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Geographic distribution and ecology of two species of *Orsillus* (Hemiptera: Lygaeidae) associated with cones of native and introduced Cupressaceae in Europe and the Mediterranean Basin

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Abstract—In the Mediterranean Basin, two species of true seed bugs in the genus *Orsillus* Dallas, *O. maculatus* Fieber and *O. depressus* Mulsant et Rey, share the exploitation of seed cones of Cupressaceae for feeding and reproduction and may carry spores of the pathogenic fungus *Seiridium cardinale* Sutton & Gibson, responsible for the cypress bark canker disease. We compared the life history of the two species. A total of 89 cone collections carried out in 10 Mediterranean countries confirmed that *O. maculatus* is most closely associated with *Cupressus sempervirens* L., although it may also infest some other *Cupressus* L. species and, more rarely, species of *Chamaecyparis* Spach. *Orsillus depressus* appears to be less host-specific, being capable of feeding and ovipositing on most of the native and exotic species of *Juniperus* L., *Cupressus*, and *Chamaecyparis*. On *C. sempervirens*, the abundance of each *Orsillus* species follows an inverted longitudinal gradient along the Mediterranean Basin, probably corresponding to the pathway of introduction of this tree species from its native eastern range towards western Europe. The dominance of *O. maculatus* progressively decreases from east to west, whereas *O. depressus* becomes dominant in the Iberian Peninsula. By contrast, *O. depressus* is the dominant seed bug on other Cupressaceae all over Europe and the Mediterranean Basin. In the area in which *C. sempervirens* has been introduced, both *Orsillus* species may live on the same tree, but morphological variability of both adults and nymphs often hinders accurate identification of the species. Based on frequency distribution, the relative length of the rostrum compared with that of the body seems to be a diagnostic character for specific identification of nymphs of the last two instars.

Résumé—Dans le Bassin Méditerranéen, deux punaises des graines du genre *Orsillus* Dallas, *O. maculatus* Fieber et *O. depressus* Mulsant et Rey, se partagent l'exploitation des cônes de Cupressacées pour leur nutrition et leur reproduction. Ces deux espèces transportent les spores d'un champignon pathogène, *Seiridium cardinale* Sutton & Gibson, responsable de la maladie du chancre cortical du cyprès. Le cycle biologique respectif des deux espèces est comparé. L'analyse de 89 récoltes de cônes réalisées dans 10 pays méditerranéens a confirmé que *O. maculatus* est plus étroitement associée à *Cupressus sempervirens* L. bien qu'elle puisse aussi se retrouver sur d'autres espèces de Cyprès et, mais plus rarement, de *Chamaecyparis* Spach. *Orsillus depressus* semble plus oligophage, étant capable de se nourrir et de se reproduire sur la plupart des espèces natives et exotiques de Cupressacées. Sur *C. sempervirens*, l'abondance relative de chaque espèce suit un gradient longitudinal inversé le long du Bassin Méditerranéen, qui correspond vraisemblablement à la route d'introduction de l'essence-hôte depuis son aire d'origine orientale vers l'Europe occidentale. La dominance d'*O. maculatus* décroît progressivement d'est

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en ouest, *O. depressus* devenant l'espèce principale dans la Péninsule Ibérique. Sur les autres Cupressaceae, *O. depressus* est l'espèce prédominante dans l'ensemble de l'Europe comme dans le Bassin Méditerranéen. Dans l'aire d'introduction de *C. sempervirens*, les deux espèces cohabitent parfois sur un même arbre, mais l'identification de chaque espèce est rendue difficile par des variations morphologiques chez les adultes et les larves. Sur la base de la distribution des fréquences, la longueur relative du rostre comparée à celle du corps semble constituer un caractère diagnostique pour la différenciation des larves des deux derniers stades de chaque espèce.

Introduction

Trees and shrubs of Cupressaceae cover a large part of Europe, especially in the Mediterranean Basin, where evergreen cypress, *Cupressus sempervirens* L., has been introduced on a large scale since the time of the ancient Greeks (Baumann 1982). However, several Cupressaceae species (*e.g.*, *Cupressus atlantica* Gaussen, *Cupressus dupreziana* A. Camus, and *Juniperus thurifera* L.) face extinction in parts of their native range, whereas *C. sempervirens* largely suffers from the disastrous spread of an exotic disease, cypress bark canker (Teissier *et al.* 1999). Since the 1990s, several studies have attempted to establish the part played by cone and seed insects in limiting the potential for natural regeneration of Mediterranean cypresses and junipers (Guido *et al.* 1995; Garcia 1998; Roques *et al.* 1999b; Roques and Battisti 1999; Battisti *et al.* 2000; Blasco-Zumeta 2000; Garcia *et al.* 2000) and in disseminating pathogenic fungi (Battisti *et al.* 1999, 2003; Ramos and Caetano 1999). Seed bugs have always appeared to constitute a key element of the cone entomofauna, but their bionomics have remained unclear because they are external predators of seed cones and thus are usually difficult to sample.

