Relationship between *Nola innocua* BUTLER (Lepidoptera: Nolidae), a Kleptoparasite, and Aphids which Cause Galls on *Distylium racemosum* Trees¹

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A nolid moth, *Nola innocua* BUTLER is a kleptoparasite of aphids which form galls on *Distylium racemosum* trees. Larvae of this species bore into galls of *Nipponaphis distyliicola* MONZEN in summer and *Monzenia globuli* (MONZEN) or *Metanipponaphis* sp. A in autumn, and feed on the internal tissues leaving faeces in the gall. The aphids in attacked galls consequently die. A single moth larva consumes more than two galls and pupates outside the galls on twigs. *Nola* is the most important mortality agency of these aphids within their galls. The moth larvae cannot feed on mature leaves of *Distylium*. Young leaves are also avoided. The moth successively attacks *N. distyliicola* galls in its summer generation and galls of *M. globuli* or *Metanipponaphis* in the autumn generation. These observations suggest that *Nola innocua* has become highly specialized to its kleptoparasitic life within the aphid galls.

INTRODUCTION

Distylium racemosum SIEB. et ZUCC. is an evergreen broad-leaved tree native to East Asian subtropical and warm-temperate forest ecosystems. The tree has been planted, especially in Japan, as an ornamental tree but the northern limit of its distribution does not extend beyond the range of warm-temperate broad-leaved forests. At least 11 species of aphids form galls of different sizes and shapes on *Distylium racemosum* (SORIN, 1977 and personal communications). During the course of studies on the population dynamics of gall aphids on *Distylium* trees, we found that a nolid moth, *Nola innocua* BUTLER (= *Clema innocua japonibia* STRAND; INOUE, 1976), is a kleptoparasite of two gall aphids, *Nipponaphis distyliicola* MONZEN and *Monzenia globuli* (MONZEN); that is, the larvae of this moth are almost obligatory feeders on the gall tissues and the host aphid consequently die (ITÔ and HATTORI, 1982). This paper reports the bionomics

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of the moth and the effect of kleptoparasitism on the population dynamics of the two gall aphids.

MATERIALS AND METHODS

Field observations and the collection of materials were made at two sites within Nagoya University, Chikusa-ku, Nagoya. The first (Station I) consisted of 11 *Distylium* trees planted on the campus of the Faculty of Agriculture, and the second (Station II), two *Distylium* trees growing in front of the University headquarters, is about 230 m distant from the Station I.

We have found, to date, 5 species of aphid galls on these trees (Table 1). Sampling and field observations were mainly made on two common galls, of *Nipponaphis distyliicola* MONZEN and *Monzenia globuli* (MONZEN).

Nipponaphis distyliicola forms fig-shaped green galls on the tips of twigs of *Distylium* in May and June. The size of the galls reaches about 30 mm in length and 20 mm in diameter at the upper (broader) part. The wall of the gall is juicy but tough except when very young, and about 2 mm thick. Most alate aphids emerge in September, but a few galls contain living apterae till November. The alate aphids migrate to the second host, species of *Quercus* including *glauca* and *stenoplula*.

Monzenia globuli is a non-migratory species which forms green globular galls, 10 to 15 mm in diameter, on the base of buds or fruits of *Distylium* in September. Its wall remains soft even after the emergence of the aphid and is about 1 mm thick. The emergence of alate sexuparae takes place from October to November and they oviposit on *Distylium*.

Another aphid which forms galls in September is *Metanipponaphis* sp. A (SORIN, personal communications). Its gall is like a comma-shaped bead, 15 to 20 mm in length and 5 mm in diameter, and the wall is soft as in the galls of *M. globuli*. As we found exuviae of *Nola* in some of these galls, additional observations were made on this species.

