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The diversity of species of Ceutorhynchinae captured in traps in the region of Sofia, Bulgaria

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Abstract

Sticky PALs and "hat" KLP+ traps (CSALOMON®) were used for studying species diversity of Ceutorhynchinae weevils (Coleoptera Curculionidae). The catches in transparent and yellow, and in unbaited and baited with 2-phenylethyl isothiocyanate traps were compared. A total of 17 species from the subfamily Ceutorhynchinae were trapped in Sofia Basin area in 2006 and 2007. They belonged to the six genera: *Amalus*, *Ceutorhynchus*, *Microplontus*, *Rhinoncus*, *Sirocalodes* and *Thamiocolus*. Several economic pests on cruciferous crops including *Ceutorhynchus pallidactylus* (Marsham), *Ceutorhynchus obstrictus* (Marsham), *Ceutorhynchus napi* Gyllenhal, *Ceutorhynchus picitarsis* Gyllenhal and *Ceutorhynchus erysimi* (F.), were found in low numbers. Fourteen weevil species are newly recorded for the Sofia Basin area. The most abundant species was *Ceutorhynchus typhae* (Herbst). Significantly high number of adults of this species was found in yellow sticky traps. The number of the beetles caught was not affected by the presence or absence of the chemical cue, 2-phenylethyl isothiocyanate. We suggest that yellow PALs traps can be effective tools for faunistic researches on Ceutorhynchinae weevils in various habitats and for detection and seasonal monitoring of harmful *Ceutorhynchus* spp.

Key words: Ceutorhynchinae, catch composition, trap design, 2-phenylethyl isothiocyanate, *Ceutorhynchus typhae*, Bulgaria.

Introduction

The Ceutorhynchinae weevils are relatively small beetles with body length ranging from 1.3 to 7 mm (mostly 2-3 mm long), and distributed almost worldwide (Korotyaev, 2006). They are associated with the seed-bearing plants: gymnosperms (Ephedraceae and Gnetaceae) and angiosperms (Amaranthaceae, Apiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Ericaceae, Fabaceae, Fagaceae, Geraniaceae, Haloragaceae, Iridaceae, Lamiaceae, Liliaceae, Linaceae, Myricaceae, Onagraceae, Oxalidaceae, Papaveraceae, Polygonaceae, Portulacaceae, Primulaceae, Resedaceae, Rosaceae, Salicaceae, Scrophulariaceae, Saxifragaceae, Urticaceae) (Angelov, 1979; Poiras, 1998; Bürki *et al*., 2001; Colonnelli, 2004; Tóth and Cagán, 2005; Korotyaev, 2006; Majka *et al*., 2007a; 2007b). More than one third of the existing Ceutorhynchinae are monophagous or oligophagous on Brassicaceae species (Korotyaev, 2006). Several *Ceutorhynchus* are among the most important pests on cruciferous crops as their larvae and adults damage plants. Larvae of members of this genus tunnel into stems [*Ceutorhynchus napi* Gyllenhal 1837, *Ceutorhynchus pallidactylus* (Marsham 1802) and leaf stalks (*Ceutorhynchus picitarsis* Gyllenhal 1837), feed on seeds within pods of the host plants (*Ceutorhynchus obstrictus* (Marsham 1802)] or cause root-gall formation [*Ceutorhynchus assimilis* (Paykull 1800)]. *Ceutorhynchus* adults gnaw leaves, stems, buds and flowers of the cruciferous plants (Grigorov, 1972). Some Ceutorhynchinae species feed on weeds and therefore are used or considered as biological control agents against weeds (Peschken and Wilkinson, 1981; Bürki *et al*., 2001; Colpetzer *et al*., 2004; Korotyaev, 2006; Newman *et al*., 2006; Gerber *et al*., 2007; Visalakshy, 2007).

One of the way for studying species diversity and distribution of insects, and in particular Ceutorhynchinae beetles, is trap catching. Ceutorhynchinae could be attracted either by chemical or visual (colour) stimuli, which are used by many herbivorous insects in host location process (Prokopy and Owens, 1983). Among Ceutorhynchinae species, field attraction to isothiocyanates, biologically active derivates found in several plant families including Brassicaceae (Fahey *et al.*, 2001), is reported for the cabbage seed weevil *C. obstrictus* (Smart *et al.*, 1993; Smart and Blight, 1997; Smart *et al*., 1997, all under *C. assimilis*), *C. pallidactylus* and *C. napi* (Walczak *et al*., 1998). During field screening of synthetic isothiocyanates, attraction of several species of *Ceutorhynchus* to single isothiocyanates or their mixtures has been found in Hungary (M. Tóth, unpublished data).

