

## Behavioral responses to parasitized and unparasitized hosts of *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae)\*

Cem ÖZKAN\*\*

M. Oktay GÜRKAN\*\*

### Summary

When presented with unparasitized *Ephestia kuehniella* Zeller larvae to *Venturia canescens* (Gravenhorst), the wasp parasitized them within five minutes. However, the parasitoid did not parasitize previously parasitized larvae in a five minute period. In addition to these results, using chronometer, the ratios of different behavioral reactions of the parasitoid to parasitized and unparasitized larvae were recorded. Both "stabbing" and "cocking" behaviors were not observed when presented with parasitized hosts, and for this reason superparasitism did not occur. In case of "probing" and "not searching" behaviors, no significant differences were found between parasitized and unparasitized hosts. The parasitoids spent significantly more of their time in "contact with host", "searching for host" and "cleaning" behaviors when presented with unparasitized rather than parasitized ones. However, they spent significantly more of their time in "escape from hosts" and "avoidance from host" behaviors when presented with parasitized rather than unparasitized ones.

**Key words:** foraging behavior, superparasitism, *Venturia canescens*, *Ephestia kuehniella*

**Anahtar sözcükler:** parazitlenme davranışı, süperparazitizm, *Venturia canescens*, *Ephestia kuehniella*

### Introduction

The parthenogenetic ichneumon wasp *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) is a solitary internal parasite of the caterpillars of various species of phycitid moths and has often been used in studies of

\* This study is a part of PhD. thesis of Cem ÖZKAN

\*\* Department of Plant Protection, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, Turkey

e-mail: cozkan@agri.ankara.edu.tr

Alınış (Received): 18.10. 2000

superparasitism (Salt, 1961). Many hymenopteran parasitoids are able to distinguish parasitized and unparasitized hosts, through the application of external or internal markers at oviposition (Salt, 1937; Guillot & Vinson, 1972; van Lenteren, 1976; Hubbard et al., 1987; Wolk & Mackauer, 1990; van Alphen & Visser, 1990).

Solitary wasps usually reject host that have been previously marked by themselves or by conspecifics (Harvey et al., 1993). Superparasitism occurs when a host contains more parasitoid eggs or larvae than able to develop successfully through to adult emergence (Wylie, 1965; van Lenteren, 1976; van Alphen & Nell, 1982; Waage, 1986; van Alphen, 1988; Bai & Mackauer, 1990; van Alphen & Visser, 1990; Harvey et al., 1993).

*V. canescens*, an endophagous solitary parasitoid of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is able to recognize the parasitized hosts, and consequently reject them as unsuitable sites for oviposition (Fisher, 1961). In order to be able to reject parasitized hosts, parasitoid must be able to recognize a change in the host after oviposition (Harrison et al., 1985). *V. canescens* can recognize the presence of an egg in the host within five minutes of the oviposition (Rogers, 1972).

Behavioral experiments with *V. canescens* have demonstrated that the secretions from its Dufour's gland are topically used as an external marker pheromone which affects the behavior of the other wasp towards host caterpillar bearing it. (Harrison et al., 1985).

The aim of this work presented in this study was to determine whether or not *V. canescens* is able to recognize parasitized *E. kuehniella* larvae and if the wasps recognize them, define behavioral responses of *V. canescens* to parasitized and unparasitized hosts.

## Materials and Methods

The stock of *E. kuehniella* and *V. canescens* were supplied from University of Ankara, Faculty of Agriculture, Department of Plant Protection. Both hosts and parasitoids were reared at  $25\pm 1^{\circ}\text{C}$  constant temperature with a 16:8h light and dark photoperiod and 60-70 % relative humidity.

*E. kuehniella* were reared in the clear plastic containers (27x37x7 cm) on a 2:1 mixture of wheat flour and wheat bran containing approximately 800 g food and 2000 *E. kuehniella* eggs. The eggs hatched approximately in 4 days. The moths completed five instars average 35 days after the eggs' hatching under the conditions of temperature and nutrition we used. Pupa stage continued approximately 8 days and adult moths lived approximately 8 days.

*V. canescens* were reared in the plastic containers (27x37x7 cm) containing 2000 29 day- old host larvae. 4-5 day old ten adult wasps fed with honey were placed in these containers. Development of the wasps from ovipositing to adult hatching completed approximately in 25 days. In order to separate wasps according

to age for experiment, hatching of the adults from the hosts was checked regularly 2 or 3 times everyday. All the adult wasps used in the experiment were 2-4 days-old. This was due to prevent different searching activity depending on the age and parasitization capacity of parasitoids. These adult wasps were fed with honey and water each day regularly before the experiment.

