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Validity of the "Greenish Gall" as an Indicator for Evaluating the Effectiveness of Parasitoids (Hymenoptera : Chalcidoidea) of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae)

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It was suggested that the chestnut galls inhabited by parasitoids would remain greenish in color until autumn (Yasumatsu, 1955). An evaluation of the effectiveness of an introduced Torymus (*Syntomaspis*) sp. had been made by the abundance of such greenish galls (Torii, 1959). In order to clarify whether it is reliable to apply this method for field experiments, greenish and brown galls were collected to examine in autumn from Mt. Aburayama. Fukuoka. The results are as follows : (1) Although the ratio of galls inhabited by live parasitoids is higher in greenish galls than in brown ones, greenish galls are not always parasitized and brown galls are frequently inhabited by parasitoids. (2) Gall color is not determined by any inhabiting species of parasitoid. (3) Greenish galls tend to be smaller in size and contain fewer emergence holes. (4) Galls withered rapidly after cynipid or parasitoid wasps had emerged because they injured the gall tissue on emergence. (5) Evaluation of the effectiveness of ***Torymus (S.)*** sp. by the abundance of greenish galls in autumn is not a reliable indicator of the actual parasitoid incidence. Dissection of galls remains the only reliable method of accurate estimates of parasitoid abundance.

INTRODUCTION

The chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu was discovered in Okayama Prefecture in 1941, and spread rapidly throughout Japan and became one of the major pests of chestnut trees. It is thought to have been accidentally introduced from China, where 7 species of parasitoids were recognized recently (Murakami *et al.*, 1977). In Japan at least 13 species of indigenous parasitoids are associated with the chestnut gall wasp (Yasumatsu, 1955; Watanabe, 1957; Tachikawa, 1973). Most of them have two or more generations during a year and they associate with various gall-making cynipids, inquiline cynipids and their parasitoids of a variety of oak galls. However, *Torymus (Syntomaspis)* sp.[1] is the only parasitoid being synchronous with the chestnut gall wasp and usually univoltine. Torii (1959) attempted to introduce *Torymus (S.)* sp. to Nagano Prefecture where it was not distributed at that time.

[1] Yasumatsu (1955) regarded this parasitoid as a new species and proposed a scientific name ***"Torymus benejicus"*** tentatively, but it has not yet been validated.

Yasumatsu (1955) suggested that the chestnut galls, and also leaves growing from them, usually *got* withered rapidly after wasps had emerged from them, while the galls harboring parasitoids would remain greenish in color bearing green leaves until autumn, accordingly it was easy to distinguish parasitized galls from unparasitized ones. Torii (1959) had the thought whether or not such greenish galls found in abundance near the end of autumn could serve as an indicator of whether parasitic natural enemies were active or not, and he applied this discrimination method for his experimental release tests of *Torymus (S.)* sp. in Nagano Prefecture.

During field work in Kanagawa and Fukuoka, I noticed that on a particular variety of chestnut trees the galls are greenish until autumn even when they do not harbor any parasitoids and that some parasitoid larvae are frequently detected from inside galls which have become brownish in autumn. Therefore, doubts about the validity of the "greenish gall" as an indicator evaluating the effectiveness of parasitoids were justified. The present paper deals with such problems in evaluating the effectiveness of parasitoids in field experiments.

MATERIALS AND METHODS

A total of 227 galls of the chestnut gall wasp were collected from four trees of the wild Japanese chestnut, ***Castanea** crenata* Sieb. et Zucc. in Mt. Aburayama, Fukuoka in September of 1976 and 1977 (Table 1). Galls were opened and examined for the number of cells within each inhabited by live parasitoid larvae, pupae or adults and dead parasitoids or gall wasps as well as the number of empty cells from which parasitoids or gall wasps had already emerged. Most parasitoids were identified by the larval or pupal appearance, but two *Megastigmus* species were indistinguishable since they were morphologically indistinct.

Table 1. Collection data of chestnut gall wasp, *Dryocosmus kuriphilus* galls collected in Mt. Aburayama, Fukuoka for examination.

Trees	Altitude (above sea level)	Date of collection	No. of galls collected	
			greenish	brown
A	290m	Sept. 5, 1976	16	24
B	380	Sept. 5, 1976	2	38
C	100	Sept. 18, 1977	40	40
D	500	Sept. 18, 1977	31	36
Total	—		89	138

RESULTS

Interrelation between the external appearance and the existence of parasitoids in galls in autumn

Many galls were inhabited by live parasitoids in both greenish and brown galls examined in autumn (Table 2). In the majority of greenish galls, more than 90%, were inhabited by live parasitoids, whereas in brown galls the percentages of those harboring parasitoids were generally lower. However, greenish galls were not always parasitized, that is, approximately 9% of greenish galls did not contain any parasitoids. In the case of brown galls, as in those collected from tree C, a high percentage (95 %) of galls were inhabited by live parasitoids, and 68% of the 138 brown galls examined were inhabited by parasitoids. Therefore, it can be said that although the ratio of galls harboring live parasitoids is higher in greenish galls than in brown ones, greenish galls are not always parasitized and also brown galls are frequently inhabited by parasitoids.

