

Effect of Ecofriendly Pesticides Against *Adoxophyes orana* (Lepidoptera: Tortrididae) on Tea Tree (*Camellia sinensis* L.)

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Abstract

A study was carried out to identify the life cycle of *Adoxophyes orana* (Lepidoptera: Tortrididae) that inflicts tea tree leaves in Korea and selected three ecofriendly pesticides (Common name for commercial: Essential oil, Nemaatch, and Wormstop in Korean Farmers' Market) of *A. orana* for pest control. *A. orana* appeared to follow four life cycle phases a year; each presenting varying developmental periods dependent on seasonal and environmental factors. The fecundity of *A. orana* female was 24.6 ± 4.10 for 1st, 36.7 ± 12.77 for 2nd, and 27.9 ± 4.22 for 3rd phase during 2011, while it was 65.0 ± 32.72 , 49.7 ± 30.27 , 63.8 ± 27.22 for corresponding phases during 2012. The average longevity of adult *A. orana* was 7.72 days. The average number of eggs deposited by each female in this study group was 44.62 with an average of 2.47. In three selected ecofriendly pesticides, the mortality of *A. orana* on treating with the Essential oil [The essential oil of *Chamaecyparis obtuse* (100%)] and Nemaatch [Azadirachtin 800-900 ppm (75%)] were 36.67% and 43.33% after 3 days and were 48.30% and 56.67% after 7 days, respectively. Besides, the mortality of *A. orana* on treating with Wormstop [Azadirachtin 500 ppm (5%) and Salannin+Liminoids (95%)] was 61.67% and 78.33% after 3 and 7 days, respectively. Therefore, the application of Wormstop was the most useful to control the diseases caused by *A. orana*.

Key Words: *Adoxophyes orana*, ecofriendly pesticides, life cycle, mortality, tea tree

Introduction

Tea tree (*Camellia sinensis* L.) is a small perennial evergreen arboreal shade tree belonging to family of *Theaceae* (Kim et al. 2010). Tea family members are sub-divided into two subspecies and two other variants. Tea leaves are present as alternate, long oval, serrate, lanceolate, coriaceous, and lustrous and inflorescence is a cyme consisting of five calyx and six to eight petals (Lee et al. 2010; Ko et al. 2013).

Tea leaves are a rich sources of a various phytochemicals such as polyphenols, amino acids, caffeine, sugars, saponins, organic acids, various minerals, vitamin C, etc. that contribute to the characteristic tea flavor and incense. Green tea that is prepared from the extract of *C. sinensis* leaves that have undergone processing with minimal oxidation. Green tea extract has several nutritional and health benefits. Its phytochemicals have been proven to participate as antioxidants, anticancer, anti-obesity, blood pressure strengthen, learning, and enhancing memory sub-

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stances (Chang et al. 2012). In the light of these unique benefits the tea cultivation area in Korea and elsewhere is rising each year. Green tea consumption among Korean population has now increased owing to its potential health benefits (Kim et al. 2010; Jung et al. 2013). Thus the demand for tea cultivation by alternative methods has increased. The increased tea cultivation area has also led to the incidence of several insect pests in tea tree plantations (Graham et al. 1992; Lee et al. 1998; Chen et al. 2001).

