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Carabidae as natural enemies of the raspberry beetle (*Byturus tomentosus* F.)

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Abstract

The raspberry beetle (*Byturus tomentosus* F.) is a widespread pest of raspberry in Europe. Although it is being controlled chemically, alternative strategies should be developed, based on biological control by polyphagous predators. Laboratory feeding tests were used to investigate the role of raspberry beetle larvae in the diet of the carabid species, *Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* and *Harpalus rufipes*. In no-choice and choice feeding tests with aphids or raspberry beetle larvae *P. melanarius* and *P. niger* preferred to consume raspberry beetle larvae. *C. nemoralis* quickly consumed both prey items. *H. rufipes* preferred to eat raspberry beetle larvae to *Thlapsi arvense* seeds. According to the consumed biomass of each food item, larvae of the raspberry beetle were preferred by each carabid species tested. Data reported here clearly indicate that large carabids − *P. melanarius*, *P. niger*, *C. nemoralis* and *H. rufipes* could play an important role in regulating raspberry pest populations.

Key words: biological control, *Carabidae*, aphid, *Byturus tomentosus* larvae, prey preference.

Introduction

Carabids are the most common predatory insects and important natural enemies of many pests on agricultural crops (Thiele, 1977), including horticultural crops. Most species are generalist predators, consuming both animal and plant material: leaves, fruits, pollen, seeds and fungi (Toft, Bilde, 2002). Considering the predatory polyphagous nutrition of carabids, they play a key role in agro-ecosystems as natural biological control agents (Kromp, 1999).

Several studies have shown that carabids, in sufficient densities, can be efficient predators of aphids as well as the egg and larval stages of *Coleopteran*, *Lepidopteran* and *Dipteran* pests (Thiele, 1977; Sorokin, 1981; Riddick, Mills, 1996; Kromp, 1999; Büchs, 2003; Horgan, Myers, 2004). A review of manipulative field studies showed that, in approximately 75% of cases, pest numbers were reduced significantly by these generalist predators (Symondson et al., 2002).

In Northern Europe, the raspberry beetle (*Byturus tomentosus* F.) is the most serious pest attacking raspberries with yield losses up to 50% depending on cultivar (Tuovinen, 1997; Hanni, Luik, 2001). The damage is mainly caused by larvae browsing on the drupes of young fruits, resulting in discoloured or contaminated ripe fruits, which leads to the rejection or down-grading of the crop. Due to their hidden lifestyle, raspberry beetle larvae are safe from predatory arthropods during their development in raspberries. But, after feeding in the berries, they drop to the soil for pupation and are then possible targets for carabids and other ground living predators. Aphids are also important and a highly preferred prey type for carabids in many crops (Ekbom et al., 1992; Kielty et al., 1999) but in raspberry, they are less important pests in North European outdoor conditions (Gordon et al., 1997).

There have been few studies on interactions between carabids and raspberry beetle larvae. Carabids are common ground living predators in raspberry plantations (Luik et al., 2000; Hanni, Luik, 2002) with the species *Pterostichus melanarius* (Illiger), *P. niger* (Schaller), *Carabus nemoralis* Mueller and *Harpalus rufipes* (DeGeer) being common and most numerous in northern Europe. The seasonal abundance of these species, in addition to the high number of individuals, is related to the ripening time of raspberry fruits which coincides with the raspberry beetle larval stage (Arus et al., 2011). *P. melanarius*, *P. niger*, *C. nemoralis* are mainly carnivorous (Thiele, 1977; Pollet, Desender, 1985). *H. rufipes* has previously been reported as a biocontrol agent of various pests (Kromp, 1999) and is an effective seed eater (Tooley et al., 1999).

The aim of the present study was to explore with laboratory feeding experiments the potential of these carabid species for control of the raspberry beetle. Two types of feeding experiments were carried out: no-choice and choice tests. In no-choice tests the numbers of different prey items consumed by the carabids over certain time periods were measured. The choice feeding tests, offering a mixture of food items, were carried out to establish carabid preferences for the different food items.