In Europe and the Mediterranean Basin, Cupressaceae species host four species of seed bugs in the genus *Orsillus* Dallas (Hemiptera: Lygaeidae) (Dioli 1991; Schuh and Slater 1995; Péricart 1998). However, two of these species seem either localized (*O. pinicanariensis* Lindberg in the Canary islands) or scarce (*O. reyi* Puton), whereas *O. maculatus* Fieber and *O. depressus* Mulsant *et* Rey have been frequently recorded in a number of countries (for details see Péricart 1998).

These latter two species of *Orsillus* have been reported to cause significant damage to seed crops in seed orchards as well as natural stands and plantations (Roques *et al.* 1999b; Battisti *et al.* 2000). They have also been shown to carry on their body spores of a pathogenic fungus, *Seiridium cardinale* Sutton & Gibson,

responsible for the cypress bark canker disease in plantations and natural forests of *C. sempervirens* in Europe and the Mediterranean area (Wagener 1939; Colombari 1996; Cantini 1997; Graniti 1998). *Seiridium cardinale* was probably introduced from California together with a Californian cypress, *Cupressus macrocarpa* Hart., which was imported to provide windbreak hedges in western Europe. The cypress bark canker disease was first noticed in France (Barthelet and Vinot 1944) and then spread rapidly all over southern Europe (Grasso 1951; Strout 1970; Graniti 1998; Battisti *et al.* 1999). It is not yet clear how the pathogenic fungus shifted from *C. macrocarpa* to *C. sempervirens*. Battisti *et al.* (1997, 1999) showed that the occurrence of *S. cardinale* on *C. sempervirens* is strongly associated with the presence of *O. maculatus*, which seems to trigger the dissemination of the fungal spores, but *O. depressus* was recently suggested to play a similar role in Portugal (Ramos and Caetano 1999). In addition, only *O. depressus* has been reported to use *C. macrocarpa* as a host tree (Dioli 1991).

It has been reported that the adults of *O. maculatus* and *O. depressus* are easily separated by the length of the rostrum relative to that of the body. In *O. maculatus*, the rostrum usually reaches the extremity of the abdomen, whereas it reaches only the mid-abdomen in *O. depressus* (Dioli 1991; Schuh and Slater 1995; Péricart 1998). However, only mean values are available for body length and rostrum length; the range of the ratio of rostrum length to body length remains unknown. Moreover, no data exist for such a ratio in nymphs and it is not yet possible to identify to species level samples containing only *Orsillus* nymphs. Under laboratory conditions, five developmental instars have been observed for the nymphs of *O. maculatus* reared on *C. sempervirens*; the overall development from egg to adult takes approximately 61 days, and the species is considered multivoltine (Guido *et al.* 1995; Battisti *et al.* 1997; Rouault 2002). In the field, *O. maculatus* oviposited on cypress cones

preferentially in the emergence holes of a cypress seed chalcid, *Megastigmus wachtli* Seitner (Hymenoptera: Torymidae), or on the inner side of partly opened cone scales (Guido *et al.* 1995; Battisti *et al.* 1997, 2003; Rouault 2002). Chalcid holes appeared to protect bug eggs from parasitoid attacks and mechanical injuries (Rouault *et al.* 2000). In contrast, the life history of *O. depressus* remains quite unknown.

Data on the host range and distribution of the two species are confusing. The distribution range of *O. maculatus* is considered to cover essentially the borders of the Mediterranean Sea (Guido *et al.* 1995; Péricart 1998; Roques *et al.* 1999b). This species has been primarily reported on *C. sempervirens* but there are some punctual records on junipers and pines (*Pinus* L.; Pinaceae) (Lindberg 1948; Kiritchenko 1951; Roques 1983; see also references in Péricart 1998). Recently, *O. maculatus* was also reported on *Cupressus arizonica* Greene in Algeria (Bouaziz and Chakali 1997). The geographic distribution of *O. depressus* is reportedly larger, covering Europe from west to north and east until Tadjikistan (Aukema 1988; Reichling 1988; Hawkins 1989; Péricart 1998). It also seems to have a wider host range, having been recorded on Mediterranean species of *Biota* D. Don *ex* Endl., *Cupressus* L., and *Juniperus* L. (Ben Jamaa and Roques 1999; El Alaoui El Fels 1999a, 1999b) but also *Pinus* (Mulsant and Rey 1872; Péricart 1998) and several exotic species of *Chamaecyparis* Spach, *Cupressus*, and *Thuja* L. (Péneau 1927; Cleu 1950, 1953; Dupuis 1965; Roques 1983; Aukema 1988; Dioli 1991; El Alaoui El Fels 1997; Péricart 1998; Roques *et al.* 1999b; Blasco-Zumeta 2000; Rouault *et al.* 2000). However, most of these records were based on the observation of a few individuals, sometimes even a single specimen, without any clear evidence of feeding and reproduction on these tree species.

