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Study of the generation, distribution of the plum fruit moth in Mongolia. Lepidoptera: *Grapholita funebrana* Treitschke, 1835

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ABSTRACT

In recent years, in our country, the variety and amount of fruit and berry cultivation has been increasing. In connection with this, many types of pests and diseases have appeared and increased in some years, making it difficult to harvest. In 2020 and 2021, during the surveillance survey to determine the type, species, distribution, and damage of harmful organisms in the western region, there was an increase in plum fruit moths in the plum plantations in Bulgan soum, Khovd Province. In 30 percent of the surveyed area, one larva was found per tree in 30-40 percent of the fruit. In 2021, 80-90 percent of the plum trees of Sant soum, Uvurkhangai province, In 2024, 70-90 percent of the total area of Khaliun soum plums and 35-45 percent of trees in Gobi-Altai province, it was determined that 60-70 percent of the area of Biger soum, 10-20 percent of trees, and 100 percent of trees in the total area of Bulgan soum of Khovd province. Also, in 2020 was an increase in plum fruit moths in the plum field planted in Jargalant village, Songinohairkhan district, Ulaanbaatar city, and there was an increase of 2024 year, and when 300 fruits were examined, 50-60% were infected with plum fruit moth. We collected infected fruit samples from Bulgan soum, Khovd province and Songinokhairkhan district of the capital, and obtained mature moths from the lab to confirm the species identification by morphological identification and PCR.

Keywords: *Plum fruit moth, PFM, Distribution, Damage, Generation, PCR.*

Чавганы үрч эрвээхэйн Монгол орон дахь тархалт, үе удмын хөгжлийн судалгаа. Lepidoptera: *Grapholita funebrana* Treitschke, 1835

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ХУРААНГУЙ

Манай оронд сүүлийн жилүүдэд жимс, жимсгэний таримлын нэр төрөл, тариалах хэмжээ нэмэгдэж байна. Үүнтэй холбоотойгоор элдэв төрлийн хортон шавж, өвчин гарч, зарим жилүүдэд олширч байгаа нь ургац авахад хүндрэл учруулж эхэллээ. Бид 2020, 2021 онд баруун бүсийн тариалангийн хөнөөлт организмын төрөл, зүйл, тархалт, хөнөөлийг тогтоох тандалтын судалгааны явцад Ховд аймгийн Булган суманд тариалагдсан чавганы таримал дээр үрч эрвээхэйн олшрол болж, судалгаа хийсэн талбайн 30%-д, нэг модны 30-40%-д нэг жимсэнд нэг авгалдай тохиолдож байгааг илүүлсэн бол 2021 онд Өвөрхангай аймгийн Сант сумын чавганы модны 80-90%, 2024 онд Говь-алтай аймгийн Халиун сумын чавганы нийт талбайн 70-90%, модны 35-45%, Бигэр сумын талбайн 60-70%, модны 10-20%, Ховд аймгийн Булган сумын нийт талбайд 100%, модны 90-95%-д тархсаныг тогтоосон. Мөн Улаанбаатар хотын Сонгинохайрхан дүүргийн 21-р хороо Жаргалант тосгонд тариалсан чавганы талбайд 2019, 2020 онуудад үрч эрвээхэйн олшрол болсон бөгөөд 2024 дахин олшрол болж 300 жимсэнд үзлэг хийхэд 50-60% нь чавганы үрч эрвээхэйн хүрэнцээр халдварлагдсан байлаа. Энэ зүйл эрвээхэйн хүрэнцэр нь олон төрлийн яст болон ясгүй жимсэнд ялангуяа чавга, тоор, интоор,чангаазны жимсэнд орж хооллон их хэмжээний хохирол учруулдаг бөгөөд нэгэнт халдварлалт тархсан тохиолдолд ургацын алдагдал өндөр гарч, бүтээгдэхүүний чанар шууд мууддаг онцлогтой. Бид Ховд аймгийн Булган сум, нийслэлийн Сонгинохайрхан дүүргийн талбайгаас халдвар бүхий жимсний дээж авч хүрэнцрийг лабораторид бойжуулан бие гүйцсэн эрвээхэйг гарган авч зүйлийн тодорхойлолтыг морфологи шинж тэмдгээр болон ПГУ-ын аргаар баталгаажуулалт хийлээ. Энэ эрвээхэй нь халуун бүс нутагт жилд 2-3 үе удмаар хөгждөг бол манай оронд жилд нэг үе удмаар үржиж хөгжиж байгаа нь бидний ажиглалтаар тогтоогдсон. Чавганы үрч эрвээхэйн тархалт тэлж, хөнөөл нэмэгдэж байгаа нь чавганы тариалалт нэмэгдэн, суулгацыг хорио цээрийн хяналтгүйгээр тарааж байгаатай болон цаг уурын хүчин зүйлтэй холбоотой гэж үзэж байна.