All the experiments took place at  $25\pm 1^\circ\text{C}$  with an overhead illumination. An arena was constructed by inverting a sterile petri dish (9 cm diameter) over a circular Whatman filter paper (12.5 cm diameter). One wasp was placed in the arena with one caterpillar. Foraging behavior of the wasp was observed and recorded 5 minutes. After 5 minutes another wasp was given to the caterpillar which had been parasitized once and behaviors of the wasp in petri dishes were observed and recorded for 5 minutes. Both experiment were repeated 10 times.

Fresh filter paper and sterile petri dishes were used for each trial and forceps were cleaned with 70 % alcohol before picking up the caterpillar. Throughout the trial healthy adult wasps and healthy host larvae were used. The behaviors of *Venturia canescens* on parasitized and unparasitized host larvae were recorded using one channel event recorder and sound recorder.

The behavioral patterns of *V. canescens* enumerated by Harrison et al., 1985 were used as follows:

**Stabbing:** before stabbing the ovipositor is flexed downward and forward. The ovipositor is then quickly inserted into the caterpillar like a hypodermic needle.

**Contact with host:** with either legs or antennae

**Probing:** the ovipositor is unsheathed and flexed forwards beneath the abdomen. The tip of the ovipositor is repeatedly brought into contact with the host or substrate. No insertion ovipositor occurs.

**Searching:** searching behavior involves directed locomotion. The wasp turns quickly and repeatedly, presumably in response to the odor emitting from the host, until it comes, in contact with the host. The antennae are continually vibrated in the vertical plane and their terminal segments make contact with substrate or host.

**Avoidance:** this normally occurs when a wasp comes into contact with a previously parasitized and on a contact attempts to fly or host.

**Escape:** the wasp becomes positively phototactic and ceases directed searching behavior on the floor of the petri dishes. A wasp walking on the sides and top of the inverted petri dish is counted as attempting to escape from the arena.

**Cleaning:** cleaning behavior is commonly observed directly after egg-laying or after contacting on already parasitized host. The antennae, legs and ovipositor are groomed. This process may involve receptor cleaning, especially on the antennae and ovipositor.

**Cocking:** cocking can be observed prior to egg-laying or after an egg has just been laid. The ovipositor is swung above the abdomen in a characteristic movement and then returned to its normal position. The action positions a single egg in a groove at the tip of ovipositor in preparation for egg laying (Rogers, 1972).

**Not searching:** this is brood category including two main types of behavior pattern. The first is resting when the wasp remains still. This is more often observed after egg-laying and cleaning. The second is when the wasp walk around the area in a slow, undirected manner in contrast to searching behavior which is more active and directed movement.

## Results and Discussion

From the sound recorder the average values for the ten replicates were calculated for the percentage of the total time spent on each of the behavior replicates when the wasp was attacking parasitized and unparasitized host larvae. These values were showed in table 1.

Table 1. Average values for percentage of total exposure time (5 min.) spent performing each behavior pattern

Behavior	Unparasitized host		Parasitized host	
	Mean % time	S.D. (Min.- Max.)	Mean % time	S.D. (Min.- Max.)
Stabbing	0.320	(0.20 - 0.60)	-	
Contact*	5.535 ± 0.38	(4.00 - 7.80)	1.424 ± 0.21	(0.65 - 2.65)
Probing	8.725 ± 0.94	(5.34 - 14.87)	8.241 ± 0.72	(4.27 - 12.27)
Searching*	25.851 ± 1.70	(17.35 - 31.05)	3.750 ± 0.61	(1.68 - 8.12)
Avoidance*	0.050 ± 0.02	(0.00 - 0.20)	9.012 ± 0.82	(5.08 - 13.41)
Escape*	10.679 ± 1.29	(4.87 - 17.36)	42.519 ± 5.58	(35.99 - 50.33)
Cleaning*	21.013 ± 1.49	(16.18 - 31.10)	6.242 ± 0.88	(3.12 - 12.10)
Coding	0.148	(0.11 - 0.20)	-	
Not searching	27.679 ± 3.05	(10.35 - 42.43)	28.812 ± 1.68	(20.77 - 37.03)

\* =Significant at the 95 % level of probability.

