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A SEX ATTRACTANT FOR THE SCARAB BEETLE *Anomala solida* ER.

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Abstract—(*R*, *Z*)-5-(-)-(Oct-1-enyl)oxacyclopentan-2-one (*R*-buibuilactone) attracted male *Anomala solida* Er. (Coleoptera: Scarabaeidae, Rutelinae), a vineyard and orchard pest in Southeastern Europe. The presence of the corresponding (*S*) enantiomer or of 2-(*E*)-nonen-1-ol (a frequently found pheromone component in other *Anomala* spp.) in the bait did not influence catches. Traps baited with (*R*, *Z*)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one were successfully used for monitoring the flight of *A. solida*, and may have practical applications for detection, monitoring, and mass trapping of the pest.

Key Words—*Anomala solida*, Coleoptera, Scarabaeidae, Rutelinae, (*R*, *Z*)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one, sex attractant, field trapping.

INTRODUCTION

Several pheromone structures have been described among scarab beetles (Coleoptera: Scarabaeidae) in the subfamily Rutelinae, mostly from pest species occurring in Japan and North America (Leal, 1998). In Hungary, 2-(*E*)-nonen-1-ol

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(E2-9OH) has been found to attract male *Anomala vitis* Fabr. and *A. dubia* Scop. (Coleoptera: Scarabaeidae, Rutelinae), defoliating pests of grapes and some orchard trees in Europe (Tóth et al., 1994). In the course of screening tests, two specimens of another *Anomala* species, namely *A. solida* Er., were also caught, one in a trap baited with (*R*, *Z*)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one (*R*-buibuilactone) and another in a trap baited with a 1:1 mixture of the same compound and (*R*,*Z*)-5-(-)-(dec-1-enyl)oxacyclopentan-2-one (*R*-japonilure) (Tóth et al., unpublished). *A. solida* is a rare species in Hungary (Endrödi, 1956), but is reported to cause occasional damage to vineyards and other plant cultures in the southeastern part of Europe (especially on the Balkan peninsula) (Tadic, 1950; Popov, 1958; Hurpin, 1962; Bobirnac and Somogy, 1971; Manolache et al., 1974). Consequently, an attractant-baited trap would be useful for plant protection purposes. The above chance captures prompted us to investigate in more detail the activity of possible attractant candidates in geographical areas with more abundant *A. solida* populations. Because *R*-buibuilactone was the common component in the two baits showing chance captures in our previous study (Tóth et al., unpublished), tests concentrated on the verification of the activity of this compound towards *A. solida*. The influence of the (*S*) enantiomer was also tested, because among scarabs enantiomeric configuration of pheromone components can be of crucial importance (Tumlinson et al., 1977; Leal, 1996). The influence of the presence of E2-9OH was also tested because *A. solida* co-occurs in several areas with *A. vitis* and *A. dubia* (Hurpin, 1962), and we wanted to check whether there was any indication of interaction in the chemical communication of these taxa.

METHODS AND MATERIALS

Chemicals. (*R*,*Z*)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one(*R*-buibuilactone) and (*S*,*Z*)-5-(+)-(oct-1-enyl)oxacyclopentan-2-one (*S*-buibuilactone) were prepared as previously reported (Leal, 1999). The optical purity of these compounds was higher than 99.6% by GC analyses with a γ-cyclodextrin-based chiral column (Leal, 1991). 2-(*E*)-Nonen-1-ol (E2-9OH) was purchased from Bedoukian Inc. (Danbury, USA) and was >95% pure by GC.

Bait Dispensers. Two dispenser types were used. Rubber: baits for the tests were prepared by using pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6; extracted 3 times in boiling ethanol for 10 min, then 3 times in methylene chloride overnight, prior to usage). PE vial: 0.7 ml polyethylene vials with lid (No. 730, Kartell Co., Italy). The lid of the vial was kept closed when setting up the trap in the field. All types of baits were attached to 8×1 cm plastic handles for easy manipulation when assembling the traps. For loading the baits, the required amounts of compounds were administered to the surface (rubber) or into (PE vial) the dispensers in hexane solutions. After evaporation of the solvent, the lids of the PE vial dispensers were closed, and dispensers were wrapped singly in pieces of aluminum foil and stored at -65°C until use.

Traps. The traps and baits used were standard *Anomala* funnel traps (CSALOMON® VARb2) produced by the Plant Protection Institute, Hungarian Academy of Science, Budapest, Hungary. The trap consisted of a plastic funnel (top opening outer diameter: 20 cm, funnel hole diameter 3 cm, height of funnel 19 cm), under which a transparent plastic catch container was attached by a rubber band. Beetles falling through the funnel were caught in the ca. 1-l plastic container. The bait dispenser was attached on the inner wall in the middle of the funnel opening.