To estimate the rate of attacks by *Nola*, all the galls of *N. distyliicola* and *M. globuli* on randomly selected shoots were collected and brought to the laboratory. These galls were at first examined to find openings made by *Nola* larvae on the gall surfaces

Table 1. Aphids which form galls on *Distylium racemosum* trees on the campus of Nagoya University

Species	Beginning of gall formation	Opening of gall	Gall characters	Literature
<i>Sinonipponaphis monzeni</i>	May	November	Giant woody pouch gall on twig	SORIN, 1958
<i>Nipponaphis distyliicola</i>	May-June	September	Fig-shaped gall on twig	This study
<i>Monzenia globuli</i>	September	October-November	Small globular gall on tip of branchlet or fruits	SORIN, 1960
<i>Metanipponaphis</i> sp. A	September	October-November	Small comma-shaped bead-like gall on tip of branchlet	This study
<i>Neothoracaphis yanonis</i>	April	June	Closed leaf gall	This study

and were then dissected to check for faeces and exuviae of the moth remaining in the gall. All the galls formed on other selected shoots were marked with vinyl tape, and examined in the field on several occasions. Shoots were selected systematically to represent upper, middle and lower parts of the tree. In October, when the emergence of *N. distyliicola* from its gall was completed, all *N. distyliicola* galls which could be reached using a step ladder were collected. These were used not only to determine the rate of attack by *Nola* but also to count traces of unsuccessful boring by *Nola* larvae. As the number of *M. globuli* galls was too large to allow examination of them all, several hundred galls were taken randomly from trees after the emergence of aphids.

For *Metanipponaphis* sp. A, which is a rather rare species of the site, galls found occasionally during the course of study of the two species mentioned above were examined.

Some experiments were made to examine the food habits of *Nola innocua* larvae. A twig of *Distylium* with a leaf of definite age or with a gall of *N. distyliicola* or *M. globuli* was put into a small glass vial (45 mm in length and 10 mm in diameter) with water. Either or both of leaf and gall were placed in a petri dish (9 cm in diameter and 1 cm in height) with a larva, and the behaviour of the larva was recorded (see Fig. 1E).

To determine whether or not *Nola* larvae kill aphids directly, ten nymphs of *M. globuli* taken from galls and a *Nola* larva were confined in a small glass vial (20 mm in diameter and 35 mm in depth) covered with parafilm®. The aphids surviving in the vial were counted.

RESULTS

Phenology

Galls of *N. distyliicola* attacked by *Nola* were seen from July. Attacked galls could be identified easily by the openings made by larvae and the faeces remaining in the gall. The larva feeds on the inside wall of the gall and the colour of the inner surface of the gall changes from white to black (Fig. 1A). The aphids in the attacked gall consequently die. Sometimes we found larval exuviae within attacked galls and these were used to determine the number of instars (HATTORI and ITÔ, in preparation). As no parasitoid or any other guests of the gall were found in the area, galls which had round opening(s) on the surface (which are quite different from the openings indicating aphid emergence) were counted as being attacked by *Nola*. When the moth larva exhausts the gall before it is fully mature, it leaves the gall (through the opening used when it entered into the gall or by boring a new one), seeks another gall, and bores into that. The larva may use openings made by other individuals, and several larvae sometimes coexist in a gall with but a single opening. When mature, the larva leaves the gall and spins a rough cocoon on a twig and pupates. Adult emergence was seen from the end of July, and the moth has at least two generations on *N. distyliicola* galls. However, the outer surfaces of *N. distyliicola* galls become quite tough in August and, accordingly, *Nola* larvae sometimes need several hours to bore into the galls or even fail to do so (see below).

In September, fresh galls of *M. globuli* become available as hosts for *Nola*. The larvae now bore into *M. globuli* galls and feed on their inside walls. As *M. globuli* galls are smaller than galls of *N. distyliicola*, a larva may consume two or more galls in completing its growth. Most aphids die from the deterioration of gall tissue, but, in some