Therefore, our objectives were to clarify the host range and distribution of both species to identify possible pathways for dissemination of *S. cardinale*. We also intended to precisely determine the life history of each bug species by rearing *O. depressus* under laboratory conditions and recording the seasonal fluctuations of *O. maculatus* abundance in cypress cones. Finally, we aimed to define diagnostic, easy-to-use morphological criteria allowing accurate identification of both nymphs and adults. For these purposes, standardized cone collections

were carried out for several years throughout western, southern, and eastern Europe, Asia Minor, and northern Africa. All the native species, as well as the massively planted exotic species of Cupressaceae, were surveyed.

Materials and methods

Geographical distribution and host range of *Orsillus* seed bugs

A total of 57 cone samplings were carried out on *C. sempervirens* during 1995–2003 at 56 locations in Europe, Asia Minor, and North Africa. Samples were collected from 7 sites in the native range of evergreen cypress (the Aegean Islands, Crete, and Turkey) and 49 sites in the introduced range (Corsica, continental France, continental Greece, Israel, Italy, Montenegro, Morocco, Portugal, Spain, and Tunisia) (Table 1, Fig. 1). In addition, 32 cone samples were collected from other native and introduced Cupressaceae species growing in the same geographic areas: at 7 sites where cones of *C. sempervirens* were also collected, and at 16 additional sites. For comparison, cone collections were also carried out in China, South Africa, and California to survey the presence of *Orsillus* species on introduced and native *Cupressus* species in countries where cypress canker is present.

For *C. sempervirens*, we collected 30 mature, 3-year-old cones per tree. From 1 to 170 trees were sampled per site, depending on cone abundance. Open cones, likely to contain nymphs or adult seed bugs, were immediately put in sealed plastic boxes, whereas closed cones were stored in paper bags until analysis at the laboratory. Similarly, for *Chamaecyparis* and *Juniperus* spp. we collected 30 mature cones per tree, but we also collected foliage where *Orsillus* species may hide. In these species, 2–5 cone-bearing branches per tree were each beaten 5 times.

Cone analysis was conducted within 10 days following collection. Each cone was first examined for the presence of nymphs and adults of *Orsillus* spp. and for emergence holes of a cypress seed chalcid, *M. wachtli*. *Orsillus* species were identified based on the length of the rostrum compared with the body length, but also using the body color (*i.e.*, darker for *O. maculatus* than for *O. depressus*, and with more reddish parts for *O. depressus*) and the shape of the pronotal spot (*i.e.*, usually a black dot at the center of the pronotum for

Table 1. Results of a survey of cypress seed bugs, *Orsillus maculatus* and *O. depressus*, in Europe and the Mediterranean Basin.

Site No.	Site	Coordinates	Elevation (m)	Type	Host	<i>O. maculatus</i>	<i>O. depressus</i>	Cones
Morocco								
1	Agadir	9°36'W, 30°24'N	n.a.	Ornamental	<i>C. sempervirens</i> (10)	0	6*	+
2	Idni (High Atlas)	8°17'W, 30°54'N	1590	Natural stand	<i>C. atlantica</i> (10)	6*	71	+
3	Oukaimeden-Tizrag	7°51'W, 31°12'N	2350	Natural stand	<i>J. thurifera</i> (5)	0	0	+
4	Ifrane (Middle Atlas)	5°06'W, 33°32'N	n.a.	Plantation	<i>C. arizonica</i> (10)	0	15*	+
Portugal								
5	Lagos	8°40'W, 37°06'N	50	Hedge row	<i>C. lusitanica</i> (3)	0	0	+
		8°40'W, 37°06'N	50	Hedge row	<i>C. sempervirens</i> (5)	8*	33*	+
6	Buçaco	8°20'W, 40°21'N	512	Plantation	<i>C. lawsoniana</i> (2)	1*	38*	+
7	Lisbon-Monsanto	8°11'W, 38°44'N	450	Plantation	<i>C. lusitanica</i> (5)	0	12*	+
8	Alto Espinho	7°56'W, 41°18'N	760	Plantation	<i>C. lawsoniana</i> (2)	0	11*	+
		7°56'W, 41°18'N	580	Hedge row	<i>C. lawsoniana</i> (3)	0	100*	+
Spain								
9	Segovia	4°08'W, 40°57'N	993	Ornamental	<i>C. arizonica</i> (2)	0	7*	+
10	Navacerrada	4°01'W, 40°43'N	1000	Plantation	<i>C. sempervirens</i> (5)	0	1	+
11	Madrid	3°52'W, 40°30'N	300	Plantation	<i>C. sempervirens</i> (5)	2	3	+
12	Manaria	2°38'W, 43°06'N	571	Plantation	<i>C. lawsoniana</i> (3)	0	1*	+
13	Fuenterabia	1°47'W, 43°21'N	10	Hedge row	<i>C. sempervirens</i> (5)	1	2	+
14	Pamplona	1°39'W, 42°48'N	700	Plantation	<i>C. sempervirens</i> (5)	1	1	+
France (continental)								
15	Saint-Michel en Grèves	3°34'W, 48°41'N	n.a.	Hedge row	<i>C. macrocarpa</i> (5)	0	26*	+
16	Lavercaillère	1°24'E, 44°36'N	350	Natural stand	<i>J. communis</i> (3)	0	14	+
17	Prénouvellon	1°31'E, 47°58'N	100	Ornamental	<i>C. sempervirens</i> (2)	0	33	+
18	Livernon	1°50'E, 44°37'N	400	Natural stand	<i>J. communis</i> (3)	0	20	+
19	Orléans La Source	1°02'E, 47°84'N	100	Ornamental	<i>C. sempervirens</i> (2)	0	20*	+
20	Chilly-Mazarin	2°18'E, 48°42'N	n.a.	Ornamental	<i>C. sempervirens</i> (2)	0	19*	+
21	Brétigny sur Orge	2°18'E, 48°36'N	n.a.	Hedge row	<i>C. arizonica</i> (5)	0	0	+
22	Carcassonne	2°19'E, 43°12'N	n.a.	Plantation	<i>C. sempervirens</i> (5)	11	3	+
23	Saint-Christophe	2°24'E, 44°22'N	n.a.	Plantation	<i>C. sempervirens</i> (5)	0	6	+

