Preliminary Research on Biological Characteristics of *Scopula sub*punctaria in Shandong Tea Region

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Abstract [Objectives] The paper was to observe the life history and living habits of Scopula subpunctaria in Shandong tea region. [Methods] The occurrence period and duration of each insect state, as well as duration of each instar larvae in various generations were investigated and statistically analyzed. [Results] There were 6 generations of S. subpunctaria per year in Shandong tea region. Different from southern tea region, S. subpunctaria survived the winter as pupae and larvae in tea trees in Shandong, and the duration of overwintering eggs was greatly affected by temperature. The generations of S. subpunctaria overlapped seriously. [Conclusions] The results provide a basis for the comprehensive treatment of S. subpunctaria in Shandong tea region.

Key words Shandong tea region; Scopula subpunctaria; Life history; Living habits

1 Introduction

Since the introduction of tea from the south to the north, the tea produced in Shandong Province has been favored by tea lovers inside and outside the province due to the characteristics of "thick leaves, brewing resistance, strong flavor and high aroma", and the area of tea gardens is increasing year by year. But with the introduction of tea tree seedlings, Scopula subpunctaria (Herrich-Schaeffer) has also been brought to Shandong tea gardens. S. subpunctaria occurs light in general years, causing mild damage to tea trees. However, it occurred severely in Shandong tea region from 2005 to 2007, especially from May to June, when the leaves of tea trees in some plots were eaten, while remaining tea leaves were covered with the excreta of S. subpunctaria, and the buds and leaves were seriously polluted and lost their economic value. From the winter of 2007 to the early spring of 2008, tea trees in Shandong suffered severe freeze injury. Due to the lack of food, the overwintering larvae of S. subpunctaria died in large numbers in the spring of 2008, and the occurrence level was always at a low level in the following years. In the corresponding period in Fujian, Sichuan and other provinces. S. subpunctaria broke out in some areas.

As previously reported, *S. subpunctaria* was mainly distributed in Zhejiang, Hunan, Sichuan, Guizhou, Jiangsu, Anhui and other places in the Yangtze River basin. In 1977, the Plant Protection Laboratory of Tea Research Institute, Chinese Academy of Agricultural Sciences conducted a detailed study on the biological characteristics of *S. subpunctaria*^[1]. Hu Wenjing *et al.* ^[2-3] observed the antennae of male moth of *S. subpunctaria* under scanning electron microscope, and studied the ultrastructure of gonads of female moth at two different developmental stages. Geng Shubao *et al.* ^[4] studied the relationship of adult life span and spawning quantity with supplemental nutrition of *S. subpunctaria*. Zhang Wei *et al.* ^[5] reported the causes and prevention measures of the

outbreak of *S. subpunctaria* in Sichuan Province in 2009. However, there is no detailed study on *S. subpunctaria* in Shandong tea region. Therefore, the author began to observe and study the life history and biological characteristics of *S. subpunctaria* in 2005, in order to provide a basis for the comprehensive control of *S. subpunctaria* in Shandong tea region.

2 Materials and methods

- **2.1 Observation sites** The experimental tea garden is located in Dougou Village, Zhengshan Street, Linshu County, Shandong Province. The tea garden covers an area of 15. 34 hm². Anhui population species are planted in the garden, which are under relatively extensive management.
- **Observation of life history** On the basis of perennial field observation, 126 larvae of S. subpunctaria were collected in the field from September to October in 2005, and were hydroponically raised on tea shoots outdoors. The tea shoots were placed in beverage bottles and updated every 3 - 5 d, and the water was replaced every 7 d. According to the characteristics of small activity range of S. subpunctaria larvae, tea branches with bifurcation were selected, and there were 2-3 larvae feeding in each bottle of tea branches according to the conditions of branches. The instar and pupal stage of larvae were observed by unified number. Observation was made 2 - 3 times a day according to the development of larvae, and once every 2 - 3 h in the critical period. After adult emergence, male and female were paired, and put into a small mesh bicycle basket loaded with hydroponic tea branches and cotton balls. Cotton balls were added with water once a day, and tea branches were replaced every day, to observe the fecundity and egg duration in the basket and tea branches. According to different overwintering states, more than 100 larvae were fed. The larval duration of different generations and instars were counted to find the longest and shortest duration of each instar of larvae in each generation, and the average duration of larvae were calculated with all the data of larvae. According to this method, the duration of

other insect states were calculated. In 2018 - 2019, verification observation was carried out in Jufeng Town, Lanshan District, Rizhao City, and the incubation and development of overwintering eggs were observed and studied.