Fig. 1. A: galls of *Nipponaphis distyliicola*; the one to the right is healthy and that to the left, attacked by a *Nola innocua* larva. X indicates an opening made by the larva. B: a mature larva of *Nola innocua* and its fecal pellets in a gall of *Monzenia globuli*. C: galls of *Monzenia globuli* showing openings from which aphids emerged on the left and those made by *Nola innocua* larvae on the right. D: trace of feeding by a *Nola innocua* larva on young leaf of *Distylium racemosum*, showing that *Nola* larva did not continue to feed on the leaf (48 hr after confinement of a large larva with a young leaf). E: an experiment on feeding preference by *Nola* larvae between galls and leaves. F: *Nipponaphis distyliicola* gall showing a large number of traces of unsuccessful probes by *Nola* larvae. Y is the opening for aphid emergence and Z is a gall of *Neothoracaphis yanonis* which sometimes forms its galls on *Nipponaphis* galls.

galls which have been attacked by *Nola* at the last stage of aphid development, at least some aphids can emerge (see Table 3). The rate of attack by *Nola* was again determined easily by the characteristic openings and faeces remaining in the gall (Fig. 1 B, C). To date neither parasitoids nor any other guests have been found in *M. globuli* galls.

Metanipponaphis sp. A also provide an autumnal host for *Nola* as does *M. globuli*. Although the density of *Metanipponaphis* galls was low in the area, about 20 percent of galls were attacked by *Nola* larvae in 1981 (Table 3).

We found remains of fine (0.4 mm in diameter) globular egg-shells on the outer surfaces of *M. globuli* galls. When such a gall was cut open, we often found the first instar larva of the moth within. We obtained similar egg-shells from laboratory-reared moths. We conclude that *Nola* females can lay eggs on the galls directly and hatched larva can bore into the gall by themselves, or use openings made by earlier pioneers. It was found that larvae which attacked galls of *M. globuli* or *Metanipponaphis* sp. A earlier (beginning of September) could pupate in September and October, and some adults even emerged from some of these pupae, although other individuals hibernated as pupae. Larvae produced later in the season, on the other hand, suspended their growth and stayed in the empty galls they had attacked or hide in other empty galls till spring. Thus the moth can overwinter in either larval and pupal stages.

Relationship to aphids

We found that, when starved for two or three days and confined in a small vial with ten apterous aphids taken from galls, mature larvae of *Nola* preyed on nymphs but younger ones did not (Table 2). When a larva was confined with a cut fresh gall of *N. distyliicola* or *M. globuli* and corresponding aphids, the aphids always remained untouched. Also when we cut open a gall at the initial stage of feeding by the moth larva on gall tissue, we found no sign of direct attack on the aphids, despite traces of feeding on gall-tissues. Living aphids were sometimes found in galls left by *Nola* larvae. Even in these cases, the aphids soon died due to deterioration of the internal tissue of gall, except in the one case where *M. globuli* galls were attacked at the last stage of the development of aphid.

Table 2. Results of experiments where a *Nola* larva was confined in a glass vial with 10 *M. globuli* nymphs. Numerals in the Table indicate the number of aphid nymphs surviving

Stage	Time after confinement		
	0 hr	5 hr	24 hr
Early instar larva ^a	10	10	10
Early instar larva ^a	10	— ^b	10
Middle instar larva	10	0	escaped ^c
Late instar larva	10	10	9 ^c
Late instar larva	10	—	10
Late instar larva	10	—	1

^a Possibly 2nd instar.

^b No observation.

^c Larva escaped by boring the parafilm® cover.

Table 3. Role of kleptoparasitism by *Nola innocua* in mortality of gall aphids. 'Other causes' mean mortality from physiological deterioration of galls and fungal infection

	Station I						Station II			
	<i>N. distylitcola</i>			<i>M. globuli</i>			<i>M. globuli</i>		<i>Metanipponaphis</i> sp. A	
	July	Aug.	Sept.	Nov.	Oct.	Oct.	Oct.	Oct.	Oct.	Oct.
	21	18	30	1	1-6	16	27-30	1	26	26
No. galls examined	82	170	99	543	511	188	565	85(5) ^b	182	31
No. galls attacked by <i>Nola</i>	53	138	81	489	126	35	192	12	54	6
% attacked	64.6	81.2	81.9	90.1	24.7	19.6	34.0	14.1	45.2	19.4
No. galls died from other causes	—	5	5	13	—	10	11	—	6	—
No. galls surviving, or from which aphid emerged	—	27	13	41	—	143	363(22) ^a	—	122(3) ^a	25
% survivals	—	15.9	13.1	7.6	—	76.1	64.0	—	67.0	80.6
No. trees examined	6	3	3	9	6	3	7	1	1	1

^a Number of galls from which aphids emerged despite attack by *Nola* larvae.