The aim of this study was to establish the species composition of Ceutorhynchinae, using different traps, aside of a field where cabbage (*Brassica oleracea* L.) crop was grown in the previous years. In addition, for the most abundant species *Ceutorhynchus typhae* (Herbst 1795), the preference to different kinds of traps and the seasonal flight were also investigated.

Materials and methods

Field investigations were conducted in the Experimental Station of University of Forestry, "Vrazhdebna", in Sofia. Experiments were arranged at the border between alfalfa (*Medicago sativa* L.) field (0.5 ha) and a field with mixed crop of oat (*Avena sativa* L.) and common wetch (*Vicia sativa* L.) (1 ha). Cabbage was grown in 2005-2006 as a second crop after mixed crop harvest. The main weed species in the study area were *Capsella* *bursa-pastoris* (L.) Medik., *Sinapis* sp. (Brassicaceae), *Amaranthus retroflexus* L. (Amaranthaceae), all possible Ceutorhynchinae hosts (Angelov, 1979; Bürki *et al*., 2001), *Stellaria* sp. (Caryophyllaceae), *Erodium* sp. (Geraniaceae), *Veronica* sp. (Scrophulariaceae), *Hordeum murinum* L. and *Dactylis glomerata* L. (Poaceae).

In 2006, two types of traps, yellow sticky CSALOMON® PALs traps (Tóth *et al*., 2003) (figure 1) and CSALOMON® KLP+ "hat" traps (Tóth *et al*., 2006) (figure 2), baited with 2-phenylethyl isothiocyanate (100 µl) were used. A small piece (1 cm x 1 cm) of insecticide with active ingredient 20% dichlorvos (Compack de RT, Hungary) was placed in the collection container of the KLP+ traps as a killing agent. Traps were placed at soil level suspended on wood stakes. They were grouped in five blocks. Each block consisted of one PALs and one KLP+ traps, both with chemical lure. The distance between the traps in a block was 5-6 m, and between blocks – at least 20-30 m.

In 2007, only PALs traps were used. Four replicates of the following four variants were tested: transparent unbaited, transparent baited, yellow unbaited and yellow baited traps. Traps were arranged in a similar way as in 2006.

Experiments were conducted from 20 April to 1 June, 2006 and 3 April to 22 May, 2007. Traps were inspected 1-2 times per week in both 2006 and 2007. Sticky surfaces of PALs traps were replaced weekly. Baits were replaced with fresh ones at 3-weeks intervals in both years.

Maximum and minimum temperatures were recorded daily at a site 100 m apart of the experimental field. Average temperature over each catching period was calculated on the base of these records.

Trapped beetles were washed in xylene (Vocational Gymnasium for chemical technology®, Bourgas, Bulgaria) for 24 hours and identified using Angelov (1979), Arnoldi *et al*. (1965) and Hoffmann (1954). The scientific names and systematic position of the weevil species captured are according to Alonso-Zarazaga (2004). The material collected is deposited in the collections of the Institute of Zoology (Sofia). Taxa are listed in alphabetical order.

Statistics

Statistical analyses were performed using Statistica for Windows 4.3. (StatSoft Inc., 1993). Significant difference between mean catches of all Ceutorhynchinae beetles caught in PALs and KLP+ traps was established by performing t-test. Catches of *C. typhae* in different traps in 2007 were analyzed by ANOVA followed by Duncan's NMRT.

Capture data were transformed to log (x + 1) before the analyses.

The χ^2 goodness of fit test was applied to test for differences in numbers of male and female *C. typhae* captured in each trap combination (colour/presence of bait).

Results

During two-year investigations, a total of 522 beetles of the subfamily Ceutorhynchinae belonging to six genera were captured in traps. The catches are presented on table 1.

In 2006, two genera, *Ceutorhynchus* and *Microplontus*, with seven and one species, respectively, were recorded. PALs traps captured significantly greater numbers of Ceutorhynchinae weevils than KLP+ traps (t-test, $P < 0.001$) and that is why only PALs traps were used in 2007. However, *C. napi* specimens were found only in KLP+ traps.

