Korean J. Ecol., 23(2): 175~180, 2000

Interactions between Insect Species Feeding on *Rumex obtusifolius*: the Effect of *Philaenus spumarius* Feeding on the Ecology of *Gastrophysa viridula*

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Abstract : In order to study the insect-insect interaction of the insect community associated with *Rumex obtusifolius*, this experiment was designed in such a way that the feeding of one insect could indirectly affect the subsequent insect species through the changes in host plant (plant mediated insect-insect interaction). *Philaenus spumarius* and *Gastrophysa viridula* were selected for the experiment. To investigate the effect of *P. spumarius* feeding on the ecology of *G. viridula*, first, statistical analyses were carried out. As results, no significant difference between Control and Experimental was found in the development patterns (Repeated Measures ANOVA, F=0.744, p=0.667) and survivorships (F=0.373, p=0.990). As the results from this experiment show, there was no effect on the ecology of *G. viridula* due to the previous feeding by *P. spumarius* on *R. obtusifolius* leaves.

Key Words: Coexistence, Hervibory, Insect-insect interaction, Interspecific competition.

INTRODUCTION

Although there are many studies about the interaction between individual insect species and *Rumex obtusifolius* (Bentley and Whittaker 1979, Bentley *et al.* 1980, Cottam *et al.* 1986, Hatcher *et al.* 1994, Hatcher *et al.* 1995, Hopkins and Whittaker 1980, Renner 1970a, Salt *et al.* 1995, Smith and Whittaker 1980a, 1980b, Speight and Whittaker 1987, Whittaker *et al.* 1979, Whittaker 1994), there has been no published study on insect-insect interactions of the insect community associated with *R. obtusifolius*.

A study on an insect-insect interaction can be very complex. Coexistence of two or more insect species on a host plant would present many factors for the comparison such as competition of food resources, physical disturbances, and competition for ovipositional space. Also the effect of the insect species to the others can be very complex even in three species interactions. The combination of three species interaction for each factor is six (A↔B, B↔C, A↔C, A+B↔C, A+C↔B, B+C↔A). The experiment was designed in such a way that the feeding of one insect could indirectly affect the subsequent insect species through the changes in host plant, *R. obtusifolius* (plant mediated insect-insect interaction). Direct coexistence of the two insect species was avoided to restrict the effect of feeding to nutritional changes in food.

Philaenus spumarius (Homoptera: Cercopidae) and *Gastrophysa viridula* (Coleoptera: Chrysomelidae) were selected for the experiment. *P. spumarius* was selected because of its xylem sucking strategy of feeding on *R. obtusifolius* (Horsfield 1977, 1978). In contrast to phloem, sugars are not a major constituent in the xylem sap. Instead, the N concentration as a form of amino acid in xylem sap is often a limiting factor for the growth of heterotrophic insects (cercopids and cicadas) (Raven 1983). A hypothesis was made that the survivorship of *G. viridula* would change as the nutritional value of *R. obtusifolius* leaves change due to the previous feeding by *P. spumarius*.

MATERIALS

A full description of the ecology of *Rumex obtusifolius* L. (Polygonaceae) had been published by Cavers and Harper (1964). The plant has a world-wide distribution following introduction from Europe. It can be found on almost any grassland that is disturbed or badly managed, as well as on the field borders and hedgerows. It is usually refused by most animals, but is a favourite food plant of deer (Amphlett and Rea 1909). The plant is an erect perennial herb with a stout tap root. An individual plant may live 5 or more years. Once established, it is extremely difficult to eradicate. It grows on a wide range of soils except very acidic ones. Annual growth cycle has three stages: rosette, elongation and inflorescence (Chabot-Jacquety 1960). The plant overwinters as a rosette stage. In spring, the root grows to produce a large, fleshy and fanged rootstock system. The root often extends to a depth of 100~150 cm in some soils. The basal and lower cauline leaves grow to sizes measuring 13~30 cm × 8~5 cm (length × width). At elongation stage, the shoot grows to 40~150 cm high. Flowering occasionally occurs in the seedling year, but usually not until the second year. A large plant can produce many inflorescences and ripe seeds twice in one growing season (May~June, August-September).

The seeds were collected from the plants around the campus of Lancaster University in 1989 and 1990. They were sown into John Innes Compost No. 2 in late January, 1992, and grown until the cotyledons were separated from each other. Then, they were initially transplanted into 9 cm-diameter pots for experimental uses. The unheated greenhouse in which the plants were kept had no artificial ventilation or shading. To prevent the extreme overheating inside the greenhouse, the ventilation windows were left open from June to August period. The plants were watered daily unless otherwise stated. Both Malathion treatment and Nicotine fumigation on plants and of the greenhouse were carried out to kill aphids from 22 April to 25 April. The plants were then transplanted into 13 cm diameter pots on 10 May.

