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The co-occurrence of an introduced biological control agent (Coleoptera: *Coccinella septempunctata*) and an endangered butterfly (Lepidoptera: *Lycaeides melissa samuelis*)

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Abstract

Whether a biological control agent presents a non-target risk to a native species depends if they co-occur spatially and temporally, and if the agent will harm the native species. We sampled two study sites during 1993 in Minnesota and Wisconsin to survey predators and parasitoids of the extant populations of the United States federally endangered Karner blue butterfly, *Lycaeides melissa samuelis*. We found the introduced coccinellid *Coccinella septempuntata* co-occurring spatially and temporally with eggs, larvae and adults of *L. m. samuelis*. The two species were also observed together on the latter's sole host plant, *Lupinus perennis*, and in Wisconsin, an adult *C. septempunctata* was observed consuming second instar larvae of *L. m. samuelis*. Using a simple model to hypothesize the risk that *C. septempunctata* presents to *L. m. samuelis*, we showed that increases in predator density could greatly increase mortality to *L. m. samuelis*. At these sites, *C. septempunctata* were reproducing and had access to summer aphids and suitable overwintering habitat. Nearby agricultural crops could provide spring aphids for oogenesis, and assist with *C. septempunctata* population build-up. Maintaining a minimum isolation distance between agricultural crops known to harbor aphids and extant *L. m. samuelis* populations may need to be considered as part of the butterfly management program.

Introduction

Biological control has been an appealing and beneficial means of pest control. However, one potential adverse effect of introduced insects is that they attack non-target flora and fauna (Pimentel et al. 1984; Howarth 1991; Andow et al. 1995; Pemberton 1995; McEvoy 1996; Louda et al. 1997). Until the 1990s there were no quantifiable cases where the introduction of an arthropod agent had been damaging to a specific conservation program or to a native fauna or flora (Samways 1988; Howarth 1991; Pemberton 1995; Louda et al. 1997; Johnson and Stiling 1998). This was due to both the difficulty in quantifying the effect under natural conditions (Andow et al. 1995), and the lack of post-release monitoring (Simberloff and Stiling 1996). Whether a biological control agent presents a risk to a native species can be evaluated by examining whether the agent:

(1) co-occurs with the native species spatially and temporally (exposure), and (2) harms a native species in a way that affects its population dynamics (effect) (Andow et al. 1995). We examined the co-occurence of the seven-spot ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), and the United States federally endangered Karner blue butterfly, *Lycaeides melissa samuelis* Nabokov (Lepidoptera: Lycaenidae; USFWS 1992). In addition, we hypothesized the risk that *C. septempunctata* presents to *L. m. samuelis*, and potential management strategies that may reduce risk.

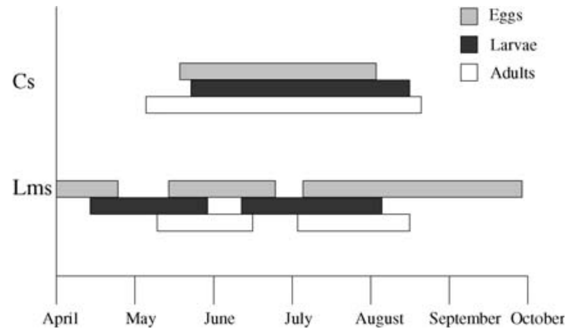

Figure 1. The seasonal phenology of *C. septempunctata* and *L. m. samuelis*. Cs = *C. septempunctata* eggs, larvae and adults. Bar for adults from mid-June to mid-July, and bar for larvae at one time period in July are based on observations by authors; eggs, and additional time periods for adults and larvae are based on literature by Horn (1991) and Hagen (1962). Lms = *L. m. samuelis* eggs, larvae and adults (Swengel and Swengel 1996; Lane 1999).

Natural history

In the United States of America, Karner blue butterfly (*L. m. samuelis* Nabokov) populations have declined in number and distribution across its range, prompting its listing as federally endangered in 1992 (USFWS 1992). A species is listed depending on the degree of threat that it faces. An 'endangered' species is one that is in danger of extinction throughout all or a significant portion of its range (USFWS 1992). In Minnesota, USA, *L. m. samuelis* is extant at only one site (Lane and Dana 1994; USFWS 2003). Wisconsin, USA still supports several large and many small populations (Bleser 1994; USFWS 2003). The primary cause of this decline is believed to be habitat destruction and degradation (Schweitzer 1990; Andow et al. 1994). In particular, the reduced abundance of the sole larval food plant, lupine (*Lupinus perennis* L.), and adult nectar plants have been suggested as key factors (Andow et al. 1994). However, resource limitation does not explain the high early instar larval mortality for this butterfly (Lane 1999). Some species of native generalist predators have been observed to prey on larvae of *L. m. samuelis* and include a pentatomid stink bug (*Podisus maculiventris*), Polistes wasps (*Polistes fuscatus*, and *P. metricus*, Hymenoptera: Vespidae), *Formica* ants (*F. schaufussi* and *F. incerta*; Savignano 1990) and spiders (Packer 1987). Predation explains some of this mortality, but key predators and rate of predation are yet to be quantified.