In the past, about 30 different pests were known to inflict tea leaves and the major insects that attack tea tree are *Adoxophyes orana*, *Caloptilia theivora*, *Tetranymphus kanzarwai*, *Euproctis pseudoconspersa*, and *Empoasca vitis* (Minakawa 1960; Kim et al. 1984; Lee et al. 1993). However, root and stem of tea are also subject to attack by some pests. The ecological study of *A. orana* on tea tree in Japan was reported that *A. orana* usually attacked new shoots and affecting too new growing leaf (Minakawa 1960). *A. orana* was officially identified by Honma (1970) according to standard classification criteria and is called as the summer fruit tortrix (a moth of the family *Tortricidae*). Later these criteria have been adopted for identification of tea pest *A. orana* (Fisher von Roeslerstamm) (Lee et al. 1993). *A. orana* appears four times in a year and over-winters as larva (Kim et al. 1984). Occurrence density of *A. orana* on tea tree is the highest during the middle of June. Therefore, it has been reported to cause more damage during summer (Lee et al. 1988; Yang et al. 2009; Byun et al. 2012; Wu et al. 2013). The pest larva preferably target young tea leaves. Generally tender bud is spoiled by 1st, 2nd and 3rd instar larva of *A. orana*, whereas 4th instar larva causes leaf damage rendering leaf tissues useless. The later instars of larva do not cause any further damage, however they roll leaves and feed on them, fill them with their feces, thus affecting the quality of tea extracts.

The major pests of tea in Japan were identified on the basis of epidemiological studies. This study resulted in establishment of an effective control system employing natural agents. The chemical structure of the sex pheromone of *A. orana* was determined by Tamaki et al. (1971) and this pheromone lure has been widely used for monitoring, communication disturbance, and mating inhibition in order to control the pest infections.

Ecofriendly pesticides, also called ecological pesticides

or green pesticides, are pesticides derived from organic sources (Lee 2009). However, others pesticides are consisting of synthetic minerals or toxic chemical compounds. Ecofriendly pesticides are used in organic farming and organic gardening because they are safer than synthetic pesticides. Thus, synthetic pesticides might be effect on destruction of ecosystems and reduction of natural enemies (Neelay et al. 1983).

In recent years, consumers are interested in ecofriendly agricultural products and the market of ecofriendly products are getting larger (Gradish et al. 2011). The application and the selection of ecofriendly pesticides to control the diseases were very important. Therefore, this study was trying to provide the effect information of ecofriendly pesticides for the effective control for tea tree from *A. orana*.

Materials and Methods

Investigation of life cycle

The main purpose of this study is to select ecofriendly pesticides against *A. orana* on tea tree (*C. sinensis* L.). Therefore, the life cycle of *A. orana* were basically investigated. The survey area for investigations of tea pest as *A. orana* life cycle was in the tea tree plantation at Gyeongnam National University of Science and Technology campus, Jinju, Republic of Korea. 50 adults of *A. orana* were collected, all waking up from a young larva after the over-wintering of *A. orana* larvae were collected, and inoculated on new tea shoots (length 1.5-2.0 cm) when tea sprout was following by spring (Starting on April). The field inoculation experiment for 2 years used by the white mesh cloth (1x1 m) was conducted and the fruit nectar was used for adult feeding during mating period. The developmental stage parameters such as; adult longevity, the number of eggs laid, and voltinism were investigated. Fifty adults were collected from the first developmental stage. Fifty larvae were used for next developmental stages. The average of egg, larva, and pupa were investigated 5 times duplicately counted.

Ecofriendly pest control

To be selected ecofriendly pesticides, three common agents were used in this study. Those are commonly used in Korean Farmers' Market (Lee 2009). Essential oil [The essential oil of *Chamaecyparis obtuse* (100%)], Nematicatch

Table 1. Average developmental periods of reared *A. orana* on new shoots of tea tree

Year	Generation	Average developmental period (days±SD)				Time
		Egg	Larva	Pupa	Total period	
2011	I	9.2±0.52	20.9±1.02	9.2±1.62	39.3±3.16	May 2-Jun. 20
	II	5.7±0.42	17.5±1.20	6.2±0.62	29.4±2.24	Jul. 2-Aug. 2
	III	6.2±0.72	20.5±2.70	7.1±2.20	33.8±5.62	Jul. 28-Sep. 12
	IV	7.5±0.72	Diapause			Sep. 10- onwards
2012	I	14.2±2.10	29.7±2.10	9.7±1.10	53.6±5.30	Apr. 1-Jun. 16
	II	8.7±0.80	25.2±2.12	7.9±1.39	41.8±4.31	Jun. 5-Aug. 1
	III	5.2±0.42	22.7±2.42	7.7±1.12	35.6±3.96	Jul. 25-Sep.18
	IV	7.2±0.72	Diapause			Aug. 27-onwards