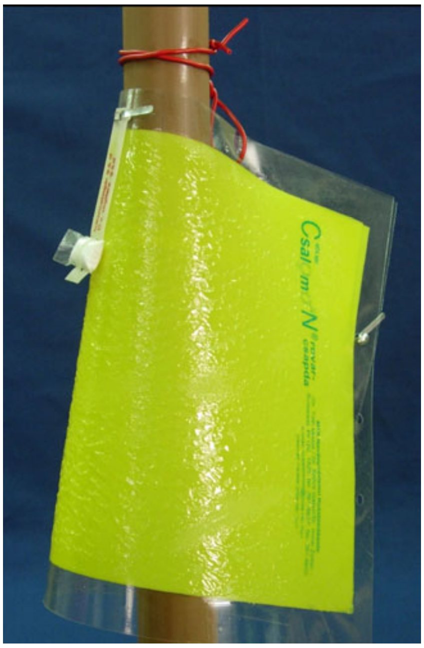

Figure 1. The PALs trap.
(In colour at www.bulletinofinsectology.org)

Figure 2. The KLP+ trap.
(In colour at www.bulletinofinsectology.org)

Table 1. Ceutorhynchinae species caught in different types of traps: KLP baited (KLP B), PALs transparent unbaited (PALs TU), PALs transparent baited (PALs TB), PALs yellow unbaited (PALs YU), PALs yellow baited (PALs YB); Vrazhdebna, Bulgaria, 2006-2007.

Species	Number of beetles caught in respective trap type, catching period					Seasonal occurrence (months) and distribution in Bulgaria[1], literature source
	KLP+ B	PALs TU	PALs TB	PALs YU	PALs YB	
**Amalus scortillum* (Herbst 1795) [= *haemorrhous* (Herbst 1795)] (Ceutorhynchini)			1♀ 8-11.V.2007			V-IX; Black Sea coast, Eastern Danubian Plain, Rhodopi Mts, Tracian Lowland; Vitosha Mt. (Angelov, 1979; Angelov and Metodiev, 2006)
**Ceutorhynchus coarctatus* Gyllenhal 1837 (Ceutorhynchini)			1♀ 11-16.V.2007			V-VI, Black Sea coast (Smreczynski and Cmoluch, 1961)
**Ceutorhynchus constrictus* (Marsham 1802) (Ceutorhynchini)			2♂ 8-22.V.2007	1♂ 8-11.V.2007	1♂ 19-25.V.2006	III-VI; Black Sea coast, Eastern Danubian Plain, Tracian Lowland (near Plovdiv), Pirin Mt., Rhodopi Mts, Stara planina Mts, Vitosha Mt. (Angelov, 1979; Anonymous, 1999)
Ceutorhynchus erysimi (F. 1787) (Ceutorhynchini)			1♀ 11-16.V.2007			III-IX; Black Sea coast; Eastern Danubian Plain, Tracian Lowland, Lyulin Mt., Osogovska Mt., Sofia Basin, Vitosha Mt., Rila Mt., Rhodopi Mts (Angelov, 1979; Angelov and Metodiev, 2006)
**Ceutorhynchus napi* Gyllenhal 1837 (rape stem weevil) (Ceutorhynchini)	4♂♂ 20-27.IV.2006					III- ; exact localities unknown (Buresh and Lazarov, 1956; Popov and Nikolova, 1958; Angelov, 1979)
**Ceutorhynchus obstrictus* (Marsham 1802) [= *C. assimilis* (F. 1792)] (cabbage seed weevil) (Ceutorhynchini)	1♀ 20-27.IV.2006			2 ♀♀, 1♂ 3-17.IV.2007	6♀♀, 2♂♂ 20.IV-19.V.2006 2♀ 3-17.IV.2007	IV-VIII; Black Sea coast, Eastern Danubian Plain, Lyulin Mt., Rhodopi Mts, Vitosha Mt. (Angelov, 1979; Angelov and Metodiev, 2006)
Ceutorhynchus pallidactylus (Marsham 1802) [= *C. quadridens* (Panzer 1795)] (cabbage stem weevil) (Ceutorhynchini)			1♀ 11-16.V.2007		1♀ 3-10.IV.2007	III-VII, Danubian Plain, Pirin Mt., Sofia Basin, Stara Planina Mts, Strandzha Mts, Tracian Lowland (Buresh and Lazarov, 1956; Popov and Nikolova, 1958; Angelov, 1979; Petryszak and Mazur, 1986)
**Ceutorhynchus picitarsis* Gyllenhal 1837 (Ceutorhynchini)					1♂ 3-10.IV.2007	IV-XI; Black Sea coast, Srednostrumska Valley, Rhodopi Mts (Angelov, 1979; Angelov and Metodiev, 2006)
**Ceutorhynchus rapae* Gyllenhal 1837 (Ceutorhynchini)			1♂ 3-10.IV.2007		2♀♀, 3 ♂♂ 20-27.IV.2006 3♂ 3-23.IV.2007	IV-VII; Black Sea coast, Eastern Danubian Plain, Rhodopi Mts, Tracian Lowland (Angelov, 1979; Angelov and Metodiev, 2006)