Field Trapping. Tests were set up in cherry and apple orchards of the Institute of Fruit Growing, Kyustendil, Bulgaria and in mixed fields of strawberries, raspberries, currants, and cherry orchards of the Station of Small Fruits, Kostinbrod, Bulgaria.

One replicate of each treatment was incorporated into a block so that individual treatments were 5–10 m apart, and blocks were sited 50–100 m apart. Traps were moved one position forward within a block at each occasion when the traps were inspected; at the same time, captured beetles were recorded and removed.

Capture data were transformed to $(x + 0.5)^{1/2}$ and analyzed by ANOVA. Treatment means were separated by Games-Howell tests. In case one of the treatments was catching nil, the difference of the catch of other treatments from zero catch was analyzed by Dunnet's Post hoc Procedure (one-tailed). All statistical procedures were conducted using the software packages StatView® v4.01 and SuperANOVA® v1.11 (Abacus Concepts, Inc., Berkeley, USA).

Single Field Experiments

Test 1. Kyustendil: June 14–July 26, 1999; traps were inspected each 3–4 days over 35 days, providing 22 data points (11 inspections of 2 block replicates).

Test 2. Kostinbrod: June 14–July 26, 1999; traps were inspected twice weekly over 28 days, providing 24 data points (8 inspections of 3 block replicates).

Dosage Test. Kyustendil: June 14–July 26, 1999; traps were inspected each 3–4 days over 35 days, providing 22 data points (11 inspections of 2 block replicates).

Monitoring of Flight. Kyustendil: May 30–July 27, 2000; and June 1–July 30, 2001; traps were inspected at 2–3 days intervals; in each test 2 traps baited with 1 mg of *R*-buibuilactone were operated at a distance of ca. 20 m from each other.

TABLE 1. CATCHES OF *A. solida* IN TRAPS BAITED WITH SCARAB PHEROMONE COMPOUNDS IN BULGARIA IN 1999

Bait (mg)			No. of beetles caught			
			Kyustendil		Kostinbrod	
R-buibuilactone	*S*-buibuilactone	E2-9OH	Mean catch/trap/ inspection ± SE	Total	Mean catch/trap/ inspection ± S.E.	Total
1.0	—	—	10.2 ± 1.7 a	224	0.5 ± 0.2 a	13
1.0	0.1	—	10.1 ± 1.8 a	222	0.2 ± 0.1 a	5
1.0	—	0.1	14.2 ± 2.5 a	313	0.4 ± 0.2 a	10
—	—	—	0.0 ± 0.0	0	0.0 ± 0.0	0
—	—	10.0[a]	0.0 ± 0.0	0	0.0 ± 0.0	0

Note: Means with same letter within one column are not significantly different at $P < 0.05$ by ANOVA followed by Games-Howell test. All means were significantly different from zero catch (unbaited and E2-9OH-baited) at $P < 0.05$ by Dunnett's Post hoc Procedure (one-tailed).

[a] *A. vitis/dubia* commercial bait (CSALOMON®; Plant Protection Institute, Budapest, Hungary).

RESULTS AND DISCUSSION

In the preliminary tests, *R*-buibuilactone was tested alone and in mixtures with the *S*-enantiomer or E2-9OH vs. unbaited control traps. Another treatment was included with E2-9OH alone to check for the presence of the related *A. vitis* or *A. dubia*. At both sites, all baits containing *R*-buibuilactone caught significantly more *A. solida* beetles than the unbaited controls (Table 1). Catches in traps with the binary mixtures of *R*-buibuilactone and 10% of *S*−buibuilactone or E2-9OH were similar to those with *R*-buibuilactone alone. Unbaited traps or those baited with the *A. vitis/dubia* attractant (E2-9OH) on its own caught no beetles. The results clearly show that *R*-buibuilactone attracts male *A. solida* confirming the indication in the earlier trials in Hungary.

In a second test, doses of 0.1 to 5.0 mg of *R*-buibuilactone were tested vs. unbaited controls. Increasing doses attracted increasing numbers of *A. solida* (Table 2). with the highest catches recorded with 5.0 mg. All doses attracted significantly more beetles than unbaited traps, which captured nil. Of the sample of ca. 100 beetles checked, all were male.