Түлхүүр үг: *Чавганы үрч эрвээхэй, Тархалт, Хор хөнөөл, Үе удам, ПГУ*

1. INTRODUCTION

The plum fruit moth (FPM), *Grapholita funebrana* Treitschke (Lepidoptera, Tortricidae), is an important agricultural pest that seriously affects fruit production across the Palearctic region [10]. It is an oligophagous species, feeding on the fruits of several hosts typically within the plant family Rosaceae. PFM is the key pest of plum (*Prunus* spp.) in most parts of Europe. In Italy, it has three generations per year (Molinari, 1994), while in many areas of central and eastern Europe, it has one or two (Vernon, 1971) [16]. All of them were members of four families and 16 genera: Tortricidae (Apotomis, Celypha, Cnephasia, Dichrorampha, Epiblema, Eucosma, Grapholita, Gypsonoma, Orhrotaenia, Pammene, Phiaris, Pristerognatha, Syricoris), Argyresthiidae (Argyresthia), Gelechiidae (Gelechia) and Ypsolophidae (Ypsolopha). The share of Tortricidae family members amounted to 99% of the total these (namely, 7510 moths) belonged to seven Grapholita species, subgenus Aspila. Trophically, the moths of this subgenus are exclusively associated with Rosaceae trees and shrubs [1]. This species of moth has been known to feed on many types of bony and boneless fruits, especially plums, peaches, cherries, and louvers, and once the infestation has spread, it is characterized by high crop losses and a direct deterioration in product quality. Larvae develop in fruit and often specialize in certain species of plants. The plum fruit moth is widely distributed in southern and central Europe, Asia minor, central Asia, the Far East, Siberia, and the European region of the Russian Federation, and it was first noted in our country by researcher Davaa (1999).

The plum fruit moth (*G. funebrana*) is one of the most hazardous pests in plum cultivation. The high yield is affected by the migration of larvae in the epidermis of the fruit, and by consuming their inner part; the fruits are damaged in terms of organoleptic and visual effect. Understanding the reason for climate change and global warming, the increase in distribution, the damage severity, and the economic significance of this pest is close to being predicted. To date, the extent of damage and reduced yield of fruit trees due to the moth is an estimated 30 and 70% the moth is harmful in its larval stage.

Hatchlings that have just hatched from the eggs enter the soft tissue of the fruit, feed there, and pupate after completing the hatching stage. Hatchlings that have just hatched from the eggs enter the soft tissue of the fruit, feed there, and pupate after completing the hatching stage. The length of the larva is 12-15mm, with a dark brown head, and the body is light yellow, gradually turning orange and pink. Pupae are light brown in size, 6-8 mm. The average wingspan of an adult is 12-15 mm, eeggs are flattened, slightly elliptical, about 0.6 mm wide by 0.7 mm across, they are clear white and eventually turns yellow [3], [6], [12]. *Grapholita funebrana* completes one to three generations per year d epending on climate.

The female lays 1 or 3-9 eggs on the surface of the fruit and on the underside of the leaves. After feeding, the last-stage larvae emerge outside the fruit and pupate under bark cracks and plant debris. In areas where it produces two generations per year, it hibernates in the larval stage, while in Mongolia, it reproduces in two generation, and hibernates in the pupa and adult stages in tree bark cracks, branches, and soil. Adult moths begin breeding 2 hours before sunrise and end at sunrise. Spawning usually occurs in the afternoon and evening [13], [14].

