Two Types of Overwintering Larvae in a Wild Multivoltine Bruchid, *Kytorhinus sharpianus* BRIDWELL¹ (Coleoptera: Bruchidae)²

Masakazu SHIMADA and Michihiro ISHIHARA

*Department of Biology, College of Arts and Sciences, University of Tokyo,
Komaba, Meguro-ku, Tokyo 153, Japan*

(Received July 5, 1990; Accepted April 8, 1991)

We studied the seasonal prevalence and the overwintering stage(s) of a wild multivoltine bruchid, *Kytorhinus sharpianus*, on the host, *Sophola flavescens*, in Tokyo and Tsukuba. Adult *K. sharpianus* appeared first on *S. flavescens* in late April and oviposited on the fresh pods of *S. flavescens* in mid-June. First-generation adults emerged from early to late August and oviposited until late August. A few second-generation adults emerged from mid-September to mid-October, with most females laying eggs by late October without adult feeding. However, most second-generation larvae did not grow to adulthood in fall, remained at the late final instar during winter, pupated in early April and emerged in late April. The third-generation larvae which hatched in October remained at the first instar during winter and grew to the second instar in late March and to the third instar in early April. A laboratory experiment showed that the late final-instar larvae diapaused at 24°C and 12L–12D, although they grew to adults at 24°C and 16L–8D. These results suggested that *K. sharpianus* overwinters either at the late final instar in the second generation or at the young instar in the third generation. This species seems to be basically bivoltine in the Kanto district and a few individuals show a trivoltine cycle.

Key words: larval overwintering, wild bruchid, trivoltinism, *Kytorhinus sharpianus*, photoperiod

INTRODUCTION

Bruchids have been classified into two types: field type and cross-infestation (storage, indoor) type (KIRITANI, 1956; SOUTHGATE, 1979; UMEYA, 1981a). According to these authors, the field type (e.g., *Bruchus rufimanus* (PIC.)) is univoltine, diapauses at the adult stage, oviposits on fresh host pods, and develops as larvae in a fresh seed. In contrast, the cross-infestation type (e.g., *Callosobruchus chinensis* (L.) and *C. maculatus* (FAB.)) is multivoltine, lacks diapause, oviposits on dry, matured pods and seeds, and develops as larvae in a dry, matured seed.

Recently, however, wild bruchids, *Salcobruchus sauteri* (PIC.) (WATANABE, 1985) and *Kytorhinus sharpianus* BRIDWELL (SHIMADA, 1988), were reported to oviposit and develop in both immature, fresh seeds and matured, dry ones. These characteristics allow them to have multivoltine life cycles in the field (WATANABE, 1990), and show that the dry

¹ MORIMOTO (1990) recently described *K. sharpianus* BRIDWELL as a synonym for *K. senilis* SOLSKY.

² The present study was supported in part by a Grant from the Fujiwara Foundation for Natural History Education to M. SHIMADA.

seed infestation and voltinism aspects of the aforementioned classification of bruchids are not appropriate.

SHIMADA (1988) observed the natural life history of *K. sharpianus* in Tsukuba, Ibaraki, Japan. The adults of this species come to flowers of the leguminous host plant *Sophola flavescens* AITON in mid-June. Just after *S. flavescens* bears seeds, *K. sharpianus* females oviposit on small, fresh pods. The first-generation larvae develop within growing fresh seeds, and the adults emerge in mid-August and lay eggs on drying matured pods of *S. flavescens*. The second-generation adults emerge from late September to early October and oviposit on completely dried pods of *S. flavescens* or directly on the dry, matured seeds in dehiscent pods. The third-generation larvae hatch and crawl into dry seeds in early October. Earlier, I reported adult emergence in a growth chamber in mid-February from *S. flavescens* seeds which were collected in October 1987 and placed under low temperatures followed by the conditions of 30°C and 16L-8D (SHIMADA, 1988). This report suggested that larvae winter in *K. sharpianus* and questioned the classic view that wild bruchids diapause at the adult stage. However, it did not define larval stage or generation of overwintering.

In this study, therefore, we studied the seasonal prevalence and the wintering stages of *K. sharpianus* in the field. We took censuses of the emergence and oviposition of adults in each generation (Experiment 1), and determined the stages and generation of the larvae in the dry seeds during winter (Experiment 2). We also examined whether the larvae enter into diapause under a short photoperiod in the laboratory (Experiment 3), as well as the reproductive activity level of the second-generation adults (Experiment 4).

MATERIALS AND METHODS

Experiment 1: Emergence and oviposition of adults in each generation. This experiment was conducted in our departmental research field (Meguro-ku, Tokyo) in 1989. Nineteen plants of *S. flavescens* were raised and a *K. sharpianus* population was introduced from a natural field in Tsukuba in 1987 and established.