3 Results and analysis

3.1 **Life history** According to the author's observation in Linshu tea garden in Shandong Province from 2005 to 2007, S. subpunctaria occurred 6 generations every year in Shandong tea region and survived the winter as pupae and larvae in tea leaves. S. subpunctaria, which survived the winter as pupae, spun silk to form thin cocoons with a single leaf or adjacent leaves and overwintered in the cocoons; those survived the winter as larvae mostly activated on the adaxial surface of leaves because the larvae had strong freezing resistance. The occurrence periods of each insect state of S. subpunctaria in various generations are shown in Table 1. The durations of each insect state of S. subpunctaria in various generations are shown in Table 2. The durations of each instar of S. subpunctaria larvae in various generations are shown in Table 3. As can be seen from Table 1 and Table 2, due to the long span time of the overwintering generation, the durations of egg, larva and pupa differed greatly due to the influence of temperature.

Occurrence period of each insect state of Sconula subnunctaria in various generations in Shandong tea region

Overwintering insect state	Generation	Egg	Larva	Pupa	Adult
Pupa	1	March to early May	Early April to late May	Early May to early June	Middle May to early June
	2	Late May to middle June	Late May to late June	Middle June to early July	Middle June to early July
	3	Late June to middle July	Late June to late July	Middle July to early August	Late July to early August
	4	Late July to middle August	Late July to early September	Middle August to middle September	Late August to middle September
	5	Late August to late September	Early September to middle October	Late September to late October	Early October to early November
	6	Early October to early November	Middle October to middle February of the following year	Middle December to late March of the following year	Early March to middle April of the following year
Larva	1	Late April to middle May	Early May to early June	Late May to middle June	Early June to middle June
	2	Early June to late June	Middle June to early July	Late June to middle July	Early to middle July
	3	Early July to late July	Early July to early August	Middle July to middle August	Late July to middle August
	4	Late July to late August	Early August to middle September	Late August to late September	Early to late September
	5	Early September to early October	Middle September to late October	Early October to middle November	Middle October to early December
	6	Middle October to early December	Early November to early April of the following year	Early April to early May of the following year	Late April to middle May of the following year

Table 2 Duration of each insect state of Scopula subpunctaria in various generations in Shandong tea region

Larva Pupa Adult Overwintering Egg Generation insect stage Duration Duration Average Duration Average Average Duration Average 13.0 - 16.021.7 2.5 - 4.5Pupa 1 15.3 20.6 - 26.39.3 - 11.010.7 3.0 2 6.4 - 6.514.4 - 18.36.2 - 9.23.0 - 4.06.4 16.4 8.5 3.2 3 6.0 - 7.36.1 13.6 - 15.714.4 7.2 - 8.48.2 2.3 - 4.33.1 4 5.9 - 6.514.8 - 19.78.2 - 9.12.4 - 4.06.0 16.3 8.3 3.0 5 3.0 - 16.36.4 - 7.56.9 17.9 - 19.818.4 9.5 - 12.611.4 6 11.6 - 22.953.6 - 110.2 41.5 - 88.111.0 - 14.212.6 19.6 9.4 - 10.517.9 - 22.4Lava 1 9.7 7.8 - 9.18.5 3.0 - 5.63.5 2 6.3 - 6.46.3 13.4 - 14.213.8 7.2 - 8.27.5 3.0 - 4.03.5 3 6.3 - 6.56.4 13.5 - 14.513.9 7.3 - 8.28.1 2.5 - 4.03.2 4 6.3 - 6.46.4 15.5 - 20.417.4 10.1 - 12.111.2 2.4 - 3.83.1 5 7.0 - 7.97.4 17.7 - 20.919.6 12.3 - 13.312.8 2.9 - 15.617.2 - 28.5129.4 - 139.717.9 - 21.318.3 13.2 - 16.714.3

Note: " - " indicates the data is not statistically counted because the difference between durations is too large and the average is meaningless.