^b At least on five galls *Nola* egg-shells were found.

Rate of attack

Table 3 shows the number and percent of galls which were attacked by *Nola* with consequent death of aphids. More than 80 percent of *N. distyliicola* galls could not produce progeny due to attacks by *Nola*. The mortality due to other causes (possibly due to physiological and fungal deterioration of gall tissue) was remarkably low. Thus *Nola innocua* is the most important mortality agency acting on summer generations of *N. distyliicola*. The percentage of attacks by *Nola* was not very high (about 30 percent) for galls of *M. globuli* and *Metanipponaphis* sp. A, but *Nola* was still the most important mortality agency on aphids within their galls.

Densities of galls of different aphids varied from tree to tree, possibly due to differences in physiological conditions among individual trees. As *Nola* attacks *N. distyliicola* in its summer generations and *M. globuli* or *Metanipponaphis* sp. A in its autumn generations, the density of the moth in autumn can be affected by that of summer generations. Figure 2 shows the relationship between the density of *N. distyliicola* galls per tree and the rate of *M. globuli* galls attacked by *Nola* in the following autumn. This figure suggests that, even within a small area, trees which have small numbers of the summer hosts do not produce many adults of *Nola* and the rate of attacked galls in autumn is consequently lowered.

Figure 1F shows traces of unsuccessful probes by *Nola* larvae on an *N. distyliicola* gall. Figure 3 shows the frequency distribution of the number of unsuccessful trials per gall at Station I. Several dozen such traces were seen on some galls. Thus *N. distyliicola* galls in the later stage of their development are considered to be resistant to attacks by *Nola* larvae.

Experiments on leaf-eating

Distylium racemosum sprouts new leaves four times per year; March to April, May,

Fig. 2. Relationship between density of *Nipponaphis distyliicola* galls (summer host) and percent of *Monzenia globuli* galls (autumn host) attacked by *Nola innocua*.

Fig. 3. Distribution of the number of traces of unsuccessful probes by *Nola innocua* larvae on *Nipponaphis distyliicola* galls.

Table 4. Behaviour of *Nola innocua* larvae when confined with either leaf or gall or both in petri dishes. Water was supplied to the leaf or gall from a small vial

Month	Body length of larva	Food ^a	No. larvae used	Behaviour of each larva ^b						
				1	2	3	4	5	6	7
July	4-7 mm	Mature leaf	2	0 ^c	0 ^c					
	4-7 mm	<i>Nipponaphis</i> gall	1	B						
Aug.	4-7 mm	Mature leaf	4	0	0	0	0			
	4-7 mm	<i>Nipponaphis</i> gall	2	B	B					
Sept.	4-7 mm	Sprouting leaf	2	8	6					
	4-7 mm	<i>Monzenia</i> gall	1	B						
	4-7 mm	Leaf and gall	2	B	B					
Oct.	<4 mm	Young leaf	2	0	0					
	4-7 mm	Young leaf	6	1	0	0.5	4	0	1	
	<4 mm	<i>Monzenia</i> gall	3	B	B	F				
	4-7 mm	<i>Monzenia</i> gall	7	B	B	B	B	B	B	B

^a Mature leaf: a leaf opened in April.

Sprouting leaf: a leaf sprouting; length of major vein <1 cm.

Young leaf: a leaf opened in September; length of major vein $\approx$ 5 cm.

^b B means the larva bored into the gall and F, that failed boring.

Numerals give the area (mm²) of leaf eaten 48 hr after the beginning of the experiment.

^c Three larvae died after 4 and 5 days, respectively, without feeding.

