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THE INTRODUCTION AND RECOVERY IN THE UNITED STATES OF *ANAPHES DIANA* [*HYM. : MYMARIDAE*], AN EGG PARASITE OF *SITONA* WEEVILS [*COL. : CURCULIONIDAE*]

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Anaphes diana (Girault) (= *Patasson lameerei* Debauche), a mymarid egg parasite of *Sitona* spp., was introduced from Europe beginning in 1976 and is now tentatively established in the United States. Techniques are described for the separation of eggs of *Sitona* spp. from soil, using a series of fine-mesh sieves, water, and a saturated salt solution. Data from 9 years of sampling in an alfalfa field at Newark, Del. (> 19,300 host eggs extracted), showed that the mean peak density of viable overwintering eggs of *Sitona hispidulus* (F.) was 14.6 per 100 cm^3 of 1 cm deep surface soil. At the study site, *Sitona* egg densities consistently increased during the fall as a result of oviposition, peaked during January and February and decreased during the spring as a result of egg hatch. Although the incidence of parasitism by *A. diana* remained surprisingly low (0.29 %), the fact that the species was recovered during 3 years and up to 7 years after the last release, indicates that it has colonized at the Delaware release site.

KEY-WORDS : *Anaphes diana*, biological control, natural enemy introduction, *Sitona hispidulus*, egg density.

The clover root curculio, *Sitona hispidulus* (F.), a common pest of leguminous plants in Europe, was first detected in New Jersey in 1876 (**Wildermuth,** 1910). By 1915 it was widely distributed across the United States and was causing serious injury to alfalfa, *Medicago sativa* L. (**Webster,** 1915). Although adults feed on the leaves, the most severe damage is caused by larvae feeding on the roots and root nodules. After emerging from the soil in late summer, adults begin to lay eggs in the fall and continue, weather permitting, until spring. Eggs are dropped on the soil surface near the base of plants (**Bigger,** 1930). **Melamed-Madjar** (1966) reported that ♀♀ of *S. hispidulus* laid an average of 719 eggs during their lifespan. This high fecundity and long period of egg availability makes *S. hispidulus* an attractive target for biological control by an egg parasite.

In Europe, **Aeschlimann** (1975) discovered a mymarid parasite of *Sitona* eggs while searching for natural enemies for introduction into Australia to control *S. discoideus* Gyllenhal, another pest of European origin. This parasite was first identified as *Patasson lameerei* Debauche, but recent research by **Schauff** (1984) indicates that *Anaphes diana*

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(Girault) is the correct name. The biology of *A. diana* has been reported by various authors; 12 citations are listed in a recent paper by **Aeschlimann** (1986). Although mymarids are fragile insects, *A. diana* is rather hardy. In southern France, adults are active during winter and the parasite aestivates as an advanced larva within the *Sitona* egg from May to September (**Aeschlimann,** 1977). Under fluctuating temperatures (6.7/18.3 °C), adult females can live for 10 days and produce up to 52 offspring (**Yeargan & Shuck,** 1981).

The purpose of this paper is to present details on the release of *A. diana* at several localities in the United States, and to report on its recovery in eggs of *S. hispidulus* at a release site in Delaware. Also included are data on the density of *S. hispidulus* eggs in soil of an alfalfa field over a 9 year period.

MATERIALS AND METHODS

SAMPLING METHODS

To obtain baseline information on *Sitona* egg densities and egg parasitism prior to the introduction of exotic parasites, periodic soil samples were obtained from the alfalfa study field at Newark, Del. and from alfalfa fields in Maryland, Michigan, and Pennsylvania. On each sample date, 8 soil samples per field were taken with a flat steel-sided spatula near randomly selected alfalfa plants. Each sample (1 cm deep) contained 235 cm^3 of surface soil. Following the parasite's initial introduction in 1976, egg populations were monitored in the Delaware study field during 8 additional winters, through March 1986 (omitted winters were 1980-81 and 1984-85). The results reported here were based on 720 soil samples taken on 88 dates. The study field (*ca.* 1 ha) was planted with "Saranac AR" alfalfa and was normally harvested 3 times per year, but was never treated with insecticides.