Table 2. Numbers and percentages of chestnut gall wasp galls harboring live parasitoids in greenish and brown galls collected in Mt. Aburayama. Fukuoka, September 1976-1977.

Trees	Greenish galls		Brown galls	
	No. of galls examined	No. of galls inhabited by parasitoids (percentages in parentheses)	No. of galls examined	No. of galls inhabited by parasitoids (percentages in parentheses)
		%		%
A	16	15 (94)	24	5 (21)
B	2	2 (100)	38	25 (66)
C	40	36 (90)	40	38 (95)
D	31	28 (90)	36	26 (72)
		%		%
Total	89	81 (91)	138	94 (68)

Parasitoid species detected from greenish and brown galls

Several species of live parasitoids were detected from greenish and brown galls collected from the four study trees (Table 3).

Torymus (S.) sp. was the most dominant parasitoid particularly on trees C and D. When galls collected from trees A and B were opened on September 5 and 6, 1976, all the individuals of the parasitoid were in the mature larval stage. Although 2 of these larvae pupated and became adults in October, most larvae hibernated and reached pupal stage in winter and then became adults the following March to April. The individuals of the parasitoid collected from trees C and D were mostly in the mature larval stage when opened

Table 3. Species and number of live parasitoids detected in chestnut gall wasp galls collected in Mt. Aburayama, Fukuoka, September 1976-1977.

Parasitoid species	No. of live parasitoids detected in galls			
	Trees: A	B	C	D
Torymus (Syntomaspis) sp.	18	33	152	161
Megastigmass spp. (2 spp.)	32	34	0	4
Eurytoma setigera	0	0	0	1
Hyperparasitoids(undetermined)	0	0	21	1
Total	50	67	173	167

on September 20-22, 1977, but 11 individuals were in the pupal stage.

Two species of *Megastigmus* were detected, namely, *Megastigmus* sp. A[2] and *Megastigmus* sp. B[2]. These are indistinguishable by their larval appearance. When the galls of the trees A and B were opened, most individuals were in the mature larval stage except 3 pupae. From the middle of September to the end of October, and rarely in the next February, they reached the adult stage. The individuals which became adults could be identified but those which died in rearing could not be determined. The results of the identification are as follows:

Trees	Species A	Species B	Undetermined
A	8	15	9
B	12	9	13
D	3	0	1

Eurytoma setigera Mayr is very similar with *E. brunniventris* Ratzeburg which the latter is a polyphagous parasitoid associated with various oak cynipid galls (Claridge and Askew, 1960) and emerges frequently from the chestnut gall in Japan. Only one unemerged adult was detected from the present examination.

Hyperparsitoid larvae were detected from galls on trees C and D. Of these, 7 larvae were middle stage larvae feeding ectoparasitically on *Torymus* (S.) sp. larvae, and the others were matured larvae. As they did not grow into adults, they could not be identified.

In order to determine the validity of "greenish gall" as an indicator of the parasitization by *Torymus (S.)* sp., the numbers and percentages of galls from which each species of parasitoids was detected showed no difference between greenish and brown galls (Table 4). That is, 85 or 86 % of parasitized galls are inhabited by *Torymus (S.)* sp., and almost 20 % galls by *Megastigmus* spp. for both types of galls. This means that gall color is not indicative of the species of parasitoid within that gall.

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[2] Yasumatsu (1955) recorded these parasitoids as new species and proposed scientific names '*Megastigmus japonicus*" and "*M. maculipennis*" respectively, but they have not yet been validated.

Table 4. Numbers and percentages of chestnut gall wasp galls harboring *Torymus (S.)* sp., *Megastigmus* spp. and other parasitoids collected in Mt. Aburayama, Fukuoka, September 1976-1977.