The butterfly is bivoltine with first brood larvae hatching from overwintered eggs in April (Figure 1, Dirig 1994; Swengel and Swengel 1996). Larvae feed solely on wild lupine (*Lupinus perennis)*, and ants often tend older instars (Savignano 1990; Lane 1999). The larval stage lasts about 3 weeks, at which time they pupate for 7–11 days, resulting in the first flight of the adults in late May to mid-June in the Midwest (Lane 1994, 1999; Swengel and Swengel 1996). Adults live for an average of 5 days (Schweitzer 1990). They nectar on a variety of plant species, and eggs are laid on or very near lupine plants (Lane 1999). The second brood follows the same series of events with adult flight, usually larger in number, occurring late July to mid-August (Swengel and Swengel 1996).

Angalet first introduced *Coccinella septempunctata* into the United States (California) in 1956 from India as a biological control agent against general species of aphids in agricultural crops (Angalet et al. 1979). Additional shipments, redistribution and rearing and releasing programs continued until 1990 (Angalet et al. 1979; Gordon 1985; Schaefer et al. 1987). *Coccinella septempunctata* has been documented across most of eastern, central and part of western United States and Canada (Obrycki et al. 1987; Schaefer et al. 1987; Humble 1992).

Coccinella septempunctata is univoltine in cool northern latitudes (Hagen 1962). The beetles overwinter as adults, which emerge in spring and lay eggs that produce larvae and new adults in mid to late summer (Hagen 1962). These adults aestivate in August or September (Hagen 1962). Although *C. septempunctata* are considered to be primarily aphidophagous, they have a wide range of accepted food (Hodek 1966). Apart from aphids and other Homoptera, 'acceptable or alternative' prey includes mites, small nematocerous Diptera and young instars of Lepidoptera, Coleoptera and Hymenoptera and minute larvae of Thysanoptera (Hodek 1966).

On a national scale, the present geographic distribution of *C. septempunctata* completely overlaps with that of *L. m. samuelis* (Obrycki et al. 1987; Schaefer et al. 1987; Humble 1992; Andow et al. 1994), and regionally, *C. septempunctata* has been recovered in Wisconsin and southeastern Minnesota, USA since 1986 (Anonymous 1986).

Materials and methods

Study sites

In 1993, the study was conducted at two locations: the Whitewater Wildlife Management Area (WWMA), Winona and Wabasha counties in Minnesota, USA; and in east central Wisconsin, 25 miles southwest of Waupaca in Portage county, USA. Both study areas support extant populations of *L. m. samuelis* and have a predominantly Plainfield sand soil type that supports an oak savanna, barrens- type community. This community is characterized by 10–70% cover of *Quercus velutina* Lam., *Pinus banksiana* Lamb., and *Q. ellipsoidalis* E.G. Hill, with an understory containing prairie and savanna forbs including *L. perennis*. The most notable difference between the two sites was geological. Minnesota sites are located in the Paleozoic plateau, believed to have escaped glacial overriding during the Wisconsin glaciation, and consequently the topography is very dissected, with steep slopes up to 45 degrees (Ojakangas and Matsch 1982). The Wisconsin sites were glaciated during the Pleistocene epoch and the topography is flat to rolling hills (Curtis 1959).

Sampling methods

In Minnesota and Wisconsin, we surveyed predators and parasitoids in different microhabitats created by tree canopy cover and topographic slopes. In a 0.5–1 ha area with microhabitat ranging from open-to-closed tree canopy cover and slope ranging from moderate-to-steep, we sampled 30 randomly selected 0.25 m^2 grids. Within each grid lupine plants were randomly selected, and we sampled 2–4 plants depending on the density of lupine. We recorded the number of predators, and parasitoids, but present primarily data on predatory coccinellids here.

Model parameter estimates

We developed a simple model to generate a hypothesis about the risk that *C. septempunctata* presents to *L. m. samuelis*. Parameter estimates for the model include the density of lupin, *L. m. samuelis* larvae, and *C. septempunctata* adults, the rate of increase of *L. m. samuelis*, and the attack rate of *C. septempunctata*.