We employed two field census methods. The first method was used for estimating the oviposition and emergence periods in each generation. We chose *S. flavescens* pods randomly and labeled them individually in late June. Cumulative numbers of emergence holes and eggs deposited on each pod were counted at least once every several days. Newly deposited eggs were distinguished from old ones laid by the previous generation by color (new eggs are yellow). One hundred and fifty one pods were labeled for the first-generation adults. Fifty of these pods were used for the second-generation adults and 37 pods were diverted to the other census in the second generation. The remainder was lost by rains or high winds.

The second method was used for directly determining the emergence period in each generation. We covered *S. flavescens* pods with nylon-mesh bags before adult emergence. We counted the number of adults which emerged in the bags at least once every several days. The number of pods used in the census was 84 for the first generation and 90 for the second generation.

In both methods, we continued the census from August 8th to September 3rd for the first generation and from September 23rd (from September 18th for the census with bags) to October 23rd for the second generation.

Experiment 2: Developmental stages of larvae during winter. About 30 dry pods and

30 dry seeds of *S. flavescens* bearing hatched egg shells were collected from the research and Tsukuba fields from late October to early November in 1989. The second-generation adults emerge in late September (SHIMADA, 1988) when some dry pods begin to dehisce and the seeds become exposed to the insects. Eggs which were attached to the seeds were therefore judged to have been laid by the second-generation adults. We then introduced these pods and seeds into separate bags and placed them in the research and Tsukuba fields.

The pods in those bags were brought into the laboratory about once a month from late November in 1989 to early April in 1990. We took out dry seeds from the pods, dissected them with a binocular microscope, and determined the larval stage based on the width of mouth parts. For the final (fourth) instar, we distinguished between the early and the late stages by larval body size.

The same inspection was carried out for the seeds with hatched eggs. This was done after February because only a small number of seeds were available. In this inspection, we selected the seeds without holes made by the larvae hatched from the eggs on the pod surface in the previous generations.

Experiment 3: Larval development at 24°C, 12L-12D. The development of larvae under a short photolength was studied in the laboratory from March to June in 1990. We used the adults which emerged in the laboratory from *S. flavescens* seeds collected in the Tsukuba field in mid-October 1989. Five pairs of adults after feeding (sugar + dry yeast and water) were introduced into a Petri dish with about 200 dry matured seeds of *S. flavescens*. We picked up seeds with eggs from the dish every day and added the same number of new seeds. Dead adults were replaced with new ones. More than 20 females were used for the entire experiment. We selected the seeds with only one egg, because the developmental rate of *K. sharpianus* was affected slightly by the larval density per seed (SHIMADA and MATSUDA, submitted).

Those seeds were placed under 24°C, 12L-12D conditions which are similar to those in mid-September in Japan. We dissected the seeds with a binocular microscope once every several days until the 70th day after oviposition and every five days from the 70th day to the 100th day. We determined the instar based on the width of larval mouth parts.

Experiment 4: Oviposition by second-generation adults without feeding. We examined whether second-generation adults are reproductively inactive and enter into wintering or show reproductive activity even without feeding after emergence. We captured the adults which emerged during September 18th and October 16th in 1989 from the pods covered with the mesh bags in the research field. We also used the adults which emerged later than October 19th from the pods that we collected in the research field from mid-September to early October and placed in a fully ventilated hut. No food was supplied to the insects. A male-female pair, within two days of post-emergence, was introduced into a plastic container with matured *S. flavescens* pods bearing no eggs. We placed six containers in a cluster of grass and five in the hut to avoid effects of rain. Containers were inspected once every several days. The date of the first oviposition and numbers of eggs were recorded.

We also collected non-feeding females which emerged from the pods covered with the same mesh bags in the Tsukuba field and introduced each female with a male into a container containing dry pods on October 17th and 30th. The number of eggs laid on the pods in Tsukuba was investigated on October 30th and November 11th.

RESULTS

Experiment 1: Emergence and oviposition of adults in each generation

The cumulative number of emergence holes of the first-generation adults increased rapidly from August 8th to August 19th, then reached a plateau (Fig. 1a). The cumulative number of eggs laid by the first-generation adults increased a few days later than the increase of emergence holes and reached a plateau at the end of August (Fig. 1a). Some of the second-generation adults had already emerged when the census was started on September 23rd (Fig. 1b). Adults probably began to emerge in mid-September. The cumulative number of emergence holes reached a plateau in early October. The cumulative number of eggs deposited by the second-generation adults increased until October 16th, then reached a plateau. Cumulative oviposition curves of the second-generation adults (Fig. 1b) rose ca. 15 days after the first generation reached the plateau (Fig. 1a), suggesting that the first-generation adults had died before the emergence of the second-generation adults.