Purpose of research work

1. To determine the types and species of Tortricid moth that damage the fruits of plum trees, and to determine their distribution status.
2. Identification of moth species by morphological characters and PCR methods.
3. To produce distribution points, to determine the generations to be given in the year, determining the cause of proliferation.
4. To determine how many generations a moth give per year in Mongolian conditions.

2. RESEARCH METHODS

The research was conducted in plum fields planted in Bulgan soum of Khovd aimag, Biger of Govi-Altai aimag, Sant soum of Uvurkhangai aimag, around Ulaanbaatar city, and Songinokhairkhan district as part of a surveillance effort to determine the distribution and damage of pests.

1. In the western and central regions of Mongolia in 2020, 2021, and 2024, a survey of pest surveillance was conducted and the spread and percentage of infection was calculated.
2. The percentage of trees and fruits damaged by insects was determined by analyzing 100 fruits from each tree at 10 points in each of the surveyed areas.
3. Detected insect larvae were identified by their external characteristics.

4. We conducted observations of the generations of the plum moth using the insect phenological observation method Dobrovolsky (1961) and Bei-Bienko (1966).

PCR conditions:

95°C	4 minutes	
95°C	30 seconds	
52°C	30 seconds	35 cycle
72°C	30 seconds	
72°C	7 minutes	
8°C	Save	

5. During the field study, the distribution of the moth in the local plum orchard was calculated using the formula of K.V Skufin,1979 [10].

$$N\% = \frac{a \cdot 100}{b} \quad (1)$$

PGR samples and and reagents:

10x standard buffer	2.5 µl	
10mM dNTP	0.5 µl	
10 p Mol R-primer	0.5 µl	
10 p Mol F-primer	0.5 µl	25µl
DNA template	1 µl	
Taq	0.5 µl	
dH_2O	19.5 µl	

b-number of all species found in a tree
a-number of dominant individual species

6. We identified the moth species using morphological characters using a “Key to the insects of Far East. Vol.5. Trichoptera and Lepidoptera. Vladivostok. Dal’nauka, 2005” identification book and confirmed it using molecular biology [19].
7. Identification of moth species by morphological characteristics was carried out at the Laboratory of Entomology and PCR analysis was carried out at the Laboratory of Molecular Biology of the Research Institute of Plant Protection
8. The identified species were confirmed by PCR. In this: Insect samples were crushed and ground by adding 100 µl of distilled water. Take the ground insect sample (100 µg wet/20 µg dry) in a new 1.5 ml tube, add 100 µl of BE buffer and mix well, add 40 µl of MG buffer and 10 µl of Proteinase K and vortex for 15 min. After vortexing, it was centrifuged at 12,000 rpm for 30 seconds. 600 µl of MG was added again and centrifuged at 12,000 rpm for 30 seconds. In a new “collection tube” the collection filter was assembled, the sample with MG buffer was added and centrifuged at 12000 rpm for 30 seconds. Discard the filtrate from the filter. 500 µL of BW buffer was added to the collection filter and centrifuged at 12,000 rpm for 30 seconds.

Discard the filtrate from the filter. 500 µl of B5 buffer was added to the collector filter and centrifuged at 12,000 rpm for 30 seconds. The collection filter containing the sample was dried by centrifugation at 12000 rpm for 30 seconds. 100 µl of BE buffer was added to the collection buffer, kept at room temperature for 5 minutes, and centrifuged at 12000 rpm for 30 seconds. Separated samples are stored at +4°C for a short time and -20°C for a long time.