[1] Zoogeographical subdivision of the Bulgarian territory by Hubenov (1997) is used.
* Species newly recorded for the Sofia Basin area.

(Continued)

(Table 1 Continued)

Species	Number of beetles caught in respective trap type, catching period					Seasonal occurrence (months) and distribution in Bulgaria[1], literature source
	KLP+ B	PALs TU	PALs TB	PALs YU	PALs YB	
**Ceutorhynchus similis* C. Brisout 1869 (Ceutorhynchini)			1♀ 11-16.V.2007		1♀ 20-27.IV.2006	V-VI; Eastern Danubian Plain, Rhodopi Mts, Vitosha Mt. (Angelov, 1979; Angelov and Metodiev, 2006)
Ceutorhynchus sp. (Ceutorhynchini)					1 ex. 10-17.IV.2007	
**Ceutorhynchus turbatus* Schultze 1903 (Ceutorhynchini)					1♀, 1 ♂ 20.IV.- 19.V.2006	V-VII; Black Sea coast, Eastern Danubian Plain (Angelov, 1979)
**Ceutorhynchus typhae* (Herbst 1795) (Ceutorhynchini)		20♀♀, 17♂♂ 10.IV.- 16.V.2007	17♀♀, 14♂♂ 10.IV.- 22.V.2007	87♀♀, 81♂♂ 3.IV.- 22.V.2007,	66♀♀, 45♂♂ 20.IV.- 1.VI.2006 59♀♀, 56♂♂; 10.IV.- 22.V.2007	III-VIII; Eastern Danubian Plain, Rhodopi Mts (Angelov, 1979; Angelov and Metodiev, 2006), many places but exact localities unknown (Smreczynski and Cmoluch, 1961)
**Microplontus rugulosus* (Herbst 1795) (Ceutorhynchini)		1♀ 10-17.IV.2007	1♀ 26.IV.- 2.V.2007	1♀, 1♂; 10-23.IV.2007	5♂♂ 20.IV.- 1.VI.2006, 2♀♀, 2 ♂♂ 10.IV.- 11.V.2007	IV-VI; Black Sea coast, Rhodopi Mts, Strandzha Mt., Konyavska Planina Mt. (Angelov, 1979; Behne, 1989)
***Sirocalodes* sp. (Ceutorhynchini)					1♀ 3-10.IV.2007	
**Thamiocolus pubicollis* (Gyllenhal 1837) (Ceutorhynchini)			1♂ 26.IV.- 2.V.2007			V-VI; Western Danubian Plain, Strandzha Mt, Tracian Lowland (Angelov, 1979)
Rhinoncus bruchoides (Herbst 1784) (Phytobiini)				1♀ 17-23.IV.2007	1♀ 23-26.IV.2007	IV-VII; Black Sea coast, Sofia Basin, Tracian Lowland (Angelov, 1979)
**Rhinoncus pericarpius* (L. 1758) (Phytobiini)				1♀ 2-8.V.2007		IV-VII; Black Sea coast, Eastern Danubian Plain, Rhodopi Mts, Stara Planina Mts, Tracian Lowland, Vitosha Mt.(Angelov, 1979)

[1] Zoogeographical subdivision of the Bulgarian territory by Hubenov (1997) is used.
* Species newly recorded for the Sofia Basin area.
** Genus newly recorded for the Sofia Basin area.
ex. - Sex of the specimen is not determined.

Table 2. Catches of *C. typhae* beetles in PALs traps with different combination of visual and chemical stimuli. Vrazhdebna, Sofia, April 3 - May 22, 2007, four replicates. Catches marked with the same letter are not significantly different at P < 0.05 by ANOVA followed by Duncan's NMRT.