[Azadirachtin 800-900 ppm (75%)], and Wormstop [Azadirachtin 500 ppm (5%) and Salannin+Liminoids (95%)] were used as ecofriendly pesticides. The essential oil of *Chamaecyparis obtusa* was extracted from Japanese cypress and Namacatch & Wormstop were purchased from Korea Hubas products (Humans Basic Agriculture & Seed co. Korea, <http://www.hubaskorea.com>). The treatment procedure of three ecofriendly pesticides to tea tree was; First: *A. orana* larvae were inoculated on newly sprout buds of tea and covered with cloth. Second: The ecofriendly pesticides were sprayed at 1/500 and 1/1,000 dilutions. Third: 3 and 7 days after treatment, the number of surviving larvae were counted. Three ecofriendly pesticides were tested on 30 numbers of tea trees. All experiments were conducted in field conditions. Each field had 330.579 m² and 5 times dublicately tested on total area (1652.893 m²). The detail information of three ecofriendly pesticides was well described in previously published paper (Lee 2009).

Additionally, the non-treatment was described in the effect of natural mortality (*A. orana*) as non-treatment control. It was the control compared to each three ecofriendly pesticides. To calculate two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT), we required the results of non-treatment control. The statistical analysis in this study was performed by ANOVA and DMRT using SAS program (SAS 9.1, SAS Institute Inc., Cary, NC).

Table 2. Average longevity and fecundity of reared *A. orana* adults on new shoots of tea tree

Year	Generation	Longevity	No. of egg mass	No. of eggs
2011	I	8.2±0.42	1.7±0.82	24.6±4.10
	II	6.4±1.47	2.9±1.37	36.7±12.77
	III	7.7±1.82	2.2±1.21	27.9±4.22
2012	I	9.2±1.22	3.1±1.12	65.0±32.72
	II	5.7±1.72	2.7±0.32	49.7±30.27
	III	9.1±2.12	2.2±2.22	63.8±27.22

Results and Discussion

Life cycle of A. orana

Life cycle of *A. orana* on tea tree was investigated during May 2011 to September 2012 at Gyeongnam National University of Science and Technology campus. *A. orana* showed 4 generations a year (Table 1). The developmental period of *A. orana* during 2011 was 39.3 days for I generation (May 2nd to Jun 20th), 29.4 days for II generation (July 2nd to August 2nd), and 33.8 days for III generation (July 28th to September 12th), and IV generation (September 10th). In 2012, the developmental period was different with 53.6 days for I (April 1st to May 16th), 41.8 days for II (June 5th to August 1st), and 35.6 days for III (July 25th to September 18th) generation. The IV generation started from August 29th. The results of *A. orana* adult longevity, the egg mass number, and the number of eggs laid are shown (Table 2). The average longevity of adults was 8.2 day (I generation), 6.4 days (II generation), and 7.7 days (III generation) during 2011. The average longevity of

adult was 9.2, 5.7, and 9.1 days for I, II and III generations, respectively during 2012 (Table 2).

A. orana laid eggs with an average mass of 1.7 (I generation), 2.9 (II generation), and 2.2 (III generation) during 2011, however the egg mass was 3.1, 2.7, and 2.2, respectively for I, II, and III generation during 2012. At the same time, the number of eggs per egg mass was found to be 24.6 (I), 36.7 (II), and 27.9 (III) generation of 2011. This parameter in 2012 was 65.0, 49.7, and 63.8, respectively for I, II, and III generations. The data presented in Table 1, clearly indicates that summer conditions favor rapid development of *A. orana* (2011 and 2012). The longevity of II generation adults was shorter than other generations of 2011 and 2012 period (Table 2). This characteristic feature of arthropods corresponded well with that of cold-blooded organisms (Behrens et al. 1983).