Living habit Most of the S. subpunctaria adults emerge from evening to midnight, and reach the peak emergence period from 20:00 to 23:00, while a few also emerge during the daytime. After emergence, the adults hide immediately on the back of the leaves during the daytime, sometimes the body do up and down push-ups, with wings standing on the back of the body, like a butterfly, and the wings reach the maximum about 0.5 h later. After emergence for 1 h, the wings change from upright to flat, and the wings are flat hidden on the back of the leaves when they are still. Adults have strong phototaxis and weak body,

Table 3 Duration of each instar of Scopula subpunctaria larvae in various generations in Shandong tea region

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Overwintering insect stage	Generation -	The 1 st instar		The 2 nd instar		The 3 rd instar		The 4 th instar		The 5 th instar		Prepupa		Total	
		Duration	Average	Duration	Average	Duration	Average								
Pupa	1	5.9 -6.4	6.2	2.6 - 3.8	3.6	2.0 - 2.4	2.3	2.9 - 3.1	2.9	3.4 - 5.3	4.1	1.9 - 3.2	2.6	20.6 - 26.3	21.7
	2	3.0 -4.3	3.4	2.2 - 3.3	2.6	2.0 - 2.7	2.4	2.1 - 3.0	2.7	2.9 -4.1	3.9	1.1-1.6	1.4	14.4 – 18.3	16.4
	3	2.4 - 2.9	2.6	1.9 -2.2	2.1	1.9 -2.7	2.2	1.9 - 3.1	2.6	3.1 -4.7	3.8	1.0 - 1.2	1.1	13.6 – 15.7	14.4
	4	2.3 - 3.0	2.6	2.0 - 3.1	2.3	1.9 - 3.2	2.8	2.7 - 3.6	3.1	3.9 - 5.1	4.3	1.2 - 1.3	1.2	14.8 – 19.7	16.3
	5	2.9 - 3.7	3.4	2.7 - 3.8	3.0	2.4 - 3.6	2.9	2.8 - 3.7	3.2	4.0 -4.8	4.4	1.4 - 1.8	1.5	17.6 – 19.2	18.4
Larva	1	4.0 - 5.6	5.2	2.5 - 3.1	2.6	3.2 - 4.7	3.7	2.6 - 3.4	3.0	3.3 -4.1	3.8	1.2 - 2.2	1.3	17.9 - 22.4	19.6
	2	3.2 - 4.3	3.5	2.0 - 2.5	2.3	1.9 - 3.1	2.2	1.9 - 2.8	2.5	2.8 -4.0	3.3	1.1 - 1.2	1.2	14.6 – 15.4	15.0
	3	2.1 - 2.6	2.2	2.2 - 2.5	2.3	2.0 - 3.1	2.5	2.4 - 2.9	2.7	3.0 - 3.4	3.1	1.1-1.2	1.1	13.5 – 14.5	13.9
	4	3.6 - 6.1	4.5	1.6 - 2.7	2.3	1.5 - 2.4	2.1	2.1 - 3.3	2.6	3.5 - 5.3	4.3	1.3 - 2.4	1.6	15.0 - 20.4	17.4
	5	3 3 - 4 1	3.8	27-45	3 4	2 9 - 4 2	3 1	3 0 - 3 8	3.2	2 9 - 4 9	4 4	16-19	1 7	17 7 - 20 9	19.6

Note: Because of the long duration and great difference, the duration of the 6th generation larvae is not listed in this table.

and are often affected by wind and drift with the wind. Adults are more active in the evening, and search for the opposite sex to mate according to sex pheromones, and the activity decreases after 21:00. Adults usually mate in the evening of the second day after emergence, and begin to lay eggs in the evening of the third day. The adult life span of the 5th and overwintering generations is longer, while that of other generations is shorter. Adults in the 5th and overwintering generations lay more eggs, generally 177 – 221 eggs. The eggs of *S. subpunctaria* are light green at the beginning of birth, then turn to yellowish green, and are light gray near hatching, with concave body surface in individuals. An unmated female adult can lay eggs that can not hatch; eggs are scattered in the axillary of buds and branches, generally one or several eggs at each place.