3. RESULT AND DISCUSSION

To date, the presence of *G. Funebrana* has been recorded across the Palearctic region and in North Africa (Algeria). In Europe, this species has a wide distribution, it was first described in 1835 by Treitschke, using type specimens collected from Germany/Czech Republic. Typically, in some other European countries such as Italy and Switzerland, this pest receives intense research attention because of its economic importance. All of Europe has a suitable habitat for *G.*


Figures 1,2. Adult moth, white larvae, purple larvae. Photos by Munhktsesteg.B

*funebrana.*In China, *G.funebrana* has been recorded in Heilongjiang in northeast China; Ningxia, Gansu, and Xinjiang i northwest China; and Hebei in central China. The prediction results show that the pest's suitable habitat covered the abovementioned regions or provinces in China. Moreover, some parts of South China, southwest China, and northeast China, such as Liaoning and Sichuan, in which the presence of *G. funebrana* has not been recorded. In Japan, *G. funebrana* had once been considered to be present, but this finding is now regarded as a misidentification. In Chines, from the perspective of biogeography, simulated the ensemble models to predict the worldwide spatiotemporal distribution pattern of *G. funebrana* for the first time [10].In the former USSR, the plum friut moth was first recorded in Krasnodar Krai in 1964. In public and private orchards of Krasnoyarsk Krai, seven species of tortrix moths were caught: *Grapholita molesta, G. inopinata, G. funebrana, G. tenebrosana, G. rosana, G. andabatana and G. cotoneastri.*

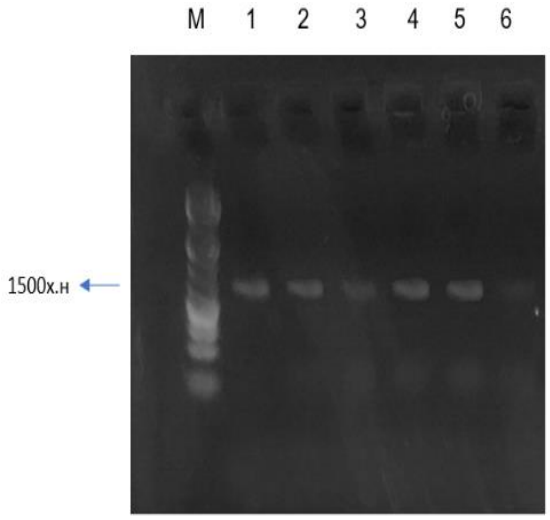
The species composition varied slightly in some areas. Thus, all of these species were recorded in the southern regions. G. cotoneastri was not recorded in the central regions, and *G. molesta and G. cotoneastri* in the western regions. In public and private orchards of the Republic of Khakassia, *G. molesta, G. tenebrosana and G. cotoneastri* were not recorded in the catches. *G. molesta and G. funebrana* were recorded in the catches at wholesale/ retail and storage facilities for domestic a and imported fruits in Krasnoyarsk and Abakan. Among the seven tortrix moths species, two species *Grapholita molesta* and *G. tenebrosana* were detected in Siberia for the first time. Five species *G. molesta, G. inopinata, G. funebrana, G. tenebrosana, G. rosana* are known as pests in their present habitats. Three species *G. molesta, G. inopinata, G. funebrana* are quarantine pests in a number of countries. To date, it has been recorded in 47 countries quarantine pests in a number of countries. In Russia, *G. funebrana* is a widely spread species. The moth is particularly severe in the southern regions (Crimea, the Caucasus)[1]. In the Trans-Baikal, fruits of some cherry and plum species are often almost completely destroyed. *G. funebrana* is on the List A1 of RPPOs (Regional Plant Protection Organizations) as a species absent in Turkey, Israel, Jordan, and the majority of the American countries.The moth was second in abundance among all Grapholita species in the catches [1].

During the pheromone monitoring for the Oriental fruit moth in southern Siberia in 2010-2013, in russian researchers were registered seven species of the genus Grapholita were found in traps (*G. molesta, G. inopinata, G. funebrana, G. tenebrosana, G. rosana, G. andabatana and G. cotoneastri*). However, research and control work using pheromone traps against fruit moths has not yet been carried out in our country. No detailed study of the distribution of this moth in Mongolia, the damage it causes to plum trees, and its biological ecology have been conducted in our country, and it has only been recorded.