Trap colour/ presence of bait	Total number of beetles caught	Mean number ± SE	Male-female ratio	χ^2	P-value[a]
Transparent unbaited	37 b	1.03 ± 0.35	1: 1.2	0.243	0.622
Transparent baited	31 b	0.86 ± 0.20	1: 1.2	0.290	0.590
Yellow unbaited	168 a	4.67 ± 0.84	1: 1.1	0.214	0.643
Yellow baited	115 a	3.19 ± 0.68	1: 1.1	0.078	0.780

[a] no significant difference between number of females and males (χ^2 test).

Sixteen species belonging to six genera (*Amalus*, *Ceutorhynchus*, *Microplontus*, *Rhinoncus*, *Sirocalodes* and *Thamiocolus*) were captured in 2007. *Ceutorhynchus constrictus* (Marsham 1802), *C. obstrictus*, *Ceutorhynchus rapae* Gyllenhal 1837, *Ceutorhynchus similis* C. Brisout 1869, *C. typhae* and *Microplontus rugulosus* (Herbst 1795) were common in both 2006 and 2007. Two specimens belonging to *Ceutorhynchus* and *Sirocalodes*, respectively, were unidentified.

The most abundant species, and the only species with catches allowing statistical analyses, was *C. typhae*. Adults of this species represent 80% and 92%, respectively, of the Ceutorhynchinae collected in both 2006 and 2007. In 2006, 111 specimens of *C. typhae* were caught only in the PALs traps. In 2007, the highest number of *C. typhae* beetles was found in the yellow unbaited traps but there was no significant difference between the catches in these traps and the catches in yellow traps baited with isothiocyanate lure (table 2). There was also no significant difference between the catches in the unbaited and baited transparent traps tested. No significant difference was found between the number of male and female specimens of *C. typhae* caught in each treatment (t-test, P > 0.05).

Distribution of catches of *C. typhae* during the periods of investigation is presented on figure 3 and 4. The beginning of the flight of *C. typhae* was missed in 2006 because of late installing of the traps in the field. The results obtained in 2007, when the traps were put in the field earlier, showed that adults of this species were active early in the spring (beginning of April) when the average air temperature reaches 9.4 ± 0.5 °C. Whole flight was not followed.

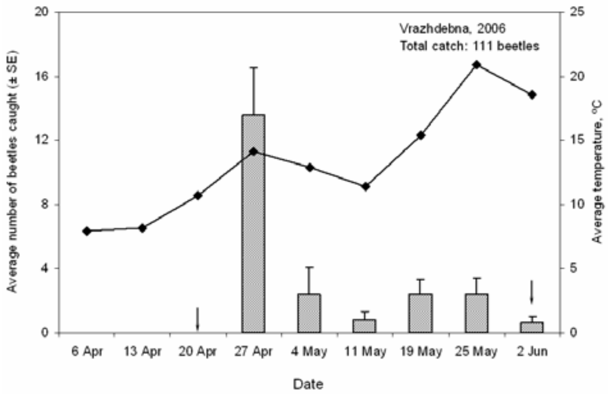

Figure 3. Catches of *C. typhae* beetles in PALs traps in Vrazhdebna in 2006. The bars show the mean catches and the curve - the mean air temperature.

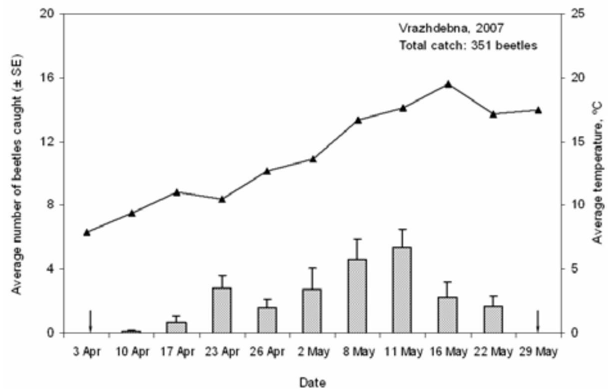

Figure 4. Catches of *C. typhae* beetles in PALs traps in Vrazhdebna in 2007. The bars show the mean catches and the curve - the mean air temperature.