Materials and methods

The feeding experiment was carried out in 2006. For feeding experiments, the carabid beetles (*Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* and *Harpalus rufipes*) were caught with dry pitfall traps (cups 9 cm in diameter and 12 cm deep) in a raspberry plantation at Polli Horticultural Research Centre, in South Estonia (latitude 58°7′ N and 25°33′ E). The traps were checked every 24 h; as the beetles are nocturnal this was done early each morning to avoid the beetles being in the traps for too long. The captured beetles were kept in a laboratory for 24 hours without food before the experiments to standardize their hunger state. They were then placed individually in small containers with water supplied on soaked cotton wool in the shade at room temperature (20ºC). The prey items, i.e. raspberry beetle fourth instar larvae (with an average biomass of 4.46 ± 0.171 mg), the large raspberry aphid *Amphorophora idaei* (Börner) (0.63 ± 0.029 mg) and dry seeds of *Thlapsi arvense* L. (1.1 ± 0.017 mg), were collected at the same locality as the beetles directly before each experiment. Average raspberry beetle larvae biomass was determined by weighing 3 batches of 160 larvae, average aphids mass was determined by weighing 3 batches of 80 aphids and average seed mass was determined by weighing 4 batches of 400 seeds.

In the no-choice feeding tests, *P. niger*, *P. melanarius*, *C. nemoralis* carabids were presented with either 10 raspberry beetle larvae or 10 aphids while the *H. rufipes* carabids were presented with either 10 raspberry beetle larvae or 10 *Thlapsi arvense* seeds.

In the choice feeding tests, *P. melanarius*, *P. niger* and *C. nemoralis* carabids were presented with 10 raspberry beetle larvae together with 10 aphids while *H.rufipes* carabids were presented with 10 raspberry beetle larvae together with 10 seeds.

A total of 135 carabids of each species were tested in each type of feeding test. All tests were made in 9 replicates. Each carabid was tested only once; it was placed together with the prey items on moist filter paper in a Petri dish at room temperature (20ºC). Tests were conducted in the dark as carabids are night active and terminated after different periods of time: 1, 2, 3, 6 and 12 hours. After the specified time, dishes were taken into the light and the number of prey items consumed was recorded.

The mean number of items consumed per defined time unit and its standard error were calculated and the significance of data differences determined using the Tukey-Kramer's test.

Results

No-choice feeding tests. *P. melanarius*, *P. niger* and *C. nemoralis* consumed most of the beetle larvae during the first hour of the experiment, whereas most of the aphids were eaten two to three hours after the experiment started. *H. rufipes* at the same time interval had eaten less food.

P. melanarius had consumed significantly more beetle larvae than aphids after both the first and second hours (Tukey-Kramer's test, $p < 0.001$ and $p = 0.01$). According to the biomass of the food, beetle larvae were consumed significantly more ($p < 0.001$) than aphids at every control point.

P. niger, *C. nemoralis* and *H. rufipes* had consumed similar numbers of the two food items. According to the biomass of the food, beetle larvae were consumed significantly more than aphids at every control point (Table 1).

Table 1. Mean number and biomass (± standard error) of raspberry beetle larvae, aphids or seeds consumed by the carabids *Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* and *Harpalus rufipes* when presented in no-choice tests for different lengths of time

Carabid species	Feeding time h	Number of food items eaten				Biomass (mg) of food items eaten			
		Larvae of *Byturus tomentosus*	*Amphorophora idaei*	*p*		Larvae of *Byturus tomentosus*	*Amphorophora idaei*	*p*	
1	2	3	4	5	6	8	9	10	11
Pterostichus melanarius	n	10	10			44.6	6.0		
	1	9.2 ± 0.68	2.2 ± 1.4	0.0006	***	40.9 ± 7.14	1.4 ± 1.25	<0.0001	***
	2	9.7 ± 0.72	4.7 ± 1.02	0.01	**	43.1 ± 3.64	2.8 ± 0.92	<0.0001	***
	3	10.0 ± 1.13	7.7 ± 1.6	0.6427	ns	44.6 ± 0.00	4.6 ± 1.39	0.0004	***
	6	10.0 ± 1.04	9.3 ± 1.48	0.982	ns	44.6 ± 0.00	5.6 ± 0.35	<0.0001	***
	12	10.0 ± 1.01	10.0 ± 1.42	1.000	ns	44.6 ± 0.00	6.0 ± 0.00	<0.0001	***
P. niger	n	10	10			44.6	6.0		
	1	8.8 ± 0.76	6.0 ± 0.88	0.1526	ns	39.0 ± 8.44	3.6 ± 1.04	0.0031	**