We took samples of larvae from inside the plum fruit and took out moths around the laboratory. It was determined to be the plum moth (*Grapholita funebrana* Treitschke, 1835) using identification letters based on body structure and morphological characteristics, species identification was confirmed by PCR and registered in the gene bank. Number registered in Genbank–OL966296 (*G.funebrana*).The plum fruit moth belongs to the *Torticidae* family of the Lepiodoptera, it belongs to the genus *Grapholita* and the official scientific name is *G. funebtana* Treitschke, 1835. [9], [18]. For insect DNA isolation, gDNA was isolated according to the factory method provided in Macherey Nagel's DNAInsect album. A control fragment of genomic DNA was amplified by polymerase chain reaction. To identify the insect species, use primers C1-J-17181 5' GGAGGATTTGGAAATTGATTAGTTCC, 3' C1-N-21911-5' CCCGGTAAAATTAA AATATA AACTTC 3' in the gDNA control region. The total PCR reagent is calculated as 50 µl, 5 µl of 10x buffer (Dream buffer), 1 µl dNTP, 1 µl of e ach primer, 3µl sample, 1µl tag polymerase (Dream taq polymerase), Dissolve in 39 µl ultrasterile water (Thermofischer ultrapure ddH_2O), predenaturing reaction conditions at 94°C for 5 min, 35 cycles: 30 sec at 94°C, 30 sec at 58°C, 30 seconds at 72°C, the last extension step is amplified at 72°C for 7 minutes (My Genie™ 32 Thermal Block, Bioneer).

PCR products were analyzed by 1.5% agarose gel electrophoresis. The resulting PCR product was subjected to gene sequencing by South Korea's Macrogen company.1 Evolutionary history was performed in MEGA-X using maximum likelihood

and Tamurei-Nei model methods. [15], [16]. We compared the 1 sequence amplified by COI primer with the gene sequences in the genebank database, similar to the research done by Zheng.Y and other researchers [15], and *G. tenebrosana* is the origin of the *G. funebrana* species. The phylogenetic tree was compared with DNA sequences from gene banks. For comparison, *G.funebrana* with genebank number KX260323.It was in cluster 1. This confirms *G.funebrana* as a single species.



Figures 3,4. Comparison of healthy fruit and larval damaged fruit. External symptoms of fruit with larval infestation. Larval samples. Photos by Munhktsesteg.B

Since 2020, the plum moth has started multiplying in Bulgan Sum of Hovd province, and its spread has been observed in the cultivated plum fields in 4 provinces and the capital (Hovd, Govi-Altai, and Uvurkhangai) of our country. In 2020, this moth spread to no more than 40% of the breeding area and infected up to 40% of the fruit of one tree. Also, in 2024, in addition to multiplying again in the area, it spread to new areas, and there was a case of 100% reduction in the yield of more than 90% of the fruits of one tree. It has been observed that the distribution of the moth has shifted from the southwestern part of Mongolia to the western part of the central region in a short period of time (**Figure 7, Tablet 1**).

Figure 3. M-Marker 10000 bp, *Grapholita funebrana* in the 2nd hole *(1500 bp) Grapholita funebrana* phylogenetic tree of insects

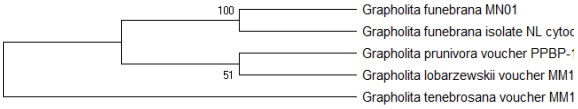
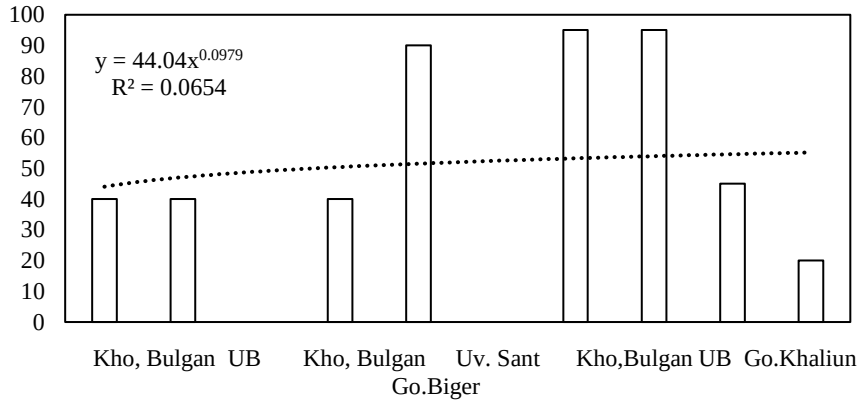


