extrafloral nectaries (Taylor & Pfannenstiel 2008). On the potential negative side, pestiferous noctuid moths and other herbivores augment their fecundity by nocturnal nectarivory and pollinivory (Wäckers et al. 2007).

Limited information available (e.g. Lundgren et al. 2006, Lundgren unpublished) suggests that mulching, reduced tillage, more closed and diversified canopies all can serve to expand the diel range of predation pressure in agricultural systems over a broader time period, typically from night into the day. These practices serve both to buffer climatic (temperature, humidity, light and wind) extremes, and to mimic more generally the later successional stages of natural ecosystems, such that a perennialization effect takes place, even in agroecosystems consisting largely of annual cash and cover crops.

ACKNOWLEDGMENTS

Zsofia Szendrei was our collaborator on the potato sentinel egg study. We also thank Eddie Bender, Leanna Kelly, and Mike Athanas for assistance with this project.

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