Eggs generally hatch at 6:00 - 9:00 in the morning, and reach the hatching peak at 7.00 - 8.00. After hatching, the larvae spin silk and drift, and do not feed on egg shells. The larvae are photophobic and stay on tea buds or the back of young leaves. When the wind is strong, the larvae will cling to the base of buds or hide between unspread leaves and buds. When there is no wind, the uropods of larvae are fixed on the buds, showing mimetic lifting. Newly hatched larvae only feed on the lower epidermis and mesophyll of leaf buds. Some will eat tea buds into holes, with their heads drilling into tea buds for feeding, and can spin silk and drift. After the 2nd instar, the larvae mainly eat tea buds, and some also eat one or two leaves below the bud. When the number of larvae is large, the victim buds are eaten into a lot of tiny holes (less than 1 mm in diameter), and the bud tip becomes dark and dull. Sometimes, it will cause the bud head to wither, resulting in the loss of economic value.

At 3-5 h prior to the 3^{rd} instar, the 2^{nd} instar larvae stop on leaf back or leaf margins, with the body hanging upside down or leaning slightly. The 3^{rd} instar larvae can eat the molting, and eat the young leaves of tea tree into notches along the leaf margin. Before entering the 4^{th} instar, the larvae are all on the leaf margin or petiole, with the abdominal pleopods and uropods grasping the leaf margin or petiole in a mimetic state, and they will enter the 4^{th} instar after about 20 h. After the 4^{th} instar, the larvae have large appetite and eat the leaves into notches. The

molting of the 4th instar larvae into the 5th instar lasts 10 min. As the head shell is peeled off, the larvae molt backward from the thorax. It takes longer for head peeling, but needs only 2 – 3 min from the beginning of thorax molting to complete molting. When the first pair of abdominal pleopods begins to molt, the body changes from hanging down to being supported by the pereiopoda and first pair of abdominal pleopods, and finally the uropods break apart, leaving the molting on the branches. After the molting is completed, the larvae immediately spin silk and connect with the branches, then rest in a mimetic state, and the larvae will eat the molting after about 1 h. After the 5th instar, the larvae enter the gluttony stage and eat up mesophyll, often leaving the main vein and petiole.

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Due to the aging of tea leaves, the larvae of the 5th and 6th generation (overwintering generation) mostly feed on flower buds, stamens and young leaves, resulting in yellowish green body. The overwintering larvae have strong freezing resistance. On December 18, 2007, 7 larvae were caught in Linshu tea garden, 5 of which were found on the surface of tea canopy, and 3 were in the buds, indicating that when the weather is cold, the larvae mostly feed on the upper leaf surface of tea canopy to expose to the sun. Even from late December to January, when it is the coldest, larvae can crawl slowly, and because they do not feed for a long time, they have thin body size and dark surface. On January 7, 2021, the extreme minimum temperature in Lanshan District, Rizhao City reached – 15. 68 °C, but overwintering larvae were still found alive in the early spring of 2021.

When S. subpunctaria enters the pre-pupae state, it will spin silk to stick adjacent two leaves, but only a few filaments are emitted in the leaves, and the larvae are basically exposed. The prepupal stage is very long if S. subpunctaria survives the winter as pupae, usually 16-62 d. The pupation lasts about 10 min and undergoes the process of splitting longitudinally from the thorax of larvae, cracking at the pereiopoda, and molting at the end of pupae. The pupa is emerald green at first, then becomes yellowish green, and the wing bud becomes white near emergence. There is an obvious black spot on the wing surface, and the pereiopoda becomes reddish-brown. The pupa is dark green if S. subpunctaria survives the winter as pupae.

