Seasonal Changes in Prevalence of Viral Disease and Parasitism by Parasitic Insects in a Larval Population of the Smaller Tea Tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae) in a Tea Field

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(Received 18 April 1997; Accepted 27 June 1997)

The incidence of parasitoids and entomopathogens in a larval population of *Adoxophyes* sp. in a tea field in Tsukuba, Ibaraki Prefecture, was determined. Three species of Braconids, one species of Ichneumonid, and one species of Tachinid were identified in the collected larvae. Three entomopathogenic viruses, an entomopoxvirus (EPV), a nuclear polyhedrosis virus and a granulosis virus, were isolated from the dead larvae. Overall, 87.0% of *Adoxophyes* sp. larvae were killed by parasitoids and entomopathogens. *Ascogaster reticulatus* and EPV were the most common parasitoid and pathogen and were responsible for 37.7% and 23.1% of the deaths in the *Adoxophyes* sp. larvae, respectively. Percent parasitism by *A. reticulatus* was highest in the first generation and then decreased progressively toward the fourth generation. EPV prevalence was low in the first generation and increased through the generations. Of 333 host larvae dying from viral infection, 12.0% of host larvae contained dead parasitoid larvae. Some parasitoid larvae were able to emerge from virus-infected hosts, whereas these parasitoids died before or after pupation. The time to death of a parasitoid developing in virus-infected hosts appears to be closely related to the species of viruses.

Key words: *Adoxophyes* sp., natural enemy, *Ascogaster reticulatus*, entomopoxvirus, host-parasitoid-pathogen interaction

INTRODUCTION

The smaller tea tortrix, *Adoxophyes* sp., is a major pest of tea plants in Japan, and its control had depended on the use of synthetic pesticides. The development of resistance by this insect to the pesticides and the residue of pesticides on the harvested tea leaves have roused interest in finding ways to use biological agents for its control. YASUMATSU and WATANABE (1965), MOMOI et al. (1975) and TAMAKI (1991) have reviewed the natural enemies of this insect, and have recorded 40 species of predators, parasitoids and pathogens in Japan. However, few quantitative data are available to evaluate the impact of these natural enemies upon *Adoxophyes* sp. In addition, competitive interaction between parasitoids and entomopathogens has been shown to occur during simultaneous parasitism of a host (BROOKS, 1993; HARPER, 1986). Therefore, it is important to understand these interactions in the field in order to evaluate the role of each natural enemy. The purpose of this study was to quantify the presence of entomopathogens and parasitoids in larval populations of *Adoxophyes* sp. in a tea field during a one year period and to analyze the interactions between these two natural enemies.

MATERIALS AND METHODS

Collection of insects. Parasitic natural enemies of *Adoxophyes* sp. were surveyed by collecting host larvae in a tea field in Tsukuba-shi, Ibaraki Prefecture in 1996. The tea field was planted with a Yabukita variety. No application of chemical insecticide was made throughout the survey. At least 110 larvae were randomly collected at each generation: April 6 (overwintering generation), June 15 (first generation), July 26 (second generation), September 6 and 16 (third generation) and October 31 (fourth generation). The collected larvae were brought into the laboratory and reared individually in 30 ml plastic cups containing artificial diet (Insecta LF, Nihon Nosan Kogyo) under a 25°C, 16L8D photoperiod. Larval stadia were estimated by head-capsule width measurements at the time of collection.

Identification of pathogens. Dead larvae were dissected under the microscope to determine the presence of parasitoid larvae. The tissues of dead larvae were examined for polyhedra of a nuclear polyhedrosis virus (NPV) or spheroids of entomopoxvirus (EPV) in a wet mount by phase-contrast microscopy. Infection with the granulosis virus (GV) was determined by an enzyme-linked immunosorbent assay (ELISA) using mouse anti-occluded virion antiserum. A homogenate of dead larvae was used for the ELISA. The ELISA procedure was carried out using the method of KUBOTA and INOUE (1989).

