2-(E)-Nonen-1-ol: MALE ATTRACTANT FOR CHAFERS Anomala vitis FABR. and A. dubia SCOP. (COLEOPTERA: SCARABAEIDAE)¹

MIKLÓS TÓTH,^{2.*} WALTER SOARES LEAL,³ ISTVÁN SZARUKÁN,⁴ MÁTYÁS LESZNYÁK,⁴ and GÁBOR SZÖCS²

¹The present paper is dedicated to the memory of our coauthor, friend and colleague Mátyás Lesznyák who died a premature and tragic death in late 1993.

> ²Plant Protection Institute Hungarian Academy of Sciences Budapest, Pf 102, H-1525, Hungary

³National Institute of Sericultural and Entomological Science Ministry of Agriculture, Forestry and Fisheries Tsukuba 305, Japan

> ⁴Agricultural University Debrecen, Pf 58, H-4001, Hungary

(Received March 7, 1994; accepted May 12, 1994)

Abstract—Traps baited with 2-(E)-nonen-1-ol alone or in combination with other compounds caught large numbers of males of both the vine chafer, *Anomala vitis* Fabr. and the margined vine chafer, *A. dubia* Scop. (Coleoptera: Scarabaeidae), vineyard and orchard pests. In a dosage test, the largest numbers were caught by traps baited with 10 mg of 2-(E)-nonen-1-ol, which was the highest dosage tested. This is the first report on male attractants for chafer species occurring in Europe.

Key Words—Anomala vitis, Anomala dubia, chafer, Coleoptera, Scarabaeidae, Melolonthidae, Rutelinae, attractant, 2-(E)-nonen-1-ol.

INTRODUCTION

Among the more than one thousand species of the genus *Anomala* [Coleoptera: Scarabaeidae (Melolonthidae), Rutelinae] worldwide there are widespread defoliator pests of many agricultural plants (Endrödi, 1956). In temperate and espe-

*To whom correspondence should be addressed.

2481

cially in warmer regions of Europe, the vine chafer, Anomala vitis Fabr. and the margined vine chafer, A. dubia Scop. are regularly reported to cause leaf damage in vineyards, and, occasionally, in various orchard trees (Hurpin, 1962). In Hungary, A. vitis clearly prefers the regions with chalky sand of the Great Plain area, while A. dubia, although in many cases cooccurring with A. vitis, can also be found on areas with more acidic sand (Endrödi, 1956; Bognár and Huzián, 1974; Koppányi, 1988). The species have regular mass outbreaks a couple of years apart; in such years in heavily damaged areas vineyards can be totally defoliated (Homonnay and H. Csehi, 1990).

In order to search for an attractant that could later be used in traps for the monitoring and forecast of these pests, several compounds, described as pheromone components from other species of Scarabaeidae were screened at several sites in Hungary. The compounds screened included all compounds identified so far from *Anomala* spp. in Japan, namely, methyl (Z)-tetradec-5-enoate (Tamaki et al., 1985); (R,Z)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one (Leal, 1991), 2-(E)-nonen-1-ol (Leal et al., 1992a), as well as (R,Z)-5-(-)-(dec-1-enyl)oxacyclopentan-2-one, the pheromone of *Popillia japonica* Newman (Tumlinson et al., 1977), and the major and minor components of the pheromone of *Holotrichia parallela* Motschulsky [L-isoleucine methyl ester (Leal et al., 1992b), (R)-(-)-linalool (Leal et al., 1993a)]. Some binary mixtures of the above compounds were also tested. In the present paper we describe the discovery of a male attractant for both *A. vitis* and *A. dubia*.

METHODS AND MATERIALS

Synthesis. Methyl (Z)-tetradec-5-enoate was synthesized starting by the coupling of 1-decyne with 1-bromo-3-chloropropane by Wittig reaction. The Grignard reaction of the product, 1-chloro-4-tridecyne, with methyl chloroformate gave methyl tetradec-5-ynoate. This was reduced by Lindlar catalyst to give an isomeric mixture containing 98% of the desired Z product. 2-(E)-Nonen-1-ol and L-isoleucine methyl ester were synthesized as previously described (Leal et al., 1992a,b). (R,Z)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one was prepared as previously reported (Leal, 1991). (R,Z)-5-(-)-dec-1-enyl)-oxacyclopentan-2-one was obtained starting from D-ribose (Koseki et al., 1993).