Table 1 continued

1	2	3	4	5	6	8	9	10	11
	2	9.0 ± 1.22	7.7 ± 1.41	0.8896	ns	40.1 ± 8.92	4.6 ± 0.92	0.0038	**
	3	9.0 ± 0.89	8.3 ± 1.03	0.9596	ns	40.1 ± 8.92	5.0 ± 1.25	0.0037	**
	6	9.0 ± 0.59	10.0 ± 0.68	0.6963	ns	40.1 ± 8.92	6.0 ± 0.00	0.0046	**
	12	10.0 ± 1.01	10.0 ± 1.42	1.000	ns	44.6 ± 0.00	6.0 ± 0.00	<0.0001	***
Carabus nemoralis	n	10	10			44.6	6.0		
	1	8.7 ± 1.27	5.0 ± 1.27	0.2349	ns	38.6 ± 2.57	3.0 ± 1.2	0.0003	***
	2	9.0 ± 1.64	9.7 ± 1.64	0.9912	ns	40.1 ± 4.46	5.8 ± 0.35	0.0053	**
	3	9.7 ± 0.64	10.0 ± 0.64	0.9822	ns	43.1 ± 2.57	6.0 ± 0.00	0.0016	**
	6	10.0 ± 0.72	10.0 ± 0.72	1.000	ns	44.6 ± 0.00	6.0 ± 0.00	<0.0001	***
		Larvae of *Byturus tomentosus*	Seeds of *Thlapsi arvense*			Larvae of *Byturus tomentosus*	Seeds of *Thlapsi arvense*		
Harpalus rufipes	n	10	10			44.6	11.0		
	1	3.7 ± 0.91	0.3 ± 1.39	0.2284	ns	16.6±14.94	0.4 ± 0.64	0.029	*
	2	4.7 ± 1.17	3.7 ± 1.79	0.9600	ns	21.0±15.38	4.0 ± 4.17	0.029	*
	3	6.0 ± 1.24	4.7 ± 1.9	0.9341	ns	26.7±17.64	5.1 ± 4.17	0.018	*
	6	7.0 ± 1.08	8.0 ± 1.64	0.9554	ns	31.2±16.84	8.8 ± 2.21	0.012	*
	12	8.1 ± 0.91	10.0 ± 1.39	0.6859	ns	36.3±12.99	11.0 ± 0.00	0.021	*

*, **, *** – significance level, ns – not significant, Tukey-Kramer's test

Choice feeding tests. In the choice feeding tests, the quantity of consumed food items was less than in the no-choice feeding tests.

For *P. melanarius* and *P. niger* a significant preference for beetle larvae was seen in the choice feeding tests after the 1st hour ($p = 0.0248$ and $p = 0.0427$). Later such significant preferences for the larvae were lost. According to the biomass of the prey, larvae were preferred to the aphids at every control point.

C. nemoralis was at first confused in choice tests and consumed less food than in no-choice tests. In choice tests, there was no significant preference between consumed food items. According to the biomass of the prey, a significant preference for beetle larvae occurred after three hours.

H. rufipes showed a clear significant preference for beetle larvae in choice tests after three hours. According to the biomass of the larvae and seeds, the larvae were consumed significantly more.

Thus choice feeding tests gave clear evidence that *P. melanarius*, *P. niger*, *C. nemoralis* and *H. rufipes* prefer to consume raspberry beetle larvae over aphids.

Table 2. Mean number and biomass (± standard error) of raspberry beetle larvae or aphids/seeds consumed by the carabids *Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* and *Harpalus rufipes* in choice feeding tests after different time periods when offered a mixture of food items

Carabid species	Feeding time h	Number of food items eaten				Biomass (mg) of food items eaten			
		Larvae of *Byturus tomentosus*	*Amphorophora idaei*	*p*		Larvae of *Byturus tomentosus*	*Amphorophora idaei*	*p*	
1	2	3	4	5	6	7	8	9	10
Pterostichus melanarius	n	10	10			44.6	6.0		
	1	3.0 ± 1.01	0.33 ± 0.96	0.0248	*	13.4 ± 4.46	0.2 ± 0.35	0.0355	*
	2	4.7 ± 2.52	0.66 ± 1.02	0.055	ns	20.8 ± 11.22	0.4 ± 0.35	0.0346	*
	3	6.3 ± 0.58	4.67 ± 1.6	0.6044	ns	28.2 ± 2.57	2.8 ± 2.84	0.0003	***
	6	9.7 ± 0.58	8.3 ± 1.48	0.2667	ns	43.1 ± 2.57	5.0 ± 0.92	0.0005	***
	12	10.0 ± 0.00	10.0 ± 1.42	0.9987	ns	44.6 ± 0.00	6.0 ± 0.00	<0.0001	***
P. niger	n	10	10			44.6	6.0		
	1	7.0 ± 0.88	3.0 ± 0.88	0.0427	*	31.2 ± 4.46	1.8 ± 0.6	0.0068	**
	2	9.3 ± 1.41	6.7 ± 1.41	0.5665	ns	41.6 ± 5.14	4.0 ± 2.50	0.0017	**
	3	10.0 ± 1.03	8.0 ± 1.03	0.5447	ns	44.6 ± 0.00	4.8 ± 1.2	0.0003	***
	6	10.0 ± 0.68	9.7 ± 0.68	0.9851	ns	44.6 ± 0.00	5.8 ± 0.35	<0.0001	***
	12	10.0 ± 0.00	10.0 ± 0.00	1.000	ns	44.6 ± 0.00	6.0 ± 0.00	<0.0001	***
Carabus nemoralis	n	10	10			44.6	6.0		
	1	1.3 ± 1.1	3.8 ± 1.1	0.4154	ns	5.6 ± 6.68	2.3 ± 1.98	0.4016	ns
	2	4.5 ± 1.42	5.8 ± 1.42	0.9222	ns	22.6 ± 16.17	3.5 ± 2.42	0.0978	ns