Gastrophysa viridula (Degeer) (Coleoptera: Chrysomelidae) is a small (around 8 mm), metallic green coloured beetle native to Britain. It is an oligophagous insect (Chavin 1968) on members of the Polygonaceae but particularly associated with the genus *Rumex*. Adults usually emerge from overwintering diapause during April. Egg batches of average 40 are laid on the underside of leaves. Both larvae (3 instars) and adults normally feed on leaves though it is observed that they can consume stems as well if starved. The third instar larva moves down or drops to the ground, and pupates in the soil. A female has capability of laying over 1,000 eggs. Adult beetles disperse by crawling, and none was observed flying either in the field or when being reared in the laboratory (Smith and Whittaker 1980b, Whittaker *et al.* 1979). The whole life cycle is about four to five weeks. It is possible for three generations to occur in a year. Considerable studies have been done on interaction between *G. viridula* and *R. obtusifolius* (Bentley *et al.* 1980, Smith and Whittaker 1980a, 1980b, Cottam *et al.* 1986) as well as the life history, morphology, and physiology of the beetles (Engel 1956, Renner 1970a, 1970b, 1970c, 1970d, 1971, Whittaker *et al.* 1979). Therefore, the beetle was chosen as a primary insect of the study.

G. viridula used on the study was collected from around the campus of Lancaster University in early April 1992, when it first emerged from diapause. Pairs of insects were randomly chosen, and then reared in plastic boxes measuring 18 cm × 18 cm × 10 cm (length × width × height) with a 3 cm^2 ventilation window on each side. Abundant amounts of *R. obtusifolius* leaves from the greenhouse were put into the box every 2 days, and old leaves were removed from the box. Then the offspring from the box were reared, and used for the experiments.

Philaenus spumarius (L.) (Homoptera: Cercopidae), a spittle bug, is a xylem-feeding insect (Horsfield 1978) which is highly polyphagous (Halka *et al.* 1967, Weaver and King 1954). It overwinters as an egg and has five nymphal instars. The first adults appear during June (Whittaker 1973, Wiegert 1964). The adult is highly colour polymorphic (Whittaker 1968). *P. spumarius* nymphs need to extract a large quantity of xylem sap for growth and maintaining the spittle (Press and Whittaker 1993). The xylem sap contains water, ions, organic solutes including amino and organic acids, low molecular mass carbohydrates and plant growth substances (Press and Whittaker 1993, Raven 1983). As demonstrated by Horsfield (1977), the survival of a nymph is correlated to the amino acid concentration of the xylem sap. These characteristics of xylem feeding of *P. spumarius* on *R. obtusifolius* results in severe convolution of the infested leaves.

The insects were collected from around the campus of Lancaster University in early May 1992, when the spittle became visible usually as the second instar nymphal stage. The nymph and the spittle were transferred to the undersides of leaves of *R. obtusifolius* in the greenhouse. The insects were able to settle on the new leaves successfully, most of the time. Rearing them from eggs in laboratory conditions requires approximately 100 days of low temperature period (West and Lees 1988).

METHODS

The plants in 13 cm diameter pots were randomly chosen, and placed into the wooden cages (30 cm × 30 cm × 60 cm) on 10 June. Each cagehoused 1 plant. Total of 16 cages (8 control cages and 8 experimental cages) were then placed in the greenhouse in random orders. On 10 June, six *P. spumarius* nymphs (second instar) were released into each of 8 experimental cages for 2 weeks (Experimental). Control cages were not treated with *P. spumarius* nymphal feeding (Control). After 2 weeks, they were removed from the plants and 2 gravid *G. viridula* females were then released into each of both control and experimental cages for 3 days. After 3 days, all plants had egg clutches and *G. viridula* females were removed from the plants. The numbers of surviving individuals in each cage and their life stages were recorded. The experiment ended on 7 August, 1992.

RESULTS

Table 1 shows the mean numbers of surviving *G. viridula* individuals per plant at each life stage during the experimental period. Comparisons of the effect of *P. spumarius* feeding on the ecology of *G. viridula* were carried out.