In the experiments, the wasps parasitized all the unparasitized host larvae within five minutes oviposition period. However, after five minutes from the previous parasitization they rejected the parasitized host larvae within five minutes oviposition period. This result shows that *V. canescens* is able to recognize parasitized hosts. In addition to this result, different behavioral responses to parasitized and unparasitized larvae were recorded.

Both "stabbing" and "cocking" behaviors engaged only a very small percentage of the total time when presented with unparasitized hosts. However, these two behaviors were not recorded when presented with parasitized hosts. That's why a t-test analysis cannot be prepared on zero data and therefore no statistical evidence is available for the two behaviors.

The behavior "not searching" occupied about one fourth of the total time for both parasitized and unparasitized hosts, and no significant differences were found between the two treatments. Similarly, in the behavior "probing" no significant differences were found between parasitized and unparasitized hosts.

The parasitoids exhausted significantly more of their time in "contact with hosts", "searching for hosts" and "cleaning" behaviors when presented unparasitized rather than parasitized ones. However, they exhausted less of their time in "avoidance" and "escape" behaviors when presented unparasitized rather than parasitized ones.

The entire wasps in the experiments recognized the parasitized hosts within five minutes of oviposition and therefore superparasitism was not occurred. *V. canescens* can detect the presence of an egg in the host within 5 minutes of oviposition (Rogers, 1972). *V. canescens* is able to recognize the parasitized hosts and consequently rejects them as an unsuitable sites for oviposition (Fisher, 1961).

Both "stabbing" and "cocking" behaviors were not seen when parasitized hosts were presented. However, these two behaviors were observed when unparasitized hosts were presented. To be able to discriminate the host, the parasitoid must be able to recognize a change in the host after parasitization (Harrison et al., 1985). Many hymenopteran parasitoids are able to distinguish between parasitized and unparasitized hosts, through the application of external or internal markers at oviposition (Salt, 1937; Guillot & Vinson, 1972; van Lenteren, 1976; Hubbard et al., 1987; Wolk & Mackauer, 1990; van Alphen & Visser, 1990). This marking may be accomplished in several ways. The wasp may deposit an external pheromone on the host in its vicinity preceding, during or after oviposition, or wasp may inject an internal marker pheromone into the host during oviposition. Alternatively, the developing egg may give off a substance, or there may be a concentration change of body fluids, or the formation of "new" substance by the host as a reaction to parasitization (van Lenteren 1976, 1981).

Harrison et al. (1985) have demonstrated that the secretions from its Dufour's gland are used as an external pheromone which can affect the behavior of other wasp towards *E. kuehniella* larvae bearing it.

From the behavioral observations with *V. canescens*, it can be concluded that the high capacity of "avoidance", "escape" and "not searching" behaviors may aid in avoiding "contact" behavior when presented with parasitized host, and therefore reduce the incidence of superparasitism. Conversely, the high capacity of "searching" behavior may aid in increasing "contact" behavior when presented with unparasitized host and consequently increase the parasitization.

In the behavior "probing", no significant difference was found between parasitized and unparasitized hosts. In fact, while "probing" behavior was mostly observed on the body of hosts when unparasitized host was presented, it was mostly observed on the substrate (petri dishes and Whatman filter paper) when parasitized host was presented. The reason that the wasp prefer the substrate for

“probing” when parasitized host was presented is because of recognizing and rejecting the parasitized hosts.

The wasp spent much more time in “cleaning” behavior when unparasitized hosts were presented rather than parasitized ones. The reason that the wasp spending much more time for “cleaning” behavior when presented with unparasitized host is because of spending much more time for “contact” behavior with the unparasitized ones.

## Özet

### *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) in parasitized ve parazitlenmemiş konukçulara davranışsal tepkileri

*Venturia canescens* (Gravenhorst) beş dakikalık bir süre içerisinde parazitlenmemiş *Ephestia kuehniella* Zeller larvalarını parazitlemiştir. Fakat parazitoitler beş dakika önce parazitlenmiş konukçuların parazitli olduklarını algılamış ve parazitlemek için ret etmiştir. Ayrıca kronometre yardımı ile parazitoitin parazitlenmiş ve parazitlenmemiş konukçulara olan farklı davranış reaksiyonlarının oranları belirlenmiştir. Parazitlenmiş konukçularda “konukçuyu delme” ve “yumurtanın konukçuya hazırlanışı” davranışları gerçekleşmemiş ve süperparazitizm görülmemiştir. “Sondalama” ve “konukçuyu araştırma” davranışları bakımından parazitlenmiş ve parazitlenmemiş konukçularda istatistiki bakımdan önemli bir fark bulunamamıştır. Parazitoitler parazitlenmemiş konukçularda parazitlenmiş konukçulara göre “konukçu ile temas”, “konukçuyu araştırma” ve “temizlenme” davranışları bakımından daha fazla zaman harcamıştır. Ancak parazitoitler parazitlenmiş konukçularda parazitlenmemiş konukçulara göre “konukçudan sakınma” ve “konukçudan kaçma” davranışları bakımından daha fazla zaman harcamıştır.