Table 1 (continued).

Site No.	Site	Coordinates	Elevation (m)	Type	Host	<i>O. maculatus</i>	<i>O. depressus</i>	Cones
24	Marcillac	2°28'E, 44°28'N	552	Natural stand	<i>J. communis</i> (2)	0	266	+
25	Salles-la-Source	2°31'E, 44°27'N	600	Natural stand	<i>J. communis</i> (5)	0	20	+
26	Savigny sur Orge	2°37'E, 48°66'N	92	Hedge row	<i>C. sempervirens</i> (5)	1*	21*	+
27	Nogent/Vernisson-Barres	2°43'E, 47°50'N	100	Ornamental	<i>C. macrocarpa</i> (1)	0	7*	+
28	Sallèles del Bosc	3°25'E, 43°40'N	300	Hedge row	<i>C. sempervirens</i> (10)	4	0	+
29	Vogüé	4°29'E, 44°33'N	n.a.	Hedge row	<i>C. sempervirens</i> (5)	5	5	+
30	Saint-Crépin	6°36'E, 44°45'N	1000	Natural stand	<i>J. communis</i> (10)	0	14	+
31	Vaison-la-Romaine	6°36'E, 44°45'N	1000	Natural stand	<i>J. thurifera</i> (10)	0	0	—
		5°07'E, 44°16'N	n.a.	Natural stand	<i>J. communis</i> (5)	0	0	+
		5°07'E, 44°16'N	n.a.	Natural stand	<i>J. oxycedrus</i> (5)	0	0	+
		5°07'E, 44°16'N	n.a.	Natural stand	<i>J. phoenicea</i> (5)	0	0	+
32	Malauçène	5°08'E, 44°10'N	378	Plantation	<i>C. arizonica</i> (1)	6*	1*	+
33	Vitrolles	5°08'E, 44°10'N	378	Plantation	<i>C. sempervirens</i> (10)	24	0	+
		5°14'E, 43°27'N	n.a.	Plantation	<i>C. sempervirens</i> (5)	10	0	+
		5°14'E, 43°27'N	n.a.	Plantation	<i>C. atlantica</i> (5)	0	0	—
34	Bornes les Mimosas	6°32'E, 43°14'N	93	Plantation	<i>C. arizonica</i> (10)	0	0	+
		6°32'E, 43°14'N	93	Plantation	<i>C. funebris</i> (10)	0	0	+
		6°32'E, 43°14'N	93	Plantation	<i>C. goveniana</i> (10)	0	0	+
		6°32'E, 43°14'N	93	Plantation	<i>C. lusitanica</i> (10)	0	0	+
		6°32'E, 43°14'N	93	Plantation	<i>C. sempervirens</i> (15)	107	0	+
		6°32'E, 43°14'N	80	Hedge row	<i>C. arizonica</i> (1)	12	0	+
		6°32'E, 43°14'N	80	Hedge row	<i>C. sempervirens</i> (5)	37	0	+
35	Palayson	6°38'E, 43°28'N	330	Seed orchard	<i>C. sempervirens</i> (45)	23	0	+
		6°38'E, 43°28'N	330	Natural stand	<i>J. phoenicea</i> (1)	0	0	+
France-Corsica								
36	Sotta	9°13'E, 41°33'N	150	Hedge row	<i>C. sempervirens</i> (10)	26	0	+
37	Conca	9°19'E, 41°42'N	86	Hedge row	<i>C. sempervirens</i> (10)	14	1	+
38	San Giuliano	9°29'E, 42°18'N	20	Hedge row	<i>C. sempervirens</i> (5)	9	0	+
39	Aleria	9°30'E, 42°05'N	n.a.	Hedge row	<i>C. macrocarpa</i> (5)	0	0	+
		9°30'E, 42°05'N	n.a.	Hedge row	<i>C. sempervirens</i> (5)	5	0	+