Similar results were obtained in the census on pods covered with mesh bags: adults emerged from August 5th to August 28th in the first generation and from September 19th to October 6th in the second generation.

The emergence ratios (no. of emerged adults: no. of eggs laid on pods) in the two censuses were 62.8% (59/94 on 151 pods) and 54.6% (59/108 on 84 pods) in the first generation. The emergence ratios in the second generation were 3.4% (12/357 on 50 pods) and 0.7% (2/295 on 37 pods). Though the egg hatchability on pods and the success ratio of hatched larvae infesting seeds were not measured precisely in this experiment, the net emergence ratio also seems to be very low in the second generation.

Experiment 2: Developmental stages of K. sharpianus during winter

The larvae which hatched from the eggs laid on pod surfaces were in the late fourth instar from late November to late February, and pupated in early April (Table 1, left). They emerged in late April, which was compatible with the first

Fig. 1. Changes in the cumulative numbers of emergence holes (Δ) of *K. sharpianus* adults and eggs deposited on *S. flavescens* pods (●). a: first generation, b: second generation.

Table 1. Developmental stages of larvae during winter, 1989–90

Sampling date	No. of larvae in each stage											
	Eggs laid on pod surface						Eggs attached to seed surface					
	Developmental stage						Developmental stage					
	I	II	III	IVE	IVL	P	I	II	III	IVE	IVL	P
Nov. 27	—	—	—	—	4	—						
Dec. 12	—	—	—	—	4	—						
Dec. 15	—	—	—	—	2	—						
Jan. 16	—	—	—	—	12	—						
Jan. 22	—	—	—	—	7	—						
Feb. 22	—	—	—	—	5*	—	7*	—	—	—	—	—
Mar. 24	—	—	—	—	—	—	—	4*	—	—	—	—
Apr. 3	—	—	—	—	2	18	—	1	3	1	—	—

I: first instar, II: second instar, III: third instar, IVE: early fourth instar, IVL: late fourth instar, P: pupae

Empty row: no observation conducted.

—: no individuals observed.

*: sampled from natural field in Tsukuba.

Fig. 2. Developmental period in each stage in *K. sharpianus* larvae reared at 24°C, 12L-12D. Roman figures indicate instar. The number of samples examined is shown in each stage (fourth instar is shown for every 10 days). All the samples remained at the late final instar until the 100th day.

observation of *K. sharpianus* adults on the growing shoots of *S. flavescentis* in the research field on April 25th in 1989 and April 27th in 1990.

On the other hand, the larvae that hatched from the eggs which were attached to the seed surface remained at the first-instar stage in late February, and grew to the second instar in late March and to the third instar in early April (Table 1, right).

Experiment 3: Larval development at 24°C, 12L-12D

It took ca. 10 days for *K. sharpianus* larvae to proceed from one instar to the next until the fourth-instar stage at 24°C, 12L-12D (Fig. 2). The fourth-instar larvae

Table 2. Oviposition test for second-generation adults without feeding after emergence

No.	Source population	Test site	Setting date	No. of eggs at first observation (date)
1	rf	rf	Sep. 21	7 (Sep. 23)
2	rf	rf	Sep. 22	4 (Sep. 23)
3	rf	h	Sep. 23	0 & died (Sep. 27)
4	rf	rf	Sep. 26	2 (Oct. 5)
5	rf	h	Sep. 28	2 (Oct. 2)
6	rf	rf	Oct. 2	13 (Oct. 9)
7	rf	rf	Oct. 2	8 (Oct. 5)
8	rf	rf	Oct. 16	0 & died (Oct. 17)
9	rf	h	Oct. 19	6 (Oct. 31)
10	rf	h	Oct. 25	5 (Nov. 2)
11	rf	h	Oct. 31	12 (Nov. 2)
12	Tsukuba	Tsukuba	Oct. 17	45 (Oct. 30)
13	Tsukuba	Tsukuba	Oct. 30	0 & died (Nov. 11)

rf: the research field of our department

h: a fully ventilated hut in the research field

which had just molted from the third instar continued to grow and filled the space inside the seed coat within ca. 50 to 55 days. All individuals then remained at this stage and did not pupate before the 100th day (the last observation). This period was much longer than the average period of 62.5 days when *K. sharpianus* reached adult emergence at 24°C, 16L–8D conditions (SHIMADA, 1988), as indicated in Fig. 2.