Trees	No. of galls inhabited by parasitoids	No. of galls inhabited by each parasitoid species (*percentages in* parentheses)		
		Torymus (S.)	*Megastigmus*	Others
		Greenish galls		
		%	%	%
A	15	5 (33)	13 (87)	0 (0)
B	2	1 (50)	1 (50)	0 (0)
C	36	36(100)	0 (0)	2 (6)
D	28	28(100)	3 (11)	2 (7)
		%	%	%
Total	81	70 (86)	17 (21)	4 (5)
		Brown galls		
		%	%	%
A	5	4 (80)	3 (60)	0 (0)
B	25	17 (68)	15 (60)	0 (0)
C	38	33 (87)	0 (0)	12 (32)
D	26	26(100)	0 (0)	0 (0)
		%	%	%
Total	94	80 (85)	18 (19)	12 (13)

Number of cells and emergence holes per gall

While examining galls it was noticed that greenish galls tend to be smaller in size and bearing less emergence holes on them than brown galls. Since gall size is difficult to measure because of irregular forms, size was represent-

Table 5. Frequency distributions of cell number per gall for greenish and brown galls of chestnut gall wasp collected in Mt. Aburayama, Fukuoka, September 1976-1977.

No. of cells per gall	Frequency (relative frequency in parentheses)		
	Greenish galls	Brown galls	Total
1	%	%	%
2	20 27 (22.5)	21 10 (7.2)	30 48 (13.2)
	(30.3)	(15.2)	(21.1)
4	21 (23.6)	32 (23.2)	53 (23.3)
5	10 (11.2)	19 (13.8)	29 (12.8)
	4 (4.5)	22 (15.9)	26 (11.5)
6	3 (3.4)	10 (7.2)	13 (5.7)
7	1	9	10
8	1	1	2
9	1 } (4.5)	8 } (17.4)	9 } (12.3)
10≤	1	6	7
	%	%	%
Total	89 (100.0)	138 (99.9)	227 (99.9)
Average	2.84±0.42	4.38±0.42	3.78±0.32

ed by the number of cells per gall. Frequency distributions of the cell number of greenish and brown galls for those collected from the four study trees (Table 5), show that greenish galls inclined towards smaller size and the average number of cells of greenish galls was significantly less than that of brown ones. More than half of greenish galls contained only one or two cells.

On the other hand, there is a significant difference when comparing the number of emergence holes on greenish and brown galls (Table 6). Most greenish galls (83 %) had no emergence hole on their surfaces, whereas only 34 % of 138 brown galls possessed no emergence hole. Of the 106 galls bearing one or more emergence holes, 91 galls (almost 86%) were brown. Galls bearing no emergence hole may be either greenish or brown.

Table 6. Frequency distributions of emergence hole number on galls for greenish and brown galls of chestnut gall wasp collected in Mt. Aburayama, Fukuoka, September 1976-1977.

No. of emergence holes per gall	Frequency (relative frequency in parentheses)		
	Greenish galls	Brown galls	Total
	%	%	%
0	74 (83. 1)	47 (34. 1)	121 (53. 3)
1	12 (13. 5)	36 (26. 1)	48 (21. 1)
2	2 (2. 2)	30 (21. 7)	32 (14. 1)
3	0 .	19 (13. 8)	19 (8.4)
4	1 (1.1)	3 (2. 2)	4 (1.8)
5	0	1 } (2.2)	1 } (1.3)
6	0	1	1
7≤	0	1	1
	%	%	%
Total	89 (99. 9)	138 (100. 1)	227 (100. 0)
Average	0. 22±0. 13	1. 36±0. 25	0. 91±0. 18

DISCUSSION

Galls were examined to determine why some chestnut cynipid galls remain greenish in color until autumn. One of the most probable causes seemed to be that the gall tissue would have less injury. Galls would get withered rapidly after wasps had emerged, as suggested by Yasumatsu (1955). It is likely that the cynipid wasps and/or their parasitoids injured the gall tissue as they tunnelled through the tissues and made emergence holes. The fact that almost 86% of galls bearing one or more emergence holes was withered suggests this is true (Table 6). Frequently, partially withered greenish galls have been observed, in which the emergence holes opened on the withered part. For instance, out of 8 greenish galls bearing one or more emergence holes collected from trees C and D, 7 galls had the holes on the withered part of each gall.

In the cases where galls were withered in spite of bearing no emergence hole could be explained as they would be more or less injured inside them by wasps which became adults in the cells but did not emerged from the galls. The fact that greenish galls were frequent in smaller galls is explained by the higher probability that small galls bear fewer emergence hole than larger ones.

The majority of greenish galls examined were inhabited by live parasitoids as previously mentioned (Table 2). These parasitoids are not always *Torymus (S.)* sp. Galls remain greenish as well when inhabited by *Megastigmus* spp. (Table 4). Therefore, it is not reliable that Torii (1959) evaluated the effectiveness of introduced *Torymus (S.)* sp. by the abundance of greenish galls. Dissection of galls remains the only reliable method of accurate estimates of parasitoid abundance.

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