The optical purity of all lactones was determined to be >97% ee by chiral chromatography (Leal, 1991). R-(-)-Linalool, kindly provided by Fuji Flavor Co. (Tokyo, Japan), was determined to be ca 100% ee by a previously described method (Leal et al., 1993b).

Trapping Tests. Dispensers for the tests were prepared by using pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 969 1/6; extracted three

times in boiling ethanol for 10 min, then also three times in methylene chloride overnight, prior to usage). For making up the baits the required amounts of compounds were administered to the surface of the dispensers in hexane (Merck) solutions. In the case of L-isoleucine methyl ester, instead of hexane dichloromethane (Merck) was used. Prepared dispensers were stored at -65° C until use. Field tests were conducted at several sites in Hungary. Trap types used included a funnel trap (Japan Tobacco Inc., Tokyo, Japan), made from green plastic (later in the paper referred to as JT), and a home-made funnel trap similar in shape and size to the JT traps, but hand made from transparent plastic bottles (later in the paper referred to as HM). Traps were suspended from branches of vine or trees at a height of 0.5-1.0 m above ground. Traps containing different baits were set up along a straight line. The distance between traps within a line was 10 m. The distance between lines ranged between 100 and 1000 m. Traps were moved one position forward within a line, and the trap positioned at the end of the line was returned to the beginning position at each occasion when the traps were inspected. At the same time, the number of captured beetles was recorded and traps were emptied. In screening tests, one replicate of each bait per trap type was set up, while in dosage tests three replicates were used.

RESULTS AND DISCUSSION

In the screening tests, A. vitis and A. dubia male beetles were captured in traps baited with 2-(E)-nonen-1-ol, or with mixtures containing this compound (Table 1). No other compound showed any attractive activity for these beetles. A. vitis was captured in very high numbers at Kunbaracs, where A. dubia was missing; on the other hand, at Ladánybene, and Józsa, where A. dubia was abundant, there were only low catches of A. vitis recorded. However, at Gödöllö, both species were present, albeit in moderate numbers. Of the ca. 100 randomly selected beetles of the catch, all were found to be males.

In a preliminary dosage test of 2-(E)-nonen-1-ol for *A. vitis*, traps baited with 10 mg, the highest dosage tested, caught significantly more beetles than lower dosages at Kunbaracs, where population density was high (Table 2). The catches of the two lower doses did not differ. In the case of *A. dubia* catches by 10 or 1 mg were not different, while catches by 0.1 mg were significantly lower (Table 2).

2-(E)-Nonen-1-ol has been identified as the sole component of the femaleproduced pheromone of *Anomala schonfeldti* Ohaus (Leal et al., 1992a) and as the major constituent of the *A. daimiana* Harold sex pheromone (Leal et al., 1993a). It has also been found that this compound is a potent attractant for the rose chafer *Macrodactylus subspinosus* F., a serious pest of fruit crops, omamentals, and flowers in eastern North America (Williams et al., 1993). Although

		Bait (mg)	(2			Total n	Total number of male Anomala vitis caught	ale <i>Anon</i> tht	nala vitis	Total nu	Total number of male Anomala dubia caught	le Anomu ht	ala dubia
(E)	(R, Z)-5- (-)-(Dec-1-	(R, Z)-5- (-)-(Oct-1-	é			Ğ	Gödöllö	Kun	Kunbaracs	Göc	Gödöllö		Ladány-
MeunyI(∠)- tetradec-5- enoate	enyı)- oxacyclo- pentan-2-one	enyı)- oxacyclo- pentan-2-one	(n)- (-)- Linalool	2-(E)-No- nen-1-ol	L-Isoleucine methyl ester	JT trap	HM trap	JT trap	HM trap	JT trap	HM trap	JT JT trap	HM trap
_						0	0	0	0	0	0	0	0
						0	0	0	0	0	0	0	0
		-				0	0	1	0	0	0	-	0
			-			0	0	0	0	0	0	0	0
				_		4	-	760	68	4	_	317	57
					10	0	0	0	0	0	0	0	0
						80	0	1110	180	10	12	184	34
	-	1				0	0	2	0	0	0	0	0
1	-					0	0	-	0	0	0	0	0
	1					12	ę	705	268	15	15	138	76
1						6	9	325	101	Ξ	7	59	LL
_		-				0	¢	-	C	c	c	c	c