Table 1 (continued).

Site No.	Site	Coordinates	Elevation (m)	Type	Host	<i>O. maculatus</i>	<i>O. depressus</i>	Cones
Tunisia								
40	Bou Abdallah (Makhtar)	9°25'E, 35°51'N	795	Naturalized	<i>C. sempervirens</i> (10)	12	2	+
41	Beni Ayeche	10°23'E, 36°41'N	50	Hedge row	<i>C. sempervirens</i> (5)	2	1	+
Italy								
42	Roselle	11°05'E, 42°40'N	5	Seed orchard	<i>C. sempervirens</i> (24)	7	0	+
	Trentino	11°07'E, 46°03'N	50–350	Plantation	<i>C. sempervirens</i> (170)	85	0	+
43	Monte Morello	11°15'E, 42°50'N	610	Plantation	<i>C. sempervirens</i> (59)	170	0	+
44	Perugia	12°22'E, 43°07'N	n.a.	Ornamental	<i>C. sempervirens</i> (5)	25	0	+
45	Barbarano-Vicentino	11°32'E, 45°24'N	250	Plantation	<i>C. sempervirens</i> (10)	30	0	+
Montenegro								
46	Budva	18°50'E, 42°17'N	100	Ornamental	<i>C. sempervirens</i> (2)	5	0	+
47	Podgorica	19°16'E, 42°26'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	11	0	+
Greece (continental)								
48	Pyllos	21°40'E, 36°57'N	10	Natural stand	<i>J. phoenicea</i> (1)	0	150*	+
		21°40'E, 36°57'N	10	Naturalized	<i>C. sempervirens</i> (5)	15	0	+
49	Methoni	21°42'E, 36°50'N	50	Hedge row	<i>C. sempervirens</i> (5)	6	0	+
50	Vassilikion	21°43'E, 36°38'N	500	Hedge row	<i>C. sempervirens</i> (5)	10	0	+
51	Megalopolis	22°06'E, 37°23'N	520	Naturalized stand	<i>C. sempervirens</i> (1)	4	0	+
52	Exochori	22°15'E, 36°53'N	750	Hedge row	<i>C. sempervirens</i> (2)	6	0	+
53	Lilea	22°30'E, 38°38'N	490	Naturalized stand	<i>C. sempervirens</i> (2)	6	0	+
54	Plaka Lithókoron	22°33'E, 40°05'N	10	Hedge row	<i>C. sempervirens</i> (1)	6	0	+
55	Almyros	22°46'E, 39°11'N	300	Hedge row	<i>C. sempervirens</i> (1)	12	0	+
56	Thírvai	23°19'E, 38°18'N	475	Naturalized stand	<i>C. sempervirens</i> (5)	7	0	+
57	Voula	23°46'E, 37°49'N	50	Hedge row	<i>C. sempervirens</i> (5)	12	0	+
58	Kavalla	24°22'E, 40°56'N	210	Plantation	<i>C. sempervirens</i> (5)	3	0	+
Greece-Crete								
59	Aghios Ioannis	24°01'E, 35°13'N	915	Natural stand	<i>C. sempervirens</i> (5)	13	0	+
60	Aradena	24°03'E, 35°13'N	677	Natural stand	<i>C. sempervirens</i> (3)	11	0	+