Figure 4. A phylogenetic tree was constructed using the maximum



Figures 7. Larva infection on the plum tree, %

Tablet 1. Distribution and infestation of plum fruit moth

Years	Ulaanbaatar		Khovd. Bulgan		Gobi-Altai. Khaluin		Gobi-Altai. Biger		Uvurkhangai. Sant	
	Distribution in the field, %	Infection on the fruit of one plum tree %	Distribution in the field, %	Larva infection on one plum tree, %	Distribution in the field,%	Larva infection on one plum tree, %	Distribution in the field,%	Larva infection on one plum tree, %	Distribution in the field, %	Larva infection on the one plum tree, %
2020	30	30-40	30	30-40	-	-	-	-	-	-
2021	-	-	30	30-40	-	-	-	-	40	80-90
2024	100	90-95	100	90-95	70-90	35-45	60-70	20-Oct	-	-

In 2020, there was an increase in Bulgan in Hovd province, and in the eastern part of the capital, it increased in Songino Khairkhan district, and after 4 years in 2024, there was a repeat increase. When counting the percentage of larval infestation in the fruit of one tree during the multiplication, it was 40-95%, making it impossible to harve. Our observations have shown that this butterfly develops in the tropics in 2-4 generations per year, while in our country it reproduces in two generation per year (**Figure 8**). Three full generations of plum fruit trees are observed on the territory of the Black Sea coast (Krasnodar region), in Armenia and Abkhazia. The third generation of Ukraine is optional. In warm climate, one generation develops in 26-35 days, in areas with complete development, the development of two generations takes 35-40 to 50-55 days [5]. In our country, there has been no detailed study of the generational observation of this moth, and we have summarized the generational continuity based on the observations we made during the study period. In the future, it will be important to calculate the sum of effective temperatures in relation to climatic facto

Figure 8. Life cycle of lum fruit moth. +adult, egg, ~ larva, 0 pupa, # mating, [ ] outbreak

Generation	V	VI			VII			VIII			IX			X-XII
		I	II	II	I	II	III	I	II	III	I	II	II	
I	(0)													
		+	#	#	+									
		#	•	•	•									
				~	[~	[~	~							
					]	]								
					0	0	0	0						
						+	#	#	+					
II							•	•	•					
							~	[~]	[~]	[~]	~			
											0	0	0	(0)
Damage period														
Control time														

It is believed that the expansion and increasing damage of the plum crumpled moth is due to the increased cultivation of plums, the distribution of seedlings without quarantine control, and climatic factors. Tortricidae family of lepidoptera group peels leaves of plants and feeds inside fruits, and this feature makes it difficult to control and prevent damage from these pests. In particular, it is necessary in our country to conduct a detailed study of insects, together with climatic features, to combat them by biological means. This is because today, crop losses are high due to the lack of time to combat this pest, the lack of resources for consulting biological preparations, and the lack of knowledge and information of farmer. In our country, control of the plum fruit moth has been mainly based in the use of chemical insecticides. In order to combat this pest, it is important to determine its distribution, damage, and study its generational development in detail, and this is the first time we have done this. In particular, this is the first study to confirm the species using PCR analysis. It is believed that continuing this research will have a significant impact on the introduction of a comprehensive approach to controlling this moth.

4. CONCLUSION

1. In the 4 years, this moth spread to no more than 40% of the breeding and infected up to 40% of the fruit of one tree and there was a case of 100% reduction in the yield of more than 90% of the fruits of one tree our research area.
2. In the climatic conditions of our country, the plum moth reproduces 2 generation a year, and so far, it has been registered in plum plantations planted in Khovd, Gobi-Altai, Uvurkhangai provinces and the capital.
3. We confirmed the species by PCR analysis, which allowed us to distinguish the plum moth species from other Grapholita genera, which is important for resolving taxonomic issues.
4. The spread and damage of the plum moth is related to the increase in plum cultivation, the distribution of seedlings without internal quarantine control, and climatic factors.
5. The biological and ecological characteristics of this pest should be studied in detail in relation to climatic factors.

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