TABLE 1. CAPTURES OF MALE A. vitis AND A. dubia in TRAPS BAITED WITH COMPOUNDS PREVIOUSLY IDENTIFIED AS PHEROMONE Components in Scarabaeidae and Their Binary Mixtures in 1993^{α}

2484

"Gödöllö, Pest County, April 22-July 9, nursery with Pirus nigra trees; Kunbaracs, Bács-Kiskun County. HM trap type May 4-June 28; JT trap type Ladańybene, Pest County, April 21-June 28; Pinus nigra stands. Traps were inspected twice weekly, baits were renewed fortnightly, except with methyl June 8-July 12; commercial vineyard; Józsa, Hajdú-Bihar County, June 2-30, backyard gardens with small vineyards and mixed orchard trees;

(Z)-tetradec-5-enoate, which was replaced weekly. At each site one replicate was set up of each bait variation per trap type.

	Mean catch		
Dore of	Anoma	la vitis	Anomala dubia,
Dose of 2-(E)-nonen-1-ol (mg)	Ladánybene	Kunbaracs	Ladánybene
0.1	0.13 a	24.27 a	0.60 a
1	0.60 ab	34.40 a	2.67 ab
10	1.13 b	72.13 b	4.20 b

TABLE 2. CAPTURES OF MALE A. vitis and A. dubia in Traps Baited with Different Amounts of 2-(E)-Nonen-1-ol^a

^aLadánybene, Pest County, and Kunbaracs, Bács-Kiskun County; June 10-28, 1993; at each site three replicates were set up of each dose, using JT traps. Traps were inspected twice weekly. In statistical analyses, catches recorded at an inspection were regarded as replicates. Capture data were transformed to $\sqrt{x} + 0.5$ and differences between means were tested for significance by ANOVA followed by Duncan's new multiple-range test (NMRT) Means followed by same letter within one column are not significantly different at P = 5%.

at present it is not known whether this compound is also produced by females of A. vitis or A. dubia, results of the present study suggest that 2-(E)-nonen-1ol can be one of the key pheromone structures in this genus. Chemically identified pheromones for phytophagous Scarabaeidae (Melolonthidae) have been described so far almost exclusively for species originating in the Pacific region: in Japan (Tumlinson et al., 1977; Tamaki et al., 1985; Leal, 1991, 1993; Leal et al., 1992a,b, 1993a,b) or in New Zealand (Henzell and Lowe, 1970). This is the first report of male attractant compounds for species occurring in Europe.

Although biological evidence is known concerning the cross-attractancy of natural pheromones in Scarabaeidae (Leal et al., 1993b), it is still surprising that males of both *A. vitis* and *A. dubia* were so strongly attracted to a single compound in the present study. The geographical distribution of the two species and their seasonal occurrence pattern broadly overlaps (Endrödi, 1956; Hurpin, 1962; Homonnay and H. Csehi, 1990). What is more, in the course of the present study, males of both species were equally frequent in approaching baits in the same period of the day, i.e., late morning (9–12 AM) (Tóth and Szöcs, unpublished). Consequently, it is highly probable that reproductive isolation between the two species is achieved by the production of secondary components in each natural pheromone. This clearly emphasizes the importance of future analyses of female-produced pheromone and the study of the influence of other synthetic pheromonal compounds added in differing ratios.

Due to the preliminary nature of the screening test in this study, we did not try to establish the influence of other compounds added to 2-(E)-nonen-1-

ol on captures. However, there have been some cases described where the addition of minor amounts of a second component had a striking effect on captures of male scarabs (Leal et al., 1993a).

For the time being, baits with 1-10 mg of 2-(E)-nonen-1-ol yield a suitable basis for the development of monitoring traps for both *A. vitis* and *A. dubia*. From the practical point of view, the mutual attraction of both species is not harmful, since both species damage the same cultures in the same period of the year. As most damage is caused by the adults (Homonnay and Csehi, 1990), trapping out of a significant part of the beetle population may have a direct diminishing effect on damage, provided the capacity and number of traps is high enough.