## Acknowledgement

Special thanks are extended to Dr. Aziz Karakaya (Department of Plant Protection, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, Turkey) for proofreading the manuscript.

## Reference

- Bai, B. & M. Mackauer, 1990. Self and conspecific host discrimination by the aphid parasitoid *Alphelinus asychis* Walker (Hymenoptera: Aphelinidae). **Can. Entomol.**, **122**: 363-372.
- Fisher, R.C., 1961. A study in insect multiparasitism. **I. J. Exp. Biol.**, **38**: 267-275.
- Guillot, F.S. & S.B. Vinson; 1972. Sources of substances which elicit a behavioral response from the insect parasitoid. *Campoletis perdistinctus*. **Nature**, **235**: 169-170.
- Harrison, E.G., R.C. Fisher & K.M. Ross, 1985. The temporal effects of Dufour’s gland secretion in host discrimination by *Nemeritis canescens*. **Entomol. Exp. Appl.**, **38**: 215-220.
- Harvey, J.A., I.F. Harvey & D.J. Thompson, 1993. The effect of superparasitism on development of the solitary parasitoid wasp, *Venturia canescens* (Hymenoptera; Ichneumonidae). **Ecol. Entomol.**, **18**: 203-208.
- Hubbard, S.F., G. Marris, A. Reynolds & G.W. Rowe, 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. **J. Anim. Ecol.**, **56**: 387-401.

- Rogers, D.J., 1972. The ichneumon wasp *Venturia canescens*: oviposition and avoidance of superparasitism. **Entomol. Exp. Appl.**, **15**: 190-191.
- Salt, G., 1937. The sense used by Trichogramma to distinguish between parasitized and unparasitized hosts. **Proc. of the Royal Entomol. Soc. of London**, **122**: 57-75.
- Salt, G., 1961. Competition among insect parasitoids. **Sym. Soc. Exp. Biol.**, **15**: 96-119.
- Salt, G., 1976. The host of *Nemeritis canescens* a problem in the host specificity on insect parasitoids. **Ecol. Entomol.**, **1**: 63-67.
- van Alphen, J.J.M. & H.W. Nell, 1982. Superparasitism and host discrimination by *Asobara tabida* Ness (Braconidae: Alysiinae), a larval parasitoid of Drosophilidae. **Netherlands J. of Zool.**, **32**: 232-260.
- van Alphen, J.J.M., 1988. Patch Time Allocation by Insect Parasitoids: Superparasitism and Aggregation. Population Genetics and Evolution (ed. by G. de Jong), pp. 215-221. Springer Berlin, 305 pp.
- van Alphen, J.J.M. & M.E. Visser, 1990. Superparasitism as an adaptive strategy for insect parasitoids. **Ann. Rev. of Entomol.**, **35**: 232-260.
- van Lenteren, J.C., 1976. The development of host discrimination and the prevention of superparasitism in the parasite *Pseudocoila hochei* Weld (Hymenoptera: Cynipidae). **Netherlands J. of Zool.**, **26**: 1-83.
- van Lenteren, J.C., 1981. Host Discrimination by Parasitoids. In: D.A. Nordlund. (ed.) Semiochemicals: Their Role in Pest Control, pp 153-179. Wiley. New York, 306 pp.
- Volkl, W. & M. Mackauer, 1990. Age-specific pattern of host discrimination by the aphid parasitoid *Ephedrus californicus* Baker (Hymenoptera: Aphidiidae). **Can. Entomol.**, **122**: 349-361.
- Waage, J.K., 1986. Family Planning in Parasitoids: Adaptive Patterns of Progeny and Sex Allocation. in: Insect Parasitoids (ed. by J.K. Waage and D. Greathead), pp. 63-95. Academic Press, London, 389pp.
- Wylie, H.G., 1965. Effects of superparasitism on *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). **Can. Entomol.**, **97**: 326-331.