Interestingly, there were differences according to each generation between average results of 2011 year and 2012 year about the average developmental period (egg, larva, pupa, and total period) including the average of longevity and fecundity (number of egg mass and number of eggs). Most of generations about the average developmental periods and the average of longevity and fecundity in 2012 were longer or higher than generations of those in 2011. According to seasonal changes and global warming, insects could make more reproduction and preservation of their species, physiologically (Hodek and Hodková 1988; Charmillot and Brunner 1989; Milonas and Savopoulou-Soultani 2006). Also, the biological adaptation of *A. orana* would increase each year because of similar environmental conditions in the same experimental field every year. Ecological characteristics and physiological descriptions of *A. orana* supported that the generations in *A. orana* each year had the different patterns based on environmental conditions (Lee et al. 1993; Byun et al. 2012).

Although the *A. orana* followed four generations per year in this study, Minakawa (1960) reported more generations (4-5) per year in Shizuoka prefecture and 5-6 in Kagoshima prefecture. The difference between this result and the report of Behrens et al. (1983) is likely to be attributed to regional environments. Lee et al. (1993) have reported the occurrence of 4-5 generations per year in reared *A. orana* an observation that correlates with our result. According to Minakawa (1960), the developmental period from egg to

emergence was 37.5-50.2 days in spring and fall and 34.3-34.8 days in the summer. Kim et al. (1984) reported that the developmental period from egg to emergence to be 41.9-42.2 days in spring and fall and 37.6 days in the summer.

In our study, the average developmental period for the II generation *A. orana* of 2011 was 29.4 days. The average developmental period *A. orana* for the II generation of 2012 was 41.8 days a result similar to Kim et al. (1984). Such an observation is seemed to be due to a drop in temperature owing to frequent rains. The adult longevity of *A. orana* is variable depending on season. Minakawa (1960) reported that the adult longevity of *A. orana* was 16.2 days in April, 10.3 days in June and 6.3-6.6 days in July and August. The fecundity of female also showed seasonal difference with respect to egg mass number being 2.9-3.1/female in spring and fall, 4.8-6.3 in summer (Table 2). Also, it was reported that the number of eggs was 20-50 regardless of the season (Minakawa 1960). In this study, adult longevity was shorter in the second generation and the average longevity was 7.72 days. This is thought to be originated from variations in the climatic conditions in Korean peninsula and Japan. The average egg mass number was 2.47, which is different with Minakawa's reports, too. However it showed a similar trend with Minakawa study (1960) in the second generation. The average of egg counts in this study was 44.62 in similar result with Minakawa's study.

Overwintering stage of *A. orana* was identified as larva and it corresponded with IV generation. These results matched with the previous examination (Minakawa 1960). The previous study reported that seasonal differences in temperature play a crucial role in developmental phases of *A. orana*. However, there was no observation of any obvious results on diapause induction and the differences in development according to the generation. Our results showed that the fecundity of *A. orana* female increased twice in 2012 compared to 2011 and this lead us to guess a biennial off-year in case of *A. orana*.

Control of pest with ecofriendly pesticides

Table 3 shows the results after treating *A. orana* infections of tea tree with ecofriendly pesticides. The essential oil of *C. obtusa* was effective in offering 36.67% after 3 days and 48.30 % destruction of pests (mortality) after 7 days

Table 3. The effects of ecofriendly pesticides against *A. orana* larva inoculated on new shoots of tea tree