The density of lupine, 0.50 plants per m^2, was obtained from counting plants within our grids while sampling for predators and parasitoids. The density of *L. m. samuelis* larvae was 0.164 per plant. This was estimated by decreasing the average number of 1^{st} brood eggs found at both sites over 2 years (Lane and Andow 2003) by 37% (0.261), which is the reported mortality rates from egg to neonate larvae (Anonymous 1993). The rate of increase of the 1st brood butterfly population was 1.81 (unpublished census data from Bob Welch obtained by sampling adult females from first broods at Wisconsin). The density of *C. septempunctata* was 0.074 per plant and estimated during data collection. We calculated attack rates assuming that *C. septempunctata* preferentially searches on plants with aphids, and that *L. m. samuelis* is randomly distributed. Therefore, the attack rate on larvae was 0.845 which was the average attack rate from 3rd instar *C. septempunctata* larvae attacking aphids on beans (*Vicia faba*), 1.240, and peas (*Pisum sativum*), 0.450, (Carter et al. 1984). This assumed that *C. septempunctata* searches legumes of lupine, beans and peas similarly.

Results

Both adults and larvae of *Coccinella septempunctata* co-occur spatially and temporally with eggs and larvae of *L. m. samuelis* at the Minnesota and Wisconsin sites (Table 1, Figure 1). Within the

Table 1. The occurrence of *C. septempunctata*, *L. m. samuelis*, and aphids on lupine at different microhabitats and sites in Minnesota and Wisconsin.

Date	No. plants	Cs	Lms	Aphids	Microhabitat	Site/state
June 16	75	1	–	–	SW aspect, 35° slope, open canopy	Historic/MN
July 7	68	1	+	+	NW aspect, 30° slope, open canopy	Satellite/WI
July 7	68	2	–	+	NW aspect, 30° slope, open canopy	Satellite/WI
July 7	68	1	+	–	NW aspect, 30° slope, open canopy	Satellite/WI
July 7	68	1	–	+	NW aspect, 30° slope, open canopy	Satellite/WI
July 8	46	1	–	–	S aspect, 5° slope, open canopy	Sawyer/WI
July 10	63	1	–	+	SW aspect, 15° slope, closed canopy	Cuthrell/MN
July 14	68	1	–	+	NW aspect, 30° slope, open canopy	Satellite/WI

Cs = number of adult *C. septempunctata*; Lms = presence (+) or absence (–) of larval *L. m. samuelis*; Aphids = presence (+) or absence (–) of aphids; Microhabitat = aspect, slope and tree canopy at study site; Site/state = study site name and state.

study sites, adult *C. septempunctata* were observed in several microhabitat types of *L. m. samuelis* (Table 1). These microhabitat types ranged from almost treeless open canopied, steep southwest facing slopes, to closed canopied, moderate slopes. *C. septempunctata* was found on *L. perennis* plants supporting *L. m. samuelis* larvae. However, most of the *C. septempunctata* were observed in open canopied areas during the second flight of the butterfly when aphid density on *L. perennis* was highest. Most of the *L. m. samuelis* have also been observed in the open canopied areas (Lane 1994; Lane and Andow 2003). Three larval *C. septempunctata* were also observed on *L. perennis*, but not in our selected sampling grids. During our survey native insect predators were also observed on *L. perennis* including a species of Nabidae (Hemiptera), Reduviidae (Hemiptera), Chrysopidae (Neuroptera), four species (a total of seven individuals) of Coccinellidae (Coleoptera) and three species of Cantharidae (Coleoptera), but none were observed to prey on eggs or larvae of *L. m. samuelis* (Lane 1999).

More importantly, at the Wisconsin site we observed one *C. septempunctata* adult consume two second instar *L. m. samuelis* larvae indicating that *C. septempunctata* can eat *L. m. samuelis* feeding on lupine plants containing toxins, and they will consume more than one individual when present.

The effect on the population of the butterfly by *C. septempunctata* can be estimated with the Nicholson-Bailey predator-prey encounter model:

$$H_{t+1} F H_t \exp(-aP_t)$$

H is the density of butterflies, *F* is the butterfly's rate of increase including mortality factors from everything except the predator, and in our estimates, also excludes overwintering mortality, *a* is the attack rate of the beetle on butterfly larvae, and *P* is the density of the predator. The function $\exp(-aP_t)$ therefore, reflects the proportion of the butterfly population that escapes beetle predation.