Experiment 4: Oviposition by the second-generation adults without feeding

Nine out of 11 female replicates without feeding began to lay vital eggs within 10 days in the research field (Table 2). It seems reasonable to include the data of the five containers in the hut into this result, because it was fully ventilated. Two females died within four days without oviposition for unknown reasons. One female out of two in Tsukuba oviposited by October 30th. The other female was found dead without oviposition on November 11th. In total, 10 out of 13 females laid eggs without feeding, and even the females which emerged later than mid-October could oviposit (Table 2). All adults examined eventually died within 20 days because they were not fed.

DISCUSSION

Seasonal prevalence and the overwintering stages

Kytorhinus sharpianus adults emerge three times in a year: from late April (the first appearance of adults in 1989 and 1990) to late June (SHIMADA, 1988) in the overwintered generation, from early August to late August in the first generation (Fig. 1a), and from mid-September to mid-October in the second generation (Fig. 1b). Adults lay eggs soon after emergence in the first (Fig. 1a) and second generations (Fig. 1b and Table 2).

This study reveals that *K. sharpianus* has at least two overwintering stages of the first and late fourth instar, as seen in Fig. 3. The late fourth-instar larvae in Table 1

Fig. 3. Two stages of overwintering *K. sharpianus* larvae. Left: A second-instar larva ("L") collected on March 24. This larva grew from one which remained in the first instar during winter (see Table 1). Right: A late final (fourth)-instar larva collected on February 22nd. The fully grown larva fills the entirety of the seed coat interior.

(left) were judged to be of the second generation, because the third-generation larvae, which deposited from late September to mid-October, could not have grown to this stage by late fall (judging from the known growth rates at various temperatures; SHIMADA, 1988).

On the other hand, the first-instar larvae in Table 1 (right) were judged to be of the third generation for two reasons. First, the oviposition by the first-generation adults ended by the end of August (Fig. 1a) and high temperatures at the time would advance those hatched larvae to later stages. Second, the eggs which were directly attached to the seed must have been deposited after full dehiscence of dried pods in early October when the second-generation adults emerged and oviposited (Fig. 1b). The reason why we observed no first-instar larvae in the third generation in the seeds infested by larvae hatched from eggs on the pod surface (Table 1, left) seems to be that most *S. flavescens* seeds had been infested by the superabundant second-generation larvae laid in August (about 8 eggs laid per pod in Fig. 1a). Though we could not confirm the adult emergence of the third generation in 1990, the younger larvae in Table 1 (right) may survive under mild temperatures in April and emerge by early June when *S. flavescens* begins to blossom.

The possibility of other overwintering stages should be discussed. Our study shows that the second-generation adults were reproductively active, even without feeding, after emergence (Table 2). We did not confirm whether those adults, if fed,

stop oviposition and overwinter. However, a preliminary examination in 1988 showed that all the second-generation adults (more than 30) which were introduced into the mesh bags with food (sugar + dry yeast) and withered leaves were found dead in March, 1989 (SHIMADA, unpubl.). It seems unlikely that the second-generation adults overwinter after stopping reproduction. We collected only a few seeds containing third-generation larvae later than late October. If we could gain a larger sample earlier, we might find overwintering larvae at the second-instar or later stages in the third generation, which grew from eggs deposited in mid- or late September.

The fact that many fourth-instar larvae in the second generation overwinter in the field indicates that *K. sharpianus* basically has a bivoltine life cycle in the Kanto district. However, existence of the overwintering first-instar larvae produced by the second-generation adults suggests that *K. sharpianus* is likely to have a partially trivoltine life cycle. Production of the third-generation larvae in fall may be an adaptation for utilizing the host seeds efficiently in warm regions. We are greatly interested in the emergence ratio of the second-generation adults in early fall along latitudinal clines in Japan.

We could confirm neither the larval stage in the seeds bearing hatched eggs from November to February in Table 1 (right) nor time of emergence because of the small number of seeds with hatched eggs in fall, 1989. A more intensive field study should be conducted for assessing the partial trivoltinism in *K. sharpianus*.

Larval diapause in the late final instar under a short photoperiod

The late final-instar larvae in the second generation (Table 1, left) seem to be in diapause, because larvae diapaused at this stage at 24°C, 12L–12D (Fig. 2) which are similar to the condition in early to mid-September in Kanto. The low emergence ratios as seen in the second-generation adults (Experiment 1) also suggest that many of the late final-instar larvae in the second generation do not reach adult emergence but remain at this stage, as seen in Table 1 (left). On the other hand, the first-instar larvae in Table 1 (right) may not have been in diapause but only stopped growing due to low temperatures during winter, because Fig. 2 showed that the first-instar larvae grew to the next instar for ca. 10 days at 24°C, 12L–12D. Non-diapausing by overwintering larvae in the third generation may be adaptive in warm regions with mild winters if winter mortality rate is not high.