Table 2 continued

1	2	3	4	5	6	7	8	9	10
	3	7.3 ± 0.56	8.5 ± 0.56	0.4277	ns	32.3 ± 4.27	5.1 ± 1.04	0.0006	***
	6	8.3 ± 0.62	8.8 ± 0.62	0.9394	ns	36.8 ± 5.61	5.3 ± 1.14	0.0011	**
	12	9.3 ± 0.41	10.0 ± 0.41	0.5883	ns	41.2 ± 6.69	6.0 ± 0.00	0.0018	**
		Larvae of *Byturus tomentosus*	Seeds of *Thlapsi arvense*	p		Larvae of *Byturus tomentosus*	Seeds of *Thlapsi arvense*	p	
Harpalus rufipes	n	10	10			44.6	11.0		
	1	5.3 ± 1.39	0.3 ± 1.39	0.1024	ns	23.8 ± 2.57	0.4 ± 0.64	0.0026	**
	2	7.3 ± 1.79	0.7 ± 1.79	0.0886	ns	32.7 ± 11.21	0.7 ± 1.28	0.037	*
	3	8.7 ± 1.9	0.7 ± 1.9	0.0493	*	38.6 ± 6.81	0.7 ± 1.28	0.0088	**
	6	9.7 ± 1.64	1.3 ± 1.64	0.0171	*	43.1 ± 2.57	1.5 ± 1.28	0.00016	***
	12	10.0 ± 1.39	3.7 ± 1.39	0.0322	*	44.6 ± 0.00	4.4 ± 2.92	0.0018	**

*, **, *** – significance level, ns – not significant, Tukey-Kramer's test

Discussion

Carabid species vary in their foraging preferences. Lindroth (1992) considered that the genus *Pterostichus* consumes chiefly animal food. It has been reported that *P. niger*, *P. melanarius*, *C. nemoralis*, and even *H. rufipes* might be efficient predators of larval stage of *Leptinotarsa decemlineata* (Thiele, 1977; Sorokin, 1981). The activity of *Pterostichus* spp. beetles coincided with the time interval in which fifth-instar *Cydia pomonella* larvae wander on the ground prior to pupation (Riddick, Mills, 1996), indicating their role in control of *Cydia pomonella. Pterostichus* spp. also has consumed winter moth (*Oteropthera brumata* L.) pupae (Horgan, Myers, 2004). In our feeding experiments, *P. melanarius* and *P. niger* liked to eat raspberry beetle larvae. This explains their higher abundance in raspberry plantations during berry ripening time when raspberry beetle larvae are available to them as prey items.

Species of the genus *Harpalus* are mostly generalists, accepting a wide range of species of seed (Honek et al., 2007), preferring *Viola* and *Cirsium* seeds (Thiele, 1977; Jorgensen, Toft, 1997; Honek et al., 2007), as well as insect prey. There are reports of *H. rufipes* as a predator of cabbage root fly eggs and larvae (Coaker, Williams, 1963) and *Pieris rapae* larvae (Dempster, 1967). Büchs (2003) and Warner et al. (2000) reported *H. rufipes* as a biocontrol agent for the larvae of the brassica pod midge in oilseed rape. Holopainen and Helenius (1992) concluded that this carabid contributed to the suppression of *Rhopalosiphum padi* L. populations in spring barley. Monzo et al. (2011) found that *H. rufipes* play an important role in regulating medfly populations in citrus orchards. In our choice feeding tests, *H. rufipes* significantly preferred raspberry beetle larvae to *Thlapsi arvense* seeds. This indicates that, if raspberry beetle larvae are available, as they are at raspberry ripening time in plantations, they could be preferably eaten by *H. rufipes* beetles. Probably some volatile signal compounds from raspberry beetle larvae which drop to the soil during raspberry ripening time attract migration of *H. rufipes*, *P. melanarius* and *P. niger* to the raspberry plantation indicating a good food source.