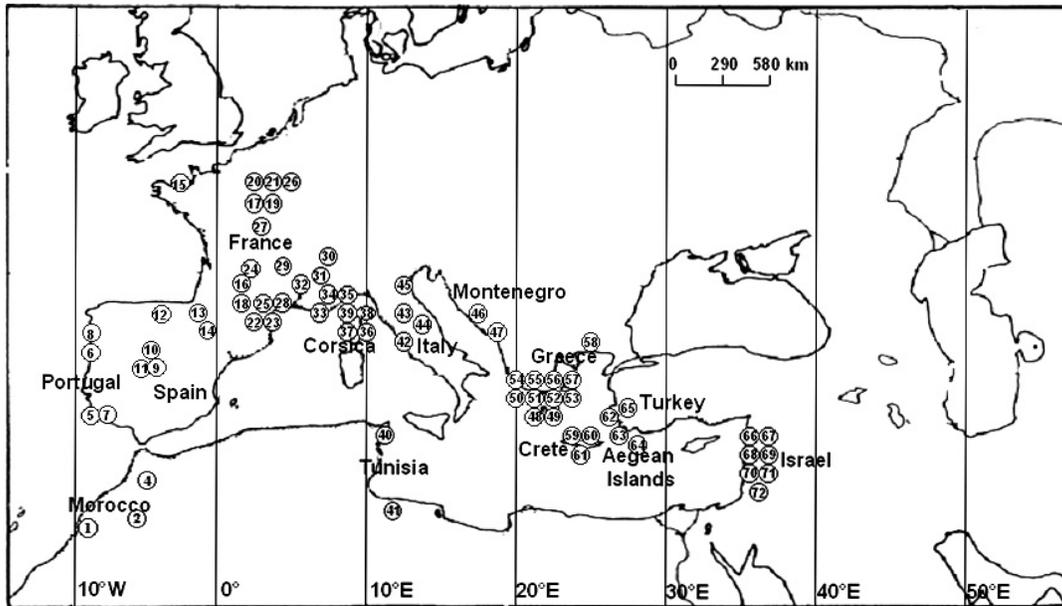
Table 1 (concluded).

Site No.	Site	Coordinates	Elevation (m)	Type	Host	<i>O. maculatus</i>	<i>O. depressus</i>	Cones
61	Frès	24°09'E, 35°23'N	105	Natural stand	<i>C. sempervirens</i> (5)	9	0	+
Aegean Islands								
62	Samos	26°42'E, 37°44'N	427	Natural stand	<i>C. sempervirens</i> (5)	5	0	+
63	Kos	27°14'E, 36°51'N	215	Natural stand	<i>C. sempervirens</i> (5)	6	0	+
64	Rhodos	27°57'E, 36°15'N	201	Natural stand	<i>C. sempervirens</i> (5)	4	0	+
Turkey								
65	Kusadasi	27°15'E, 37°51'N	50	Natural stand	<i>C. sempervirens</i> (5)	7	0	+
Israel								
66	Bequoa	34°56'E, 31°50'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	1	0	+
67	Esh'ta'ol	35°00'E, 31°47'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	1	0	+
68	Bet Me'ir	35°02'N, 31°48'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	2	0	+
69	Elyakim	35°08'E, 32°37'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	9	0	+
70	Bet Shé'arim	35°10'E, 32°41'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	16	0	+
71	Meggido	35°10'E, 32°34'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	5	0	+
72	Segev	35°15'E, 32°51'N	n.a.	Plantation	<i>C. sempervirens</i> (10)	12	0	+
China								
	Zitong (Sichuan)	105°11'E, 31°43'N	775	Natural stand	<i>C. funebris</i> (15)	0	1 nymph*	+
South Africa								
	Capetown	18°27'E, 33°57'S	300	Ornamental	<i>C. sempervirens</i> (3)	0	0	+
United States (California)								
	Point Lobos	121°57'W, 36°31'N	50	Natural stand	<i>C. macrocarpa</i> (20)	0	0	+

Note: Sites are shown by decreasing latitude within the same country. Numbers in parentheses in the host column are the number of trees sampled per site. Numbers in *Orsillus* columns correspond to the total number of adults found in cone collections in *Cupressus* spp. and in cone collections plus branch beatings in other tree species. The cone column indicates the presence (+) or absence (-) of maturing cones on the trees. n.a., no available data.

*First report in the country or on the host tree species.

Fig. 1. Locations of the Cupressaceae stands and plantations sampled during the study. The numbers correspond to the site numbers listed in Table 1.



O. maculatus and a vertical black line from the top to the bottom of the pronotum for *O. depressus*) as proposed by Mulsant and Rey (1872), Dioli (1991), and Péricart (1998). Then, the cones were dissected scale by scale. The emergence holes of seed chalcids and the inner side of cone scales were examined for the presence of bug egg masses and (or) salivary tracks indicating the insertion of a rostrum between cone scales to feed (Battisti *et al.* 1999). The presence of egg masses was interpreted as an indication that the cone of the considered Cupressaceae species was suitable for oviposition, and the presence of bug salivary tracks indicated that the cone was suitable for bug feeding.

Comparison of the life histories of *O. maculatus* and *O. depressus* under laboratory conditions

The durations of egg and nymph development have already been identified for *O. maculatus* (Battisti *et al.* 1997). To determine the corresponding values for *O. depressus*, 15 males and 15 females of this species were collected in central France on *C. sempervirens* (site 17, Table 1) and reared together in a 30 cm × 30 cm aerated plastic box for one generation. Insects were fed with foliage of *C. sempervirens* bearing 3-year-old cones. Branches were changed weekly. The box was stored in a

climatic chamber at 20 °C under 16L:8D conditions and checked daily for egg masses in cones and egg hatch to compare egg development patterns. We avoided handling of early-instar nymphs because they appeared very delicate. To assess the number of developmental stages, successive moults were recorded through daily sampling of nymphal exuviae in the box until all nymphs had reached the adult stage. For comparison, 30 males and 30 females of *O. maculatus* collected on *C. sempervirens* in southern France (site 34, Table 1) were reared under similar conditions.