Only temperature has attracted researchers' notice as a major factor controlling the development of multivoltine bruchids such as *C. chinensis* (ISHIKURA, 1941; UMEYA, 1981b) and *Bruchidius atrolineatus* (MONGE et al., 1989). Stored bean pests such as *C. chinensis* and *C. maculatus* do not diapause even in darkened growth chambers (e.g., UTIDA, 1941). Therefore, there has been no report showing the dependence of larval diapause on the photoperiod in multivoltine bruchids.

The present study, however, revealed that a short photoperiod causes the larval diapause of *K. sharpianus* at the late fourth instar, although this species is a multivoltine bruchid. Larvae may be able to sense light through the seed coat, because the late final-instar larvae of *K. sharpianus* feed on the cotyledon of *S. flavescens* seed and fill the space inside.

We have just started low-temperature treatment followed by 24°C, 16L–8D. Such treatments probably terminate the diapause as known in many insect species (TAUBER et al., 1986). However, further experiments under various combinations of temper-

atures and photoperiods are necessary for identifying the critical photolength inducing the diapause and the sensitive stage of larvae to a short photolength. We project those experiments for the next study. We are also interested to study if the variation among second-generation larvae between adult emergence and diapause in fall has a genetic basis. Such studies may reveal the evolutionary process of the complex life cycle in *K. sharpianus*.

ACKNOWLEDGEMENTS

We thank Prof. Y. INO of Waseda University for his encouragement throughout the course of this study, and also Prof. K. FUJII of University of Tsukuba for his support of the field in Tsukuba. We are grateful to Prof. Emer. S. UTIDA, Prof. K. FUJII, Drs. N. WATANABE and T. SOTA for their valuable comments on earlier manuscripts. We also express our thanks to two anonymous referees for improving the manuscript.

REFERENCES

- ISHIKURA, S. (1941) Influences of temperature and moisture on the developmental period of *Callosobruchus chinensis* (L.) *Oyo-Dobutsu* **13**: 118–131 (in Japanese with English summary).
- KIRITANI, K. (1956) Ecology and establishment as pests in Bruchidae. *Shin-Kontyu* **9**: 7–11 (in Japanese).
- MONGE, J.-P., A. LENG and J. HUIGNARD (1989) Induction of reproductive diapause in *Bruchidius atrolineatus* during the dry season in a Sahelian desert. *Entomol. exp. appl.* **53**: 95–104.
- MORIMOTO, K. (1990) A synopsis of the bruchid fauna of Japan. In *Bruchids and Legumes: Economics, Ecology and Coevolution* (K. FUJII et al., eds.) Kluwer Acad. Publ., Dordrecht, pp. 131–140.
- SHIMADA, M. (1988) Dry bean infestation and oviposition without feeding by a wild multivoltine bean weevil, *Kytorhinus sharpianus* BRIDWELL (Coleoptera: Bruchidae). *Appl. Ent. Zool.* **23**: 459–467.
- SOUTHGATE, B. J. (1979) Biology of the Bruchidae. *Ann. Rev. Entomol.* **24**: 449–473.
- TAUBER, M. J., C. A. TAUBER and S. MASAKI (1986) *Seasonal Adaptation of Insects*. Oxford Univ. Press, New York, 411 pp.
- UMEYA, K. (1981a) Bean weevils: Biology of bruchids (1). *Insectarium* **18**(1): 4–10 (in Japanese).
- UMEYA, K. (1981b) Temperature and development: Biology of bruchid (8). *Insectarium* **18**(8): 16–23 (in Japanese).
- UTIDA, S. (1941) Studies on experimental population of the azuki bean weevil, *Callosobruchus chinensis* (L.). I. The effect of population density on the progeny populations. *Mem. Coll. Agric., Kyoto Imp. Univ.* **48**: 1–30.
- WATANABE, N. (1985) Oviposition habit of *Sulcobruchus sauteri* (PIC.) and its significance in speculation on the preagricultural life of seed beetles attacking stored pulses (Coleoptera, Bruchidae). *Kontyu, Tokyo* **53**: 391–397.
- WATANABE, N. (1990) Diversity in life cycle patterns of bruchids occurring in Japan (Coleoptera: Bruchidae). In *Bruchids and Legumes: Economics, Ecology and Coevolution* (K. FUJII et al., eds.) Kluwer Acad. Publ., Dordrecht, pp. 141–147.