Our simple model suggested that *C. septempunctata* would kill 6% of *L. m. samuelis* larvae (Figure 2). Furthermore, conditions that would increase predator density such as the development of agricultural land near butterfly habitat (Horn 1991) would increase the risk that *C. septempunctata* presents to *L. m. samuelis*. For example, if the density of the beetle increased more then 5 times from 0.074 to 0.37 beetles per plant the beetle could eat 27% of the butterfly larvae if all encounters resulted in mortality (Figure 2). In

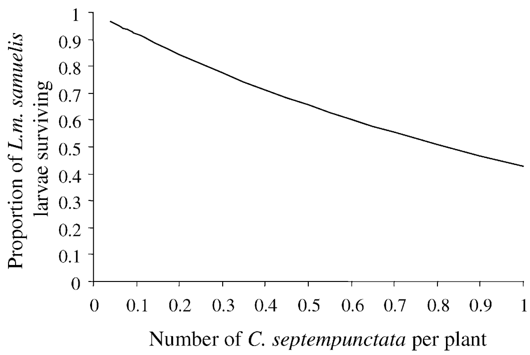

Figure 2. The proportion of *L. m. samuelis* larvae surviving with increasing density of *C. septempunctata* adults at an estimated attack rate of 0.845 *L. m. samuelis* larvae per plant.

addition, we determined that our model estimates were not sensitive to slight changes in *L. m. samuelis* population parameters.

Discussion

Exposure

Whether a biological control agent presents an environmental risk to an endangered species can be evaluated in part by examining whether the agent co-occurs with the endangered species, spatially and temporally. The present geographic distribution of *C. septempunctata* overlaps with *L. m. samuelis*, and observations indicate that *C. septempuntata* occurs in both the microhabitat and on the host plant of the butterfly.

The spatial and temporal occurrence of *C. septempunctata* at Minnesota and Wisconsin sites follow reports in the literature that the beetle is found in a variety of microhabitats providing various nutritional requirements (Maredia et al. 1992), including herbaceous plants in sunny habitats (Honk 1985) and sometimes on lupine, (*Lupinus mutabilis* Sweet) (Gruppe and Roemer 1988).

Effect

Most striking is that *C. septempunctata* adults consume *L. m. samuelis* larvae. This observation is supported by the work of Horn (1991) where he demonstrated that *C. septempunctata* prey upon eggs of a lycaenid, *Everes conyntas*, within the laboratory, suggesting that the ability to feed on lycaenids may be general.

Given spatial and temporal co-occurrence, and the ability of *C. septempunctata* to consume *L. m. samuelis* larvae, the risk that *C. septempunctata* presents to populations of *L. m. samuelis* is greater than zero. The degree of the risk depends on predator attack rates, species densities, and presence/abundance of alternative prey. Although we have used an attack rate for *C. septempunctata* larvae preying on aphids, it is unlikely that an attack rate on *L. m. samuelis* would be higher. However, if we were to use an attack rate of 2.0, as determined for the Asian lady beetle, *Harmonia axyridis* (Pallas), preying on larvae of the monarch butterfly, *Danaus plexippus* (L.) (Koch et al. 2003), *C. septempunctata* would kill 14% of *L. m. samuelis* larvae.

Angalet et al. (1979) suggested that three factors are needed for build-up of large *C. septempunctata* populations: aphids in spring for oogenesis, aphids in late summer and autumn to allow build-up of food resources for overwintering and suitable habitat. Aphids are frequently present in the spring on a variety of agricultural crops several of which are near extant populations of *L. m. samuelis*. For example, in Minnesota, agricultural fields (maize) are 0.5–0.9 km from remaining populations of *L. m. samuelis* (Andow et al. 1995). These populations are small, therefore especially vulnerable as compared with several of the much larger and isolated Wisconsin sites (Lane 1999). Out of twenty *L. m. samuelis* recovery sites in Wisconsin, nine are approximately 400 m from agricultural crops including alfalfa, maize, potato and beans (personal communication, R. Welch). This is also true for Indiana and New Hampshire, USA, where maize is grown approximately 300 m and 800 m, respectively, from *L. m. samuelis* populations (personal communication R. Grundel, and M. Amaral). These nearby agricultural crops could provide aphids in spring for oogenesis, and assist with population build-up of *C. septempunctata* that could disperse to adjacent *L. m. samuelis* habitat. The last two requirements are met in the same habitat as the remaining *L. m. samuelis*; aphids on *L. perennis* and the oak savannah habitat may be suitable for overwintering. A possible management option to reduce exposure of *C. septempunctata* to *L. m. samuelis* is to minimise dispersal of *C. septempunctata* to *L. m. samuelis* habitat. Maintaining a minimum isolation distance between agricultural crops known to harbor aphids and extant *L. m. samuelis* populations may need to be part of the butterfly management program. However the minimum isolation distance in not yet known. Research on dispersal of *C. septempunctata* from agricultural to native habitats may contribute to the *L. m. samuelis* management plan.

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