First, the index of development (Hodkinson *et al.* 1979) was calculated for each observation date on Fig. 1:

Table 1. Mean Number of surviving *Gastrophysa viridula* individuals per plant in Control and in Experimental (*Rumex obtusifolius* was subjected to the feeding by *Philaenus spumarius* nymphs in Experimental) (Unit = Mean No. / Plant)

Day	Control									
	Egg	S.E.	Larvae I	S.E.	Larvae II	S.E.	Larvae III	S.E.	Adult	S.E.
0	42.13	4.68	0	0	0	0	0	0	0	0
2	42.13	4.68	0	0	0	0	0	0	0	0
4	28.25	5.83	12.00	4.38	0	0	0	0	0	0
6	5.12	3.46	30.25	5.50		0	0	0	0	0
8	2.75	1.81	28.25	5.20	0	0	0	0	0	0
10	0	0	2.38	1.13	23.00	5.04	0	0	0	0
12	0	0	0.13	0.13	22.62	4.36	0	0	0	0
14	0	0	0	0	11.12	2.59	4.00	4.00	0	0
17	0	0	0	0	0	0	9.50	3.40	0	0
20	0	0	0	0	0	0	6.75	3.15	0	0
24	0	0	0	0	0	0	0.50	0.27	0	0
27	0	0	0	0	0	0	0.13	0.13	0	0
30	0	0	0	0	0	0	0.13	0.13	0	0
33	0	0	0	0	0	0	0	0	0.25	0.16
35	0	0	0	0	0	0	0	0	0.75	0.31
37	0	0	0	0	0	0	0	0	1.75	0.73
39	0	0	0	0	0	0	0	0	1.75	0.73
42	0	0	0	0	0	0	0	0	2.00	0.78

Day	Experimental									
	Egg	S.E.	Larvae I	S.E.	Larvae II	S.E.	Larvae III	S.E.	Adult	S.E.
0	53.50	9.44	0	0	0	0	0	0	0	0
2	53.50	9.44	0	0	0	0	0	0	0	0
4	30.50	7.18	19.62	5.86	0	0	0	0	0	0
6	2.12	2.12	93.50	9.44	0	0	0	0	0	0
8	0	0	36.75	8.24	0	0	0	0	0	0
10	0	0	10.25	3.21	22.13	6.70	0	0	0	0
12	0	0	0	0	28.62	6.81	0	0	0	0
14	0	0	0	0	17.50	5.19	1.38	1.38	0	0
17	0	0	0	0	0.88	0.64	7.75	3.57	0	0
20	0	0	0	0	0	0	7.75	3.88	0	0
24	0	0	0	0	0	0	2.75	1.16	0	0
27	0	0	0	0	0	0	0.38	0.26	0	0
30	0	0	0	0	0	0	0.13	0.13	0	0
33	0	0	0	0	0	0	0	0	0.13	0.13
35	0	0	0	0	0	0	0	0	0.25	0.25
37	0	0	0	0	0	0	0	0	0.88	0.44
39	0	0	0	0	0	0	0	0	1.38	0.53
42	0	0	0	0	0	0	0	0	1.50	0.60

$$\text{Index of Development for each date} = \frac{\sum_{i=1}^{5} n_i x_i}{N}$$

where n_i = number of individuals at i stage of life cycle; x_i = instar code at i stage of life cycle (Egg = 0, instar I = 1, instar II = 2, instar III = 3, pupae = 4, adult = 5); N = total number of individuals present.

A statistical analysis was done to see if there is a difference of the development patterns in Control and in Experimental. The data between day 0 to day 20 were used for the analysis since there is no difference between the two after day 20 (Fig. 1). No significant difference was found in the development patterns between the two (Repeated Measures ANOVA, F = 0.744, p = 0.667).

Second, the survivorships of *G. viridula* in Experimental and in Control were compared. For analysis, the data in Table 1 were transformed to cumulative percentages of the total individual numbers. Then, they were prepared for the Repeated Measures ANOVA by Arcsine transformation (von Ende 1993, Sokal and Rohlf 1981). The difference in the survivorships between the two was not significant (F = 0.373, p = 0.990). Fig. 2 shows the mean cumulative survivorship of *G. viridula* individuals per plant in Control and in Experimental cages. Even though the mortality on day 24 was greater in Control than in Experimental, it may not be accounted to be a valid figure since the pupal mortality was not added.

Third, a survival analysis based on Cox's Proportional Hazard Model (Cox 1972, Muenchow 1986, Prentice and Gloeckler 1978) was tried to see if there is a significant difference between the survival time of *G. viridula* in Control and in Experimental. This method does not make any assumptions about the nature or shape of the underlying survival distribution. The analysis showed no significant difference (Survival Analysis, χ^2 = 0.856423, p = 0.65168). Fig. 3 shows the cumulative survival (%) of *G. viridula* individuals per plant at each life stages in Control and in Experimental cages. Although the pupal mortality could not be monitored for monitoring the adult emergence, pupation may have started on around days 20~24 considering the adult emergence on day 33.

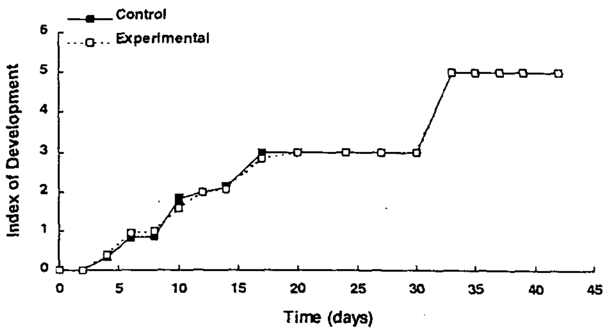


Fig. 1. Index of development for *Gastrophysa viridula* in Control and in Experimental.