Acknowledgments—The research in Hungary was partially supported by the grant OTKA 1438 of the Hungarian Academy of Sciences. We thank also Fuji Flavor Co. (Tokyo) for providing the JT traps used in field tests and optically pure (R)-linalool.

REFERENCES

- BOGNÁR, S., and HUZIÁN, L. 1974. (eds.) Növényvédelmi Állattan. Mezögazdasági Kiadó, Budapest, 436 pp.
- ENDRÖDI, S. 1956. Lemezescsápú bogarak—Lamellicornia. In V. Székessy (ed.). Fauna Hungariae Coleoptera 4. Vol. IX. Iss. 4. 188 pp.
- HENZELL, R.F., and LOWE, M.D. 1970. Sex attractant of the grass grub beetle. Science 168:1005-1006.
- HOMONNAY, F., and H.CSEHI, É. 1990. Cserebogarak---Melolonthidae, in T. Jerrny, and K. Balázs, (eds.). A Növényvédelmi Állattan Kézikönyve 3/A. pp. 156-215.
- HURPIN, B. 1962. Super-famille des Scarabaeoidea, in A. S. Balachowsky (ed.). Entomologie appliquée à l'agriculture, vol. 1. Mason et Cie, Paris, pp. 24-204.
- KOPPÁNYI, T. 1988. Növényvédelmi Állattan II/A., Részletes ismeretek (Nematoda—Coleoptera). Egyetemi jegyzet. Agrártudományi Egyetem, Debrecen, pp. 1–188.
- KOSEKI, K., EBATA, T., KADOKURA, H., KAWAKAMI, H., ONO, M., and MATSUSHITA, H. 1993. Synthesis of sex pheromone of the Japanese beetle and cupreous chafer beetle. *Tetrahedron* 49:5961–5968.
- LEAL, W.S., 1991. (R,Z)-5-(-)-(Oct-1-enyl)oxacyclopentan-2-one, the sex pheromone of the scarab beetle Anomala cuprea. Naturwissenschaften 78:521-523.
- LEAL, W.S., 1993. (Z)-Tetradec-7-en-2-one and (E)-tetradec-7-en-2-one, a new type of sex pheromone from the oriental beetle. *Naturwissenschaften* 80:86-87.
- LEAL, W.S., HASEGAWA, M., and SAWADA, M. 1992a. Identification of *Anomala schonfeldti* sex pheromone by high-resolution GC-behavior bioassay. *Naturwissenschaften* 79:518-519.
- LEAL, W.S., MATSUYAMA, S., KUWAHARA, Y., WAKAMURA, S., and HASEGAWA, M. 1992b. An amino-acid derivative as the sex pheromone of a scarab beetle. *Naturwissenschaften* 79:184– 185.
- LEAL, W.S., SAWADA, M., and HASEGAWA, M. 1993A. The scarab beetle Anomala daimiana utilizes a blend of 2 other Anomala spp sex pheromones. Naturwissenschaften 80:181-183.
- LEAL, W.S., SAWADA, M., MATSUYAMA, S., KUWAHARA, Y., and HASEGAWA, M. 1993b. Unusual periodicity of sex pheromone production in the large black chafer *Holotrichia parallela*. J. Chem. Ecol. 19:1381-1391.

- TAMAKI, Y., SUGIE, H., and NOGUCHI, H. 1985. Methyl (Z)-5-tetradecenoate, sex-attractant pheromone of the soybean beetle, Anomala rufocuprea Motschulsky (Coleoptera, Scarabaeidae). Appl. Entomol. Zool. 20:359-361.
- TUMLINSON, J.H., KLEIN, M.G., DOOLITTLE, R.E., LADD, T.L., and PROVEAUX, A.T. 1977. Identification of the female Japanese beetle sex pheromone: inhibition of male response by an enantiomer. *Science* 197:789-792.
- WILLIAMS, R.N., FICKLE, D.S., and MCGOVERN, T.P. 1993. Attractants for the rose chafer, Macrodactylus subspinosus (F.) US Patent (March 30, 1993) 5:198-219.