EXTRACTION OF EGGS FROM SOIL

Egg extraction procedures, modified after techniques used by **Waldbauer & Kogan** (1973) and **Aeschlimann** (1975), began by soaking each soil sample for 20 min in 2 l of tap water. Next a water spray was used to wash the sample through a series of sieves of decreasing mesh (U.S. Standard Testing Sieves No. 10, 18, 20, 30, and 60). Large soil particles were rubbed lightly with fingers in the top 2 sieves only; rubbing in lower sieves produced excessive amounts of fine plant material below. After washing in each sieve for 5 min., residues were discarded in all but the No. 60 sieve. Using a wash bottle filled with a saturated solution of NaCl, the final residue was washed into a 2,000 ml percolator separatory funnel containing *ca.* 1,700 ml of saturated salt solution. A long glass tube was inserted to the bottom of the funnel. An aquarium pump attached to the tube provided bubbling agitation to prevent eggs from being trapped in soil sediment. After 5 min the tube was removed slowly while being rinsed clean with a wash bottle. The sediment was drained and discarded after 10 min of settling. The remaining liquid, with all organic material including insect eggs floating on the surface, was drained into a No. 60 sieve. The residue was then washed, with a fine stream of distilled water, back into the empty stoppered funnel. After washing the sides, the remaining liquid was carefully drained into a large filter paper held in a sieve. The residue was then rinsed with distilled water several times to remove all traces of salt and was examined, while moist, under a low power microscope. Intact *Sitona* eggs were counted and removed with a fine, moistened camel's-hair brush and incubated for 2 wk at 21 °C to determine viability and to capture any emerging parasites. Accuracy of the extraction procedure was tested on 3 occasions by processing soil samples containing known numbers of eggs. The soil used in such tests was taken from cornfields where *Sitona* species were not present.

PARASITE RELEASES

All foreign exploration and field collection of *A. diana* for release in the United States or used in laboratory studies was performed by personnel of the USDA-ARS European Parasite Laboratory, Sèvres, France. *Sitona* eggs from various European collection sites were placed on moist filter paper in tightly-covered Petri dishes, and shipped in chilled containers to the USDA-ARS Beneficial Insects Research Laboratory at Newark, Delaware. Upon receipt at the quarantine facility, eggs were stored at 5 °C or held at 21 °C for emergence. Emerging parasites were counted, sexed, allowed to mate and to oviposit on *Sitona* eggs obtained from domestic collections. Unparasitized or infertile eggs of foreign origin were destroyed in quarantine. All releases in Delaware were made at the study field. Liberations in other states were made by state and university cooperators (see Acknowledgments) from parasitized *Sitona* eggs shipped from the Newark, Del. quarantine facility. All parasite releases were targeted against *S. hispidulus* except those in Idaho, which were against *S. lineatus* (L.).

To maximize the probability of establishment, several release strategies were used at the Delaware study field. The first releases of adult parasites were made in the late spring of 1976 when vials containing mated adults were opened and placed on the soil beneath alfalfa plants. Adults and parasitized host eggs were released from the fall of 1977 through spring of 1978. Frequently, unparasitized host eggs also were placed on the soil to provide oviposition sites for emerging parasites. During March and April, 1978, serial releases of both parasite adults and host eggs were made daily for 36 days. Colonies of *A. diana* were discontinued each spring and restarted each fall from fresh European stock to reduce possible deleterious effects caused by laboratory rearing. During 1979, local releases were made without laboratory rearing ; adults were released each day as they emerged from eggs collected in Europe.

RESULTS AND DISCUSSION

EXTRACTION OF EGGS FROM SOIL

The recovery of *Sitona* eggs from soil samples containing known numbers of eggs averaged 98.7 %. This rate compares well with a recovery of 98 % reported by **Aeschlimann** (1975) and a 99 % recovery reported by **Ng** *et al.* (1977). Hatchability of eggs recovered in the present study averaged 90.4 % (n = 21,366), ranging from 83.9 % in 1985-86 to 95.5 % in 1983-84. This rate of egg hatch is higher than most averages reported by **Aeschlimann** (1986), suggesting that the extraction method did not seriously affect egg viability.