An additional experiment used adults of *O. depressus* collected on *Juniperus communis* L. in southern France (site 24, Table 1). A total of 15 males and 15 females were reared under conditions similar to those described above but using branches of *J. communis* bearing 2-year-old mature cones. Feeding and egg laying were observed on cones, foliage, and branches. After 15 days of rearing, the juniper branches were replaced by branches of evergreen cypress. At the same time, adults of *O. maculatus* (15 males and 15 females from site 34) were reared on juniper branches bearing cones for 15 days.

Life history of *O. maculatus* under field conditions

This study was conducted in a plantation of *C. sempervirens* in Italy, where a large pop-

ulation of *O. maculatus* was previously recorded (Battisti *et al.* 2003). We used a family test, Fonte dei Seppi, located at Monte Morello, approximately 7.5 km north of Florence, Tuscany, Italy (42°50'N, 11°15'E, 610 m altitude), which is operated by the Institute of Forest Tree Pathology of Florence with the Italian National Research Council (CNR). The family test was planted in 1984 using 1-year-old seedlings, including 40 seedlings for each of 30 families. In 1998, we selected five half-sib families showing normally grown and apparently healthy trees. To survey the phenology of seed bug development, six trees in each family were randomly sampled every 15 days between 7 May and 30 November 1999 (*i.e.*, 14 times). Each sample consisted of a random collection of 30 mature, 3-year-old cones and 30 overmature, 4-year-old cones. Sampled cones were stored at 5 °C until analysis, which was completed within 10 days of the date of collection. The partly opened cones, which may have contained bug nymphs, were individually put into plastic boxes, whereas closed or entirely opened cones were put together into a sealed plastic bag. Finally, all the cones were examined in the laboratory for the number of egg masses, nymphs at different developmental stages, and adult seed bugs.

Comparative morphology of adults and nymphs of *Orsillus* seed bugs

The frequency distributions of body and rostrum length were assessed for males and females of both species. Body and rostrum length were measured on 138 adult females and 186 males of *O. maculatus* and on 266 females and 258 males of *O. depressus*. We then calculated a rostrum length : body length ratio (hereafter, R:Bd) for each specimen. Different populations of seed bugs were used to evaluate the influence of geographical location on morphological variables. The effect of host on rostrum length was tested between populations within the same country: females of *O. depressus* were compared between host trees in France (sites 15–20, 24, 26, 27, and 30; Table 1), Morocco (sites 1 and 2; Table 1), and Portugal (sites 5, 6, and 8; Table 1). Populations of *O. maculatus* sampled on *C. sempervirens* from France, Italy, and Greece were compared for rostrum length (sites 32, 34–38, 43, 45, 59, 60, and 61; Table 1).

The morphological characters of nymphal instars were assessed in both species, using 17 to 30 nymphs of each of the developmental stages.

The nymphs originated from France and Italy. As in adults, body and rostrum length were measured for each nymph, and the R:Bd ratio was calculated. Changes in nymph color were also recorded throughout development and compared between species.

Observations and measurements were made using a Leica® MZ12 stereomicroscope equipped with a Leica DC100 digital camera and Leica QWin® image processing and analysis software (version 2.3).