In our feeding tests, *C. nemoralis* ate raspberry beetle larvae and aphids at an equivalent level and did not show a significant preference.

The impact on raspberry beetle larvae is not necessarily associated with a high preference for larvae. Fawki et al. (2005) reported that for *P. melanarius* and *C. nemoralis* insects are high-quality, earthworms are intermediate, and slugs and seeds are low quality food for these species, whereas for *C. nemoralis*, earthworms are the preferred prey. But, it is important to note that pure diets of all prey types are nutritionally incomplete and most predators can improve their fitness and fecundity by choosing a mixed diet (Toft, 1996; Saska, 2008). Different authors have studied carabid diet and aphid control potential in the laboratory and field (Ekbom et al., 1992; Kielty et al., 1999). Even if aphids are important food for several polyphagous predators, a diet of aphids alone provides a low-quality food for a wide range of generalist insectivores (Bilde, Toft, 1994; Jorgensen, Toft, 1997).

Further, animal food in the diet of carabids could be dependent on season. For example, the intestines of *P. cupreus* contained 67% plant material in spring but at the end of summer insect material dominated (80%) (Thiele, 1977). That is apparently influenced by the greater availability of insects as a food source in summer. In raspberry plantations, aphids are available for a longer time in the vegetation period. The aggregation of *H. rufipes*, *P. melanarius*, *P. niger* and *C. nemoralis* during berry ripening time when raspberry beetle larvae drop to the soil for pupation (Arus et al., 2011) suggests that they are concentrating at the available food source. Our feeding experiments confirmed this. In choice feeding tests, *H. rufipes*, *P. melanarius* and *P. niger* significantly preferred raspberry beetle larvae to aphids. *C. nemoralis* had a tendency to consume more raspberry beetle larvae than aphids in no-choice tests.

Conclusion

These carabid beetles (*Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* and *Harpalus rufipes*) as a group of generalist predators, have, through their feeding preferences, the potential to make a significant contribution to raspberry beetle control thereby reducing the raspberry beetle population. In future, it is important to develop raspberry growing technologies which enhance the occurrence of carabids in plantations. In recent years, integrated pest management systems should focus on developing ecological practice in which the utilization of natural enemies is an important issue in the control of pests.

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Žygiai (*Carabidae*) – natūralūs paprastojo avietinuko (*Byturus tomentosus* F.) priešai

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Santrauka

Paprastasis avietinukas (*Byturus tomentosus* F.) yra Europoje plačiai paplitęs aviečių kenkėjas. Nors jo paplitimas kontroliuojamas cheminėmis priemonėmis, reikėtų kurti alternatyvias strategijas, paremtas biologine kontrole – polifaginiais plėšrūnais. Laboratorinių tyrimų metu siekta ištirti paprastojo avietinuko lervų reikšmę žygių rūšių *Pterostichus melanarius*, *P. niger*, *Carabus nemoralis* ir *Harpalus rufipes* mitybai. Tyrimų metu su ir be pasirinkimo pateikus amarus arba paprastojo avietinuko lervas, žygių *P. melanarius* bei *P. niger* rūšys teikė pirmenybę paprastojo avietinuko lervoms. *C. nemoralis* greitai suvartojo abi maisto rūšis. *H. rufipes* teikė pirmenybę paprastojo avietinuko lervoms, o ne *Thlapsi arvense* sėkloms. Pagal kiekvienos maisto rūšies suvartotą biomasę visos tirtos žygių rūšys teikė pirmenybę paprastojo avietinuko lervoms.
Straipsnyje pateikti duomenys rodo, kad didieji žygiai – *P. melanarius*, *P. niger*, *C. nemoralis* ir *H. rufipes* – galėtų būti reikšmingi reguliuojant aviečių kenkėjų populiacijas.

Reikšminiai žodžiai: biologinė kontrolė, *Carabidae*, amaras, *Byturus tomentosus* lervos, pirmenybės teikimas maistui.