Treatment materials	No. of treated tea-tree	Dilution	Mortality		Effect of natural mortality (<i>A. orana</i>)
			Pest destruction (%)	Pest destruction (%)	
			3 DAT ¹⁾	7 DAT	
Essential oil	30	500	36.67 ± 1.72 ^{c2)}	48.30 ± 1.77 ^c	17.27 ± 0.74 ⁴⁾ (As non-treatment control)
Nemacatch	30	500	43.33 ± 1.87 ^b	56.67 ± 1.87 ^b	
Wormstop	30	500	61.67 ± 2.07 ^a	78.33 ± 2.27 ^a	
Essential oil	30	1,000	27.27 ± 1.31 ^d	26.21 ± 1.02 ^d	
Nemacatch	30	1,000	28.21 ± 1.22 ^d	26.27 ± 1.27 ^d	
Wormstop	30	1,000	28.22 ± 2.05 ^d	26.37 ± 2.01 ^d	
F-value			12.2** ³⁾	8.2**	

¹⁾DAT: days after treatment; ²⁾Same letters within each column indicate no significance at $p=0.05$ (DMRT, SAS); ³⁾Significant at 95% level ($p=0.05$); ⁴⁾The natural mortality of *A. orana* without the treatment of any ecofriendly pesticides.

treatment with 500 dilutions, respectively. Nemacatch showed 43.33% after 3 days and 56.67% destruction of pests after 7 days treatment with 500 dilutions. Wormstop were 61.67% after 3 days of and 78.33% destruction of pests after 7 days treatment with 500 dilutions.

Besides, the essential oil of *C. obtusa* was effective in offering 27.27% after 3 days and 26.21% destruction of pests after 7 days treatment with 1,000 dilutions. Nemacatch showed 28.21% after 3 days and 26.27% destruction of pests after 7 days treatment with 1,000 dilutions. Wormstop were 28.22% after 3 days of and 26.37% destruction of pests after 7 days treatment with 1,000 dilutions. Thus, all three ecofriendly pesticides were more effective on pest destruction than non-treatment. Especially, Wormstop showed highest effect on pest destruction; 61.67% after 3 days and 78.33% after 7 days of treatment with 500 dilutions. F-value were 12.2 after 3 days and 8.2 after 7 days of treatment and significant at 95% level ($p=0.05$). Similarly, Wormstop had also a positive effect against the damage of chestnut (Gyeongnam Province, Republic of Korea) fruits by *Dichocrocis punctiferalis* for environmentally friendly controls. The control effect for *D. punctiferalis* was showed the highest in Wormstop treatments with 40.49% and 41.89% in early stage of harvest (Lee 2009). Additionally, Lee et al. (1993) showed insecticidal rate of 100% after 3 days of treatment, when they used Fenitrothion and Pyraclofos. However, these two compounds are toxic organic environmental chemicals and non-ecofriendly. Consequently, the demand for environ-

mentally friendly food is gradually increasing. The demand has restricted the use of chemical pesticides on tea plantations. Therefore, the application of ecofriendly pesticide using Wormstop might be useful to control the diseases caused by *A. orana* as an environmentally friendly agent in alternative to toxic chemical pesticides.

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References

- Behrens W, Hoffmann KH, Kempa S, Gäbler S, Merkel-Wallner G. 1983. Effects of diurnal thermoperiods and quickly oscillating temperatures on the development and reproduction of crickets, *Gryllus bimaculatus*. *Oecologia* 59: 279-287.
- Byun BK, Lee BW, Lee ES, Choi DS, Park YM, Yang CY, Lee SK, Cho SW. 2012. A review of the genus *Adoxophyes* (Lepidoptera Tortricidae) in Korea, with description of *A. para-orana* sp. nov. *Anim Cells Syst* 16: 154-161.