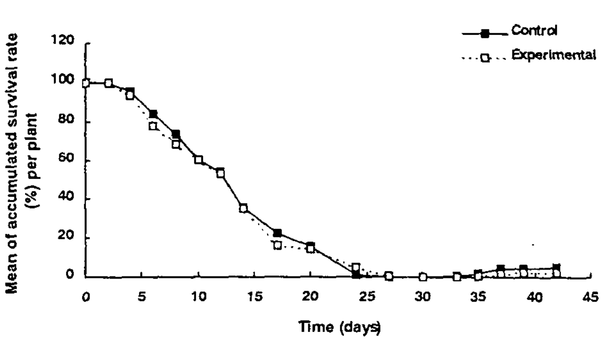
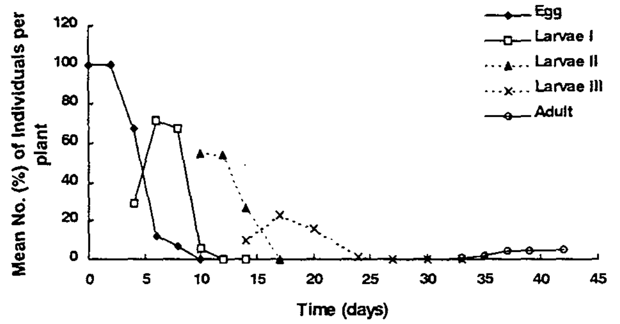
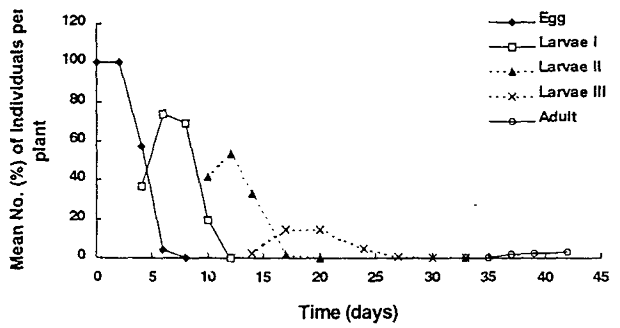


Fig. 2. Cumulative suvivorship of *Gastrophysa viridula* individuals per plant in Control and in Experimental cages.



(a) Control



(b) Experimental

Fig. 3. Mean numbers (%) of surviving *Gastrophysa viridula* individuals per plant in Control (a) and in Experimental (b) cages.

DISCUSSION

Interactions within and among herbivorous insect species have been considered important in relation to host range (Bernays and Chapman 1994). One form of interaction among insect species is interspecific competition. The other forms of interaction among insect species include predation and parasitism. Crawley (1983) suggested 4 categories of interspecific relationships between two herbivore species (symbiosis, commensalism, competition, amensalism).

Karban (1986, 1989) suggested the existence of interspecific competition between *Philaenus spumarius* and *Platyptilia williamsii* on *Erigeron glaucus*. They showed that asymmetrical interspecific competition (amensalism) was a consistently important factor for *P. spumarius*. But *Platyptilia williamsii* was not affected to feeding by *P. spumarius*, when they were placed together on host-plant. This asymmetry had been noted in other systems (Lawton and Hassell 1981, Schoener 1983).

As a result, there was no effect on the ecology of *G. viridula* due to the previous feeding by *P. spumarius* on *R. obtusifolius* leaves. Speight (1983) had a similar result on the interaction between *G. viridula* and *P. spumarius*, although he found the significant reduction only in the survivorship of *G. viridula* eggs. Both studies forced *G. viridula* females to lay eggs on the leaves which had been subjected to *P. spumarius* feeding. In a natural field condition, these two species may not even have any possibility of interspecific interaction due to their temporal ecology of host-plant utilization. Or, *G. viridula* females may actively select the leaves which has not been subjected to *P. spumarius* feeding.

However, if they are forced to share a host plant, they may exhibit some form of interspecific interaction. The assumption drawn from the study by Karban (1986) suggests that there may be amensalism on interspecific competition between *G. viridula* and *P. spumarius* feeding on *R. obtusifolius*. Further studies on the plant-mediated interspecific competition among *P. spumarius* and other insect species associated with *R. obtusifolius* are needed to find out the nature of the interaction.

ACKNOWLEDGEMENTS

We wish to thank Professor J.B. Whittaker, and Dr. A.J.C. Malloch, for their constant advice and support throughout this study.

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(*Received March 3, 2000*)