To ascertain the existence of any native egg parasites in the resident population of *S. hispidulus,* 129 soil samples were taken on 19 dates from September 1975 to June 1976 in the Delaware study field. These yielded 3,057 viables eggs, but no parasites. During the same period, 56 samples were taken from 9 alfalfa fields in Washington Co., Md. ; Berrien Co., Mich. ; and in Berks, Franklin, and York counties, Pa. No parasites were reared from the 712 viable eggs found at these sites. Based on these negative results and the fact that no hymenopterous egg parasites of *Sitona* spp. are recorded from North America (**Krombein** *et al.,* 1979), it was concluded that this ecological niche was empty and an introduction of the European mymarid was warranted.

PARASITE RELEASES

All releases of *A. diana* made in the United States are summarized in table 1. Founder colonies released in Delaware were predominantly female : parasites from France averaged

55.8 % female (n = 1,431), those from Austria were 59.1 % female (n = 252). These figures do not differ from sex ratios observed by **Aeschlimann** (1977) in France, but are reported here because new evidence indicates that in Europe there may be 2 biotypes of *A. diana*, occurring sympatrically, one reproducing thelytokously and the other bisexually (**Aeschlimann,** 1986). Based on sex ratio and the frequent matings observed, all introduced *A. diana* were assumed to be bisexual, but it is possible that some of the founder females were thelytokous.

TABLE 1

Summary of releases of Anaphes diana *made in the United States through 1986* (1)

State	County	Year	Origin	No. released
Delaware	New Castle	1976	France	1,776
	New Castle	1977	France	4,009
	New Castle	1978	Austria, France	7,396
	New Castle	1979	France	409
Idaho	Latah	1976	France	138
	Latah	1977	France	1,900
	Latah	1982	France	9,967
	Latah	1984	Austria	876
Illinois	Washington	1977	France	437
Kentucky	Fayette	1977	France	5,000
	Fayette	1979	France	1,000
	Fayette	1982	France, Greece Italy	2,050
	Fayette	1984	Austria	57
	Owen	1980	France	4,400
	Todd	1978	France	200
	Warren	1977	France	500
Total				40,115

(1) Release information from USDA-ARS shipment and release data bank at Newark, Del.

RECOVERY ATTEMPTS

To determine if *A. diana* had established in the Delaware study field, *Sitona* egg populations were monitored for several winters (table 2) using the techniques previously described. The sampling program covered the period of major *Sitona* oviposition activity, from fall to spring, with peak egg densities being recorded in mid-winter (fig. 1). Similarly, **Leibee** (1979) reported that in Kentucky, *S. hispidulus* eggs could be found in alfalfa fields throughout the year except from mid-June to mid-September. In Delaware, most of the oviposition by *S. hispidulus* took place in October and November and most of the eggs hatched in early spring. The mean densities of viable overwintering *Sitona* eggs found each year varied from 8.5 to 34.2 per 100 cm^3, but averaged 14.6 eggs/100 cm^3 over the 9 years. By comparison, a mean of *ca.* 27 viable eggs/100 cm^3 of surface soil is indicated using data reported by **Ng** *et al.* (1977) for *S. hispidulus* in alfalfa near Lexington, Ky.

A. diana was first recovered from the study field in 1982 (table 2), *ca.* 3 years after the last release was made. Positive identification was made from 2 ♀♀ and 1 ♂ reared from eggs of *S. hispidulus* extracted from soil in February 1982. During the next winter one more female was recovered, but no recoveries were made during the winter of 1983-84, suggesting that

TABLE 2

Egg populations of Sitona hispidulus *in soil of alfalfa study field at Newark, Delaware*

Winter of:	No. sample dates	Total sample area (1) (cm^2)	Total viable eggs	No. viable eggs/100 cm^3		No. parasitized eggs
				mean	peak	
1975-76	19	30,334	3,057	10.1	21.4	0
1976-77	14	26,320	2,926	11.1	25.9	0
1977-78	2	5,640	1,844	34.2	38.7	0
1978-79	10	18,800	2,294	12.2	19.9	0
1979-80	11	20,680	1,762	8.5	10.9	0
1981-82	11	26,320	2,909	10.9	21.4	3
1982-83	6	11,280	1,136	10.1	14.3	1
1983-84	6	11,280	2,350	20.8	28.7	0
1985-86	4	7,520	1,030	13.7	16.9	3

(1) Soil sampled to a depth of 1 cm.