4 Conclusions and discussion

4.1 There are little changes in the occurrence of *S. sub-punctaria* from the 1st to the 5th generations *S. subpunctaria* survives the winter as larvae in Zhejiang and other southern tea regions, and as pupae and larvae in Shandong tea region. Since the temperature in Shandong tea region is low, *S. subpunctaria* overwinters in a dormant state and can continue to develop when the temperature is appropriate. Because the ability of pupa to resist low temperature is much higher than that of larva, two insect states of pupa and larva can go through the winter in Shandong tea region. In Hangzhou, the total durations of egg, larva and pupa of *S. subpunctaria* from the 1st to the 5th generations were 37.3, 88.5 and 51.6 d, respectively^[1].

The total durations of egg, larva and pupa of S. subpunctaria that survived the winter as pupae from the 1st to the 5th generations in Shandong tea region were 40.7, 87.2 and 47.1 d, and those survived the winter as larvae were 36.2, 85.5 and 48.1 d, respectively. The total durations of eggs from the 1st to the 5th generations in Hangzhou was 3.4 d less than that of the eggs that survived the winter as pupae in Shandong Province, and 1.1 d longer than that of the eggs that survived the winter as larvae. Although the total durations of larvae and pupae of S. subpunctaria from the 1st to the 5th generations in Hangzhou were longer than those overwintered as pupae and larvae in Shandong, there was little difference (1.3, 3.0, 4.5 and 3.5 d longer), indicating that the occurrence of S. subpunctaria from the 1st to the 5th generations had little changes in different latitudes. The insect states of the 6th generation (overwintering generation) were affected by temperature, and the duration varied greatly. The prepupal stage was 15 - 27 d with an average of 21.22 d for those who pupated before December 25, 2007; the pupal stage lasted 85 - 93 d, with an average of 88.11 d. For those who pupated after February 8, 2008, the pre-pupal stage lasted for 51 - 58 d, with an average of 54.33 d; the pupal stage lasted for 38 - 46 d, with an average of 41.50 d. Compared with pupation after February 8, 2008, the total duration of pre-pupal stage and pupal stage of pupation before December 25, 2007 was about 15 d longer, and the emergence was concentrated in middle and late March 2008. No larvae pupated from December 25, 2007 to February 8, 2008, and this period was also the coldest period in Shandong. S. subpunctaria entered pre-pupal period on May 23 in 2007, which also illustrated this situation. S. subpunctaria showed high resistance to freezing if it overwintered as larvae. After 9:00 in the morning, the larvae were still slowly moved on tea branches under the sun. The development starting point and effective accumulated temperature of each state of S. subpunctaria need further study in the future.

- Duration of overwintering eggs is greatly affected by The freezing resistance of overwintering eggs of S. subpunctaria was compared from November to December in 2019. The eggs laid on November 9, 2019 were divided into 3 groups. The eggs in the first group were placed in a natural indoor state, which hatched on November 25, with an egg duration of 15.5 d. The eggs in the second group were placed outdoors, and became gray and white due to low temperature freezing, during which the lowest temperature was lower than 0 °C for 4 d, namely -4.40 °C, -6.84 °C, -3.60 °C and -5.09 °C on November 18, 19, 20 and 25, respectively. They were moved to the indoor natural state on November 25. The larvae hatched from December 9 to 16, with an egg duration of 29.0 - 36.2 d. The eggs in the third group were kept outdoors, and there were 17 d of low temperature below 0 $^{\circ}$ C, with the lowest temperature of $-9.19 ^{\circ}$ C. They were moved indoors in a natural state on December 10, and hatched from December 20 to 26, with an egg duration of 41 - 47 d. indicating that overwintering eggs had strong freezing resistance. However, whether they could overwinter as eggs remains to be studied in the future.
- **4.3** *S. subpunctaria* has serious overlapping generations In the tea region of Shandong Province, the generations of *S. subpunctaria* overlapped seriously except January and February, and four insect states of *S. subpunctaria* existed simultaneously at other time. Whether the insect state of the overwintering generation of *S. subpunctaria* changes with the duration of different insect states should be further studied in the future.

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