R-buibuilactone seems to be a common pheromone component in *Anomala*. It has been identified in the sex pheromones of *A. cuprea* Hope (Leal, 1991; Leal et al., 1993), *A. albopilosa sakishimana* Nomura (Leal et al., 1994b), and *A. octiescostata* Burm. (Leal et al., 1994a). In *A. daimiana* Harald (Leal et al., 1993) and *A. albopilosa albopilosa* Hope (Leal et al., 1996), both *R*-buibuilactone and

TABLE 2. CATCHES OF *A. solida* IN TRAPS BAITED WITH DIFFERENT DOSES OF *R*—BUIBUILACTONE IN BULGARIA

Dose of *R*-buibuilactone (mg)	Mean catch/trap/inspection ±SE	Total caught
0.1	7.7 ± 1.6 a	209
0.5	12.2 ± 2.3 ab	330
1.0	14.5 ± 2.2 bc	392
5.0	23.3 ± 3.2 c	629
Unbaited	0.0 ± 0.0	0

Note: For significance levels see Table 1. Means with different letters are significantly different at $P < 0.05$.

E2-9OH are present in the pheromone. In the case of *A. solida*, the presence of E2-9OH in the bait did not seem to influence the activity of *R*-buibuilactone at the ratio tested (Table 1).

It has long been known that responses of male Japanese beetles *Popillia japonica* Newman to their pheromone *R*-japonilure were inhibited by the *S*-enantiomer (Tumlinson et al., 1977). This was elucidated to be part of an interesting mechanism of reciprocal agonist–antagonist activities of enantiomeric pheromone (Leal, 1996). The Osaka beetle, *A. osakana* Sawada produces and responds only to *S*-japonilure, the activity of which is completely inhibited by the *R*-enantiomer (Leal, 1996). Thus, we tested whether *S*-buibuilactone might affect catches of *A. solida*. However, captures in traps baited with *R*-buibuilactone alone or in combination with *S*-buibuilactone were not significantly different, suggesting that *S*-buibuilactone is neither beneficial nor a behavioral antagonist.

The seasonal flight of *A. solida* was reliably monitored by traps baited with *R*-buibuilactone in both 2000 and 2001 (Figure 1). In both years, the flight started at the beginning of June and beetle abundance peaked between June 10 and July 5. According to Grigorov (1972) *A. solida*'s flight period begins in Bulgaria at the end of June and the flight lasts until, the end of July. In the present study, the flight in both years started ca.1 month earlier. Whether this difference with literature data is due to warmer temperatures in the given years or to other factors remains to be determined.

We conclude that traps baited with *R*-buibuilactone can be used for trapping *A. solida* in regions of Europe where it is a pest . Furthermore, such traps might be applied for beetle control through mass trapping, building on promising results obtained recently with the closely related *A. vitis/dubia* in Hungary (Voigt and Tóth, 2000, 2002).

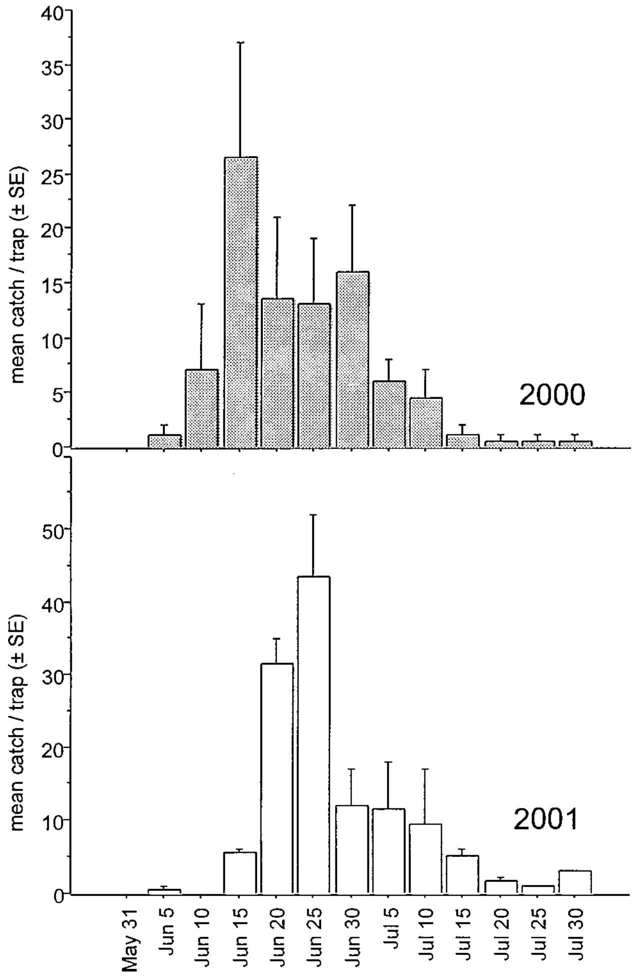

FIG. 1. Monitoring of the flight of *A. solida* by two traps baited with 1 mg of *R*-buibuilactone on rubber dispenser in 2000 and 2001 in Bulgaria.

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