September and December. In June and July, mature leaves only are seen on trees and there was no sign of biting by *Nola* larvae on leaves. Table 4 shows that, when a larva was placed in a petri dish with a mature leaf, the larva did not feed on the leaf and died after several days. On the other hand, if a larva which was deprived food for 2 days

was confined with a tiny, sprouting leaf (length of major vein <1 cm), the larva fed on the leaf. But when a larva was confined with a gall and a sprouting leaf, the larva bored into the gall without feeding on the leaf (Fig. 1E). When a fasting large larva was confined with a young leaf (about 20 days after its sprouting), it tried to feed on the leaf, but did not continue this (Fig. 1D). It was shown that this moth can complete its development only on galls.

DISCUSSION

TAKAHASHI (1935) reported that larvae of some moths were found in galls formed by Aphididae. Moth larvae have also been reported from galls of aphids, wasps and midges (e.g. WALSH, 1866; MANI, 1964; CALTAGIRONE, 1964). WALSH (1866) suggested a strong dependence of the moth, *Batrachedra salicipomonella* on galls of the wasps, *Pontania* spp. CALTAGIRONE (1964) reported that *B. salicipomonella* larvae were found only from *Pontania* galls and HODGES (1966) added *B. striolata* as another inhabitant of *Pontania* galls. CALTAGIRONE (1964) also found that *B. salicipomonella* females actually laid their eggs on the gall. However, the status of the moth with respect to gall forming insects can be as a facultative guest (which only shelters in the gall), an obligatory or a facultative cecidophage (including commensals and kleptoparasites), or a predator of the gall-forming insects, and there is no report of the obligatory cecidophagous habit of moths before ITÔ and HATTORI's (1982) description of *Nola innocua*.

As shown in Table 4, *Nola innocua* larvae customarily do not feed on leaves of *Distylium racemosum*, on which they are found. They cannot feed on leaves even when starved except in the special case where tiny sprouting leaves were presented to older larvae. This observation and the remarkably high rate of occurrence of the moth larvae in galls (Table 3) indicate that the moth is a highly specialized cecidophage of aphid galls. Although *Nola* larvae do not feed on aphids in attacked galls, the aphids, as a rule, die from deterioration of galls due to the attacks. Accordingly, ITÔ and HATTORI (1982) concluded that this moth is a kleptoparasite of gall aphids.

It is of special interest that this species exploits two kinds of aphid galls in Nagoya; *Nipponaphis distyliicola* in summer and of *Monzenia globuli* or *Metanipponaphis* sp. A in autumn (also of *Neothoracaphis yanonis* in spring; ITÔ, unpublished). In another locality (Komaba, Tokyo), where *Monzenia globuli* was apparently absent and galls of *Metanipponaphis* sp. B (SORIN, personal communications) were the only common galls in autumn, we found that the moth attacked *N. distyliicola* and *Metanipponaphis* sp. B successively (HATTORI and ITÔ, unpublished). Figure 2 indicates also this strong dependence of *Nola* on a set of summer and autumn hosts. This requirement for two (or three), sequential hosts demands that we seek an evolutionary explanation.

Major advantages of gall formation for gall aphids have been said to be the provision of a sheltered place which protects the aphids from adverse weather conditions and natural enemies, and of improved nutrition (MANI, 1964; DIXON, 1973). However, these are also advantages for insects which feed on galls. When the cecidophage is a kleptoparasite, which ultimately kills the aphids or decreases their fitness, the aphid should evolve ways to 'escape' its depredations such as the production of a soldier 'caste' as in *Colophina clematis* and *Pseudoregma alexanderi* (AOKI, 1977; AOKI et al., 1981) or by forming tougher galls. In other words, coevolution (PIANKA, 1978) between a gall-maker and the kleptoparasite is expected. The fact that *N. distyliicola* galls often

bear traces of unsuccessful attacks by *Nola* suggests that, at least in *N. distyliicola*, galls which have grown to a larger size and accordingly become tougher early in the season should be relatively freer from moth attack than those formed in later. Regular examination of moth attacks on marked galls will shed light on this suggestion.

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