- Chang MS, Park MJ, Jeong MC, Kim DM, Kim GH. 2012. Antioxidative Activities and Antibrowning Effects of Green Tea Extracts and Propolis. *Korean J Food and Cookery Science* 28: 319-326.
- Charmillot PJ, Brunner JF. 1989. Summerfruit tortrix, *Adoxophyes orana* life cycle, warning system and control. *Entomol Hellenica* 7: 17-26.
- Chen Z, Zhu QY, Tsang D, Huang Y. 2001. Degradation of green tea catechins in tea drinks. *J Agric Food Chem* 49: 477-482.
- Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G. 2011. Effect of reduced risk pesticides on greenhouse vegetable arthropod biological control agents. *Pest Manag Sci* 67: 82-86.
- Graham HN. 1992. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 21: 334-350.
- Hodek I, Hodková M. 1988. Multiple role of temperature during insect diapause: a review. *Entomol Exp Appl* 49: 153-165.
- Honma K. 1970. Morphological difference of the smaller tea tortrix, *Adoxophyes orana*, in Japan. *Jap J Appl Ento Zool* 14: 89-94.
- Jung MH, Seong PN, Kim MH, Myong NH, Chang MJ. 2013. Effect of green tea extract microencapsulation on hypertriglyceridemia and cardiovascular tissues in high fructose-fed rats. *Nutr Res Pract* 7: 366-372.
- Kim KJ, Park SK, Lee TS, Choi HS. 1984. Study on identification and classification of insect pest at tea tree. *School and Industry* 81: 1-50.
- Kim YD, Min JY, Jeong MJ, Song HJ, Hwang JG, Karigar CS, Cheong GW, Choi MS. 2010. Rapid selection of catechin-rich tea trees (*Camellia sinensis*) by a colorimetric method. *J Wood Sci* 56: 411-417.
- Ko KS, Oh SJ, Lee JH, Koh SC. 2013. Chlorophyll fluorescence and CO₂ fixation capacity of the leaves of tea plants (*Camellia sinensis* L.) grown in the field. *J Korea Tea* 19: 34-40.
- Lee CK. 2009. Study on chestnut insect pest by environmentally friendly controls in Korea. *Korean J Appl Entomol* 48: 95-100.
- Lee JS, Lee KH, Oh CJ. 2010. New woody plant flora of Korea. Academy information center, Korea. pp. 122-540.
- Lee SC, Kim DI, Kim SS. 1993. Ecological Characteristics of *Adoxophyes* sp. at tea tree plantation. *Korean J Appl Entomol* 32: 279-284.
- Lee SC, Park SK, Kim SS, Kim DI. 1988. General control and occurrence ecology of major insect pest at tea tree. *Plant Protect* 2: 22-35.
- Milonas PG, Savopoulou-Soultani M. 2006. Seasonal abundance and population dynamics of *Adoxophyes orana* (Lepidoptera: Tortricidae) in northern Greece. *Int J Pest Manage* 52: 45-51.
- Minakawa J. 1960. Study on ecological institute of *Adoxophyes orana*. *Tea Technol* 13: 32-36.
- Neelay VR, Bhandari RS, Negi KS. 1983. Effect of insecticidal and hormonal spray on the production of fruits in teak seed orchard. *Indian For* 109: 829-839.
- Tamaki Y, Noguchi H, Yushima T, Hirano C. 1971. Two sex pheromones of the smaller tea tortrix: Isolation, Identification and Synthesis. *Appl Ent Zool* 6: 139-141.
- Tomata Y, Kakizaki M, Nakaya N, Tsuboya T, Sone T, Kuriyama S, Hozawa A, Tsuji I. 2012. Green tea consumption and the risk of incident functional disability in elderly Japanese: the Ohsaki Cohort 2006 Study. *Am J Clin Nutr* 95: 732-739.
- Wu QL, Liu W, Shi BC, Gu Y, Wei SJ. 2013. The complete mitochondrial genome of the summer fruit tortrix moth *Adoxophyes orana* (Lepidoptera: Tortricidae). *Mitochondrial DNA* 24: 214-216.
- Yang CY, Han KS, Boo KS. 2009. Sex pheromones and reproductive isolation of three species in genus *Adoxophyes*. *J Chem Ecol* 35: 342-348.