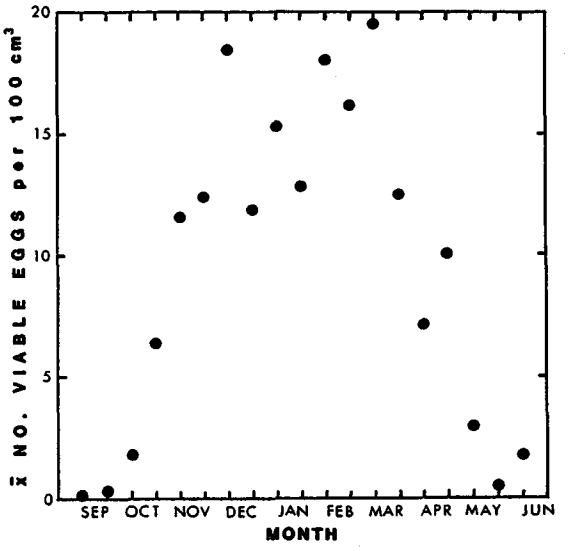

Fig. 1. Seasonal variation in mean density of *S. hispidulus* eggs in top 1 cm of soil in alfalfa study field, Newark, Del. (biweekly averages over 9 yr, 1975-1986).

the parasite had failed to persist. However, the parasite was recovered again (1 female, 2 males) from eggs collected in March 1986 and the identity was reconfirmed. Based on the number of viable host eggs collected (table 2) during the most recent sampling period (1985-86), the average level of parasitism had reached only 0.29 % (3/1,030). In contrast, **Aeschlimann** (1975) reported a mean of 7.9 % parasitism by this species in southern Europe.

Explanations for the disappointing performance of *A. diana* in Delaware are lacking. However, it is unlikely that the reason can be inadequate genetic diversity in the introduced

parasites, or severe climatic differences between the region of origin and the region of introduction. **Aeschlimann** (1986) suggests that the choice of sexual biotype used in such introductions may affect the ability of the species to colonize, but there is no clear evidence as yet to support this view. It also is quite possible that parasitism figures from the Delaware study field are underestimated and that additional parasites could have been in aestivation and were missed among the 9.6 % of the host eggs which failed to hatch. **Aeschlimann** (1977) reported aestivation in *A. diana* from the Mediterranean region.

Although the parasite has had no significant effect on *S. hispidulus,* the fact that the species was recovered 7 years after the last release indicates that it has colonized at the Delaware release site. Cooperators in Illinois and Kentucky (see Acknowledgments) have not reported any recoveries of *A. diana* to date. However, in Idaho, specimens of *A. diana* were recovered (and identified) from release fields on 2 occasions (**L. E. O'Keeffe,** personal communication).

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RÉSUMÉ

Introduction et récupération aux Etats-Unis d'*Anaphes diana* [*Hym. : Mymaridae*], parasite des œufs de *Sitona* [*Col. : Curculionidae*]

Anaphes diana (Girault) (= *Patasson lameerei* Debauche), mymaride parasite des œufs de *Sitona* spp., provenant d'Europe, a été libéré aux Etats-Unis à partir de 1976 et il a été retrouvé dans l'Etat du Delaware. Des techniques sont décrites pour l'extraction des œufs de *Sitona* du sol. Après 9 ans de prélèvements dans un champ de luzerne au Delaware (> 19,300 œufs obtenus), la densité moyenne des œufs viables hivernant de *Sitona hispidulus* (F.) a été 14,6 par 100 cm^3 du sol (1 cm de profondeur). Bien que le niveau de parasitisme par *A. diana* ne dépasse pas 0,29 %, le fait que cette espèce ait été retrouvée pendant 3 ans, et jusqu'à 7 ans après le dernier lâcher, indique qu'elle s'est probablement établie dans l'Etat du Delaware.

MOTS CLÉS : *Anaphes diana,* lutte biologique, *Sitona hispidulus,* densité des œufs.

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