RESULTS AND DISCUSSION

Of 862 *Adoxophyes* sp. larvae reared, 87% were killed by natural enemies. The percentage of larvae reaching adulthood differed between the generations: 18% for overwintering

Table 1. Percentage parasitism of *Adoxophyes* sp. larvae by each natural enemy

Natural enemy	No. collected ^b	Percentage of parasitism
<i>Ascogaster reticulatus</i>	325	37.7
<i>Apanteles adoxophyesi</i>	27	3.1
<i>Meteorus ictericus</i>	2	0.2
<i>Campoplex</i> sp.	1	0.1
Unidentified parasitic wasps	15	1.7
Tachinidae gen. sp.	3	0.3
Mermithidae gen. sp.	2	0.2
Entomopoxvirus (EPV)	199	23.1
Nuclear polyhedrosis virus (NPV)	31	3.6
Granulosis virus (GV)	19	2.2
EPV + NPV ^a	55	6.4
EPV + GV ^a	13	1.5
NPV + GV ^a	13	1.5
EPV + NPV + GV ^a	3	0.3
<i>Erynia radicans</i>	5	0.6
<i>Fusarium</i> sp.	1	0.1
Unknown pathogens	36	4.2
Total	750	87.0

^a *Adoxophyes* sp. larvae were infected with two or more different kinds of viruses simultaneously.

^b A total of 862 *Adoxophyes* sp. larvae were reared.

Fig. 1. Percentage of entomopoxvirus (EPV), nuclear polyhedrosis virus (NPV) and granulosis virus (GV) infection occurring in *Adoxophyes* sp. in different stadia collected in 1996 from a tea field in Ibaraki, Japan.

generation, 13% for the first generation, 10% for the second generation, 11% for the third generation and 15% for the fourth generation. The percentage parasitism of *Adoxophyes* sp. larvae by natural enemies is shown in Table 1. Three species of Braconids, one species of Ichneumonid, Tachinid and Nematodes were identified in the collected larvae. Three entomopathogenic viruses, EPV, NPV, GV, and two entomopathogenic fungi, *Erynia radicans* and *Fusarium* sp., were observed in the dead larvae.

The most dominant parasitic natural enemy was *Ascogaster reticulatus* (WATANABE, 1967), which caused 37.7% of all deaths in *Adoxophyes* sp. larvae. TAKAGI (1974) used a sticky suction trap and showed that the most common hymenopteran parasitoid of *Adoxophyes* sp. was *Apanteles adoxophyesi*, followed by *A. reticulatus* in a tea field in Shizuoka Prefecture. His results do not correspond to those from our study because the sticky suction trap could easily catch adult wasps, such as *A. adoxophyesi*, flying among the rows of tea bushes. In contrast to *A. adoxophyesi*, *A. reticulatus* adults spend most of the time walking on leaves rather than flying from leaf to leaf (SHIMIZU et al., 1991). As a result, *A. reticulatus* might not be caught as easily as *A. adoxophyesi* when using the sticky suction trap.

The most common entomopathogen identified in our study was EPV, which caused 23.1% of all deaths in the *Adoxophyes* sp. larvae. A high prevalence of EPV has been reported

in a natural population of *Adoxophyes* sp., in Miyazaki Prefecture (ISHIKAWA et al., 1983). Although the presence of NPV in *Adoxophyes orana* (a species closely related to *Adoxophyes* sp.) has been reported in Europe (PONSEN and DE JONG, 1964), and this NPV has been shown to infect *Adoxophyes* sp. (SATO, 1981), the relationship between *A. orana* NPV and *Adoxophyes* sp. NPV has not yet been established. Of 333 larvae with viral infection, 25% were infected with two or more of the viruses simultaneously. Among those larvae, larvae infected with a mix of EPV and NPV were the most abundant (Table 1).

The second to fifth stadia were attacked by each of these three viruses (Fig. 1). The prevalence of these viruses was similar among the stadia. These results indicate that *Adoxophyes* sp. larvae might be infected with those viruses during early stadia.

Seasonal changes in the occurrence of parasitism in *Adoxophyes* sp. by its primary natural enemies is shown in Fig. 2. EPV prevalence declined from the overwintering to the first generation. The reason for this is unknown. However, the buds of the tea leaves were harvested just before the infestation of the first generation occurred. This suggests that, as the virus population on the tea leaves decreases, the vertical transmission rate of EPV may also decline, as was seen in the change between the overwintering and the first generation. EPV prevalence increased after the first generation. Other authors have also reported that

Fig. 2. Seasonal fluctuation of percentage parasitism by natural enemies of *Adoxophyes* sp. larvae collected in 1996 from a tea field in Ibaraki, Japan. Entomopoxvirus (EPV), nuclear polyhedrosis virus (NPV), granulosis virus (GV), *Ascogaster reticulatus* (*Ascogaster*), and *Apanteles adoxophyesi* (*Apanteles*) were the primary natural enemies.