Discussion

Five *Ceutorhynchus* species [*C. pallidactylus*, *C. obstrictus*, *C. napi*, *C. picitarsis* and *Ceutorhynchus erysimi* (F. 1787)] among the weevil species trapped by us are considered as important pests on Brassicaceae crops including cabbage in Bulgaria (Buresh and Lazarov, 1956; Popov and Nikolova, 1958; Lazarov *et al.*, 1959). According to Angelov (1979), *C. rapae* also could damage cabbage plants.

Fourteen weevil species captured during this study are newly recorded for the region of Sofia Basin. Two species of them are considered as a rare (*C. similis*) or very rare [*Thamiocolus pubicollis* (Gyllenhal 1837)] species for the Bulgarian entomofauna (Angelov, 1979). About the presence of *C. napi* in Bulgaria, no exact locality records are given (Buresh and Lazarov, 1956; Popov and Nikolova, 1958; Lazarov *et al.*, 1959; Popov, 1962; Grigorov, 1972; Angelov, 1979). We report exact locality of this species in Bulgaria for the first time.

Angelov (1979) stated that *Ceutorhynchus coarctatus* Gyllenhal 1837 is distributed over the whole country but only one original locality is cited in that source – Ljaskovets (Black Sea coast) (Smreczyński and Cmoluch, 1961). Definite localities for *C. typhae* are these in the Eastern Danubian Plain and Rhodopes (Angelov, 1979) although Smreczyński and Cmoluch (1961) reported that this species has been found in many places in Bulgaria.

In our investigations the number of *C. typhae* recorded in the yellow traps was significantly higher than that captured in the transparent traps in both baited and unbaited variants. Yellow colour preference has been also reported for the cabbage seed weevil *C. obstrictus*, *C. pallidactylus* (Láska *et al*., 1986; Smart *et al*., 1997) and *C. picitarsis* (Büchi, 1986). Prokopy and Owens (1983) suggested that yellow colour represents a super-normal foliage stimulus for herbivorous insects. Studying the influence of trap design on captures of the cabbage seed weevil, *C. obstrictus*, Smart *et al*. (1997) established that the yellow water traps and sticky traps (sticky box traps, and sticky card traps, mounted vertically or on a 45°-angle compared to vertical traps), placed at ca. 1 m above the ground, have been equally effective.

Addition of a lure comprising a mixture of 3-butenyl, 4-pentenyl, 2-phenylethyl, and allyl isothicyanates to yellow traps enhances the catches of *C. obstrictus* during the periods of the weevil migration to the host plant in the spring (Smart and Blight, 1997) and dispersal to overwintering sites (Smart *et al*., 1997; Smart and Blight, 1997). Traps baited with 2-phenylethyl isothiocyanate catch significantly higher numbers of *C. obstrictus*, *C. pallidactylus* and *C. napi* than traps without (Smart and Blight, 1997; Walczak *et al*., 1998). This volatile compound seems not to be important for the attraction of *C. typhae.*

C. typhae feeds mainly on seeds of non-cultivated wild cruciferous species (Hoffmann, 1954; Angelov, 1979; Bürki *et al*., 2001; Knutelski, 2005; Cripps *et al*., 2006; Kadas, 2006) but infestations on turnip rape *Brassica rapa* ssp. *oleifera* (DC.) Metzg., (Popov, 1962; Angelov, 1979) and oilseed rape *Brassica napus* L. (Czerniakowski, 1975; Hiiesaar *et al*., 2003) were also reported. Veromann *et al*. (2006) observed a relative abundance of beetles of this species in winter oilseed rape in Estonia. There is no literature data about damages on cabbage caused by larvae or adults of this species.

Adults of the most pest species of *Ceutorhynchus* end their hibernation and activate early in the spring when the soil temperature has reached 8-9 °C and the air temperature has risen above 10-12 °C (Grigorov, 1972; Sekulić and Kereši, 1998). In 2007, we registered the first catches of *C. pallidactylus*, *C. obstrictus*, *C. picitarsis*, *C. rapae* and *C. typhae* at an average weekly air temperature of 9.4 ± 0.5 °C, which coincided with the upper data.

Our research showed an earlier appearance of *C. coarctatus* and *C. similis* than that reported in the literature for Bulgaria (Angelov, 1979).

In conclusion, our results suggest that yellow sticky PAL traps placed on the ground level can be useful for faunistic researches on Ceutorhynchinae in various habitats. Besides for detection, these traps could be also used for seasonal monitoring of *Ceutorhynchus* pests.

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