Table 2. Percentages of virus-killed *Adoxophyes* sp. larvae containing parasitoid larvae

Generation	No. of virus-killed hosts	Percentage containing parasitoid larvae
Overwintering	111	7.2
First	42	26.2
Second	38	23.7
Third	68	8.8
Fourth	74	8.1
Total	333	12.0

Table 3. Relationship between the conditions of parasitoid death and the viral species infecting *Adoxophyes* sp. larvae

Virus species infecting hosts	No. observed	Percentage of parasitoid larvae showing		
		Premature death	Death occurred after emergence	Death occurred after pupation
Entomopoxvirus	35	60.0	31.4	8.6
Nuclear polyhedrosis virus	12	33.3	66.7	0
Granulosis virus	8	25.0	75.0	0
Viruses ^a	21	61.9	33.3	4.8
Total	76	52.6	42.1	5.3

^a Host larvae were infected with two or more different kinds of viruses.

the prevalence of viral diseases appears to increase through the generations of the insects (EHLER, 1977; HUKUHARA, 1975), but the reasons for this have not been cited.

The rates of parasitism in *Adoxophyes* sp. larvae by *A. reticulatus* were lower in the generations showing a high EPV prevalence (the overwintering and fourth generations) than in those with a low EPV prevalence (the first and second generations). Regression analysis revealed a significant linear relationship between parasitism rates by *A. reticulatus* and EPV prevalence ($r = -0.99$, $p < 0.01$), where data were arcsin root transformed before analysis. This may indicate that *A. reticulatus* and EPV were in competition. KODOMARI (1987) reported that levels of parasitism in *Adoxophyes* sp. larvae by parasitic insects declined in a tea field where GV was applied to control *Adoxophyes* sp. There are some other examples where the levels of parasitism were reduced when viruses had been applied to the fields (SHIGA et al., 1973; FALCON, 1973).

The percentage of virus-infected *Adoxophyes* sp. larvae containing parasitoid larvae is shown in Table 2. The highest value was obtained in the first generation (26.2%), and these parasitoid larvae could not complete development in virus-infected hosts, resulting in premature death. Although some parasitoid larvae could emerge from the virus-infected hosts, these parasitoid larvae were killed before or after pupation. The relationship between the conditions of parasitoid death and the viral species infecting the host larvae is shown in Table 3. The relationship between the time to parasitoid death (premature death, death after emergence and death after pupation) and viral species infecting the host larvae (EPV, NPV and GV) was analyzed using a χ^2 test. Most of the parasitoid larvae developing in EPV-infected hosts died before they emerged from the host larvae, whereas those developing in GV- or NPV-infected hosts died after emergence from their hosts (Table 3). The time to parasitoid death was significantly different among host larvae infected with different viral species ($\chi^2 = 9.65$, $df = 4$, $p < 0.05$). In the laboratory experiment, more than 80% of *A. reticulatus* failed to emerge from the host when neonate parasitized *Adoxophyes* sp. larvae were inoculated with EPV (NAKAI et al., 1997). On the other hand, most of the parasitoid larvae developing in GV- or NPV-infected hosts died after emergence from their hosts (Table 3). When neonate parasitized *Adoxophyes* sp. larvae were inoculated with GV, more than 90% of *A. reticulatus* emerged from the hosts and then died before pupation (NAKAI and KUNIMI, 1997). It is clear that the results from this field study correspond to the results from the laboratory studies. In this study, we found that both NPV and GV from *Adoxophyes* sp. killed their hosts slowly (NAKAI et al., unpubl.). Therefore, the impact of NPV-infection of the hosts on parasitoid larvae may be similar to that of GV. Our results indicate that the

survival of parasitoids can be adversely affected when rates of viral infection in the hosts are high.

ACKNOWLEDGEMENTS

We thank Dr. K. MAETOH, Forestry and Forest Products Research Institute and Dr. K. KONISHI, National Institute of Agro-Environmental Science, for the determination of Braconidae and Ichneumonidae, respectively. We also thank an anonymous referee for critical reading and valuable suggestions.

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