Data analysis

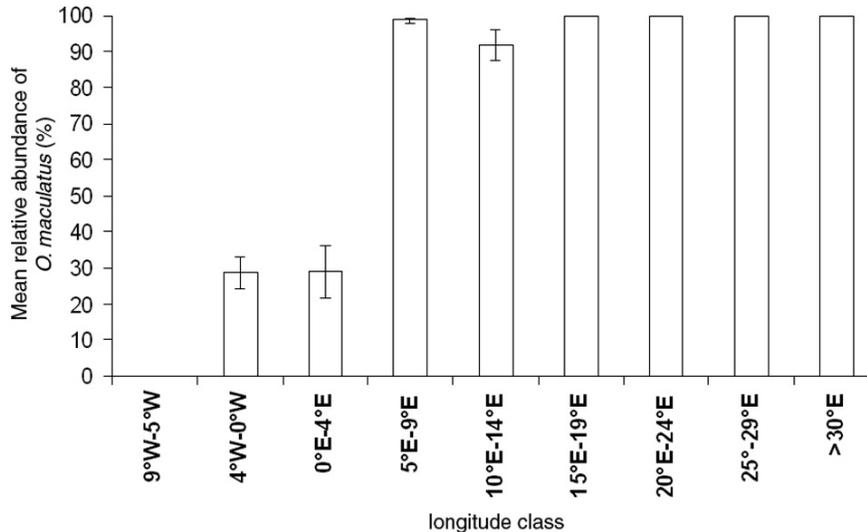
The relationship between the geographical coordinates (latitude, longitude) of sampled sites and the relative abundance (percentage of the total number of seed bugs observed) of each *Orsillus* species at those sites was tested using Pearson's correlation test (Zar 1999). Body length, rostrum length, and R:Bd ratio observed in males, females, and nymphs at each developmental stage were compared between the two species using Student's *t* test (Zar 1999). In parallel, the relationship between body length and rostrum length of individuals was tested using Pearson's correlation test. ANOVA was used to test for variation in adult size of both species throughout their distribution range. In this analysis, we used only the individuals sampled on *C. sempervirens* and pooled the data per country and per *Orsillus* species before analysis. ANOVA was also used to evaluate the effect of host tree on adult size. When necessary, data were transformed using either $\log(x + 1)$ or arcsine transformation to satisfy normality and homoscedasticity requirements (Zar 1999). Analyses were conducted with the statistical software STATISTICA® for Windows, version 5.5 (2000) (StatSoft Inc., Tulsa, Oklahoma).

Results

Geographical distribution and host range of *Orsillus* seed bugs

Table 1 presents the numbers of *Orsillus* species sampled at each location. One of the two species was present at most sites. All samples from *C. sempervirens* in its native range (the eastern Mediterranean) and in continental Greece, Montenegro, and Israel contained only specimens of *O. maculatus* (Table 1). In the remainder of the introduction area of *C. sempervirens*, the relative abundance of *O. maculatus* decreased from east to west of the

Fig. 2. Relationship between the mean (\pm SE) abundance (percentage of total number of seed bugs sampled) of *Orsillus maculatus* on *Cupressus sempervirens* and longitude (increasing from west to east of the Mediterranean Basin).



Mediterranean Basin, being negatively correlated with longitude (Pearson's correlation test: $r = 0.77$, $P < 0.05$, $n = 51$; Fig. 2). In the central part of the Basin (Corsica, southeastern France, Italy, and Tunisia), *O. maculatus* was still largely dominant on *C. sempervirens*, representing an average of 99.4% of the sampled bugs, but this percentage decreased to 17.7% in the western part of the Basin (western France, Spain, Portugal, and Morocco), where *O. depressus* was the dominant species, as well as in northern France (Table 1). During this study, *O. maculatus* and *O. depressus* were observed for the first time on *C. sempervirens* in northern France and Portugal, and *O. depressus* was observed for the first time in Morocco.

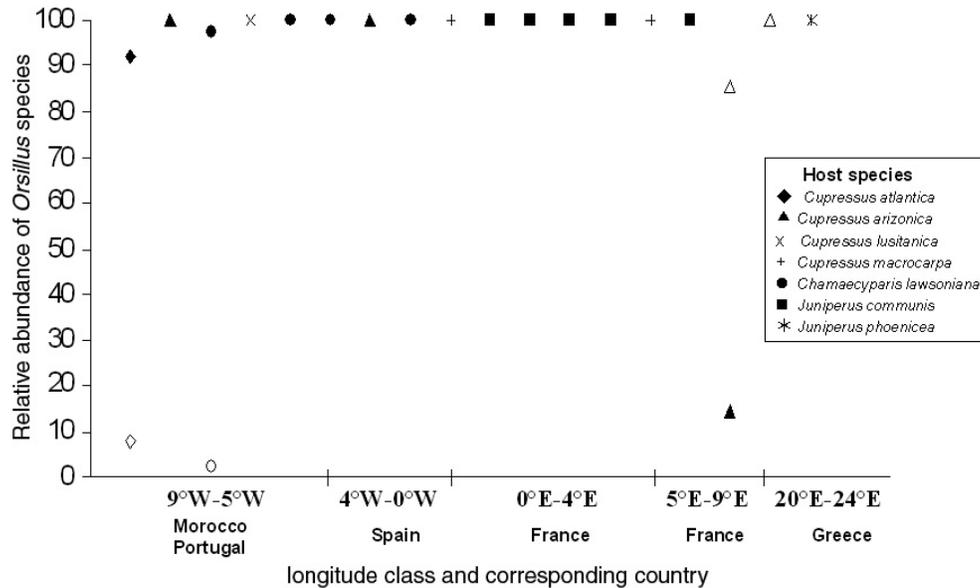
Samples from other species of Cupressaceae were dominated by *O. depressus* (Fig. 3). *Orsillus depressus* was found on a native Moroccan cypress (*C. atlantica*), introduced cypresses (*C. arizonica*, *Cupressus lusitanica* Mill., and *C. macrocarpa*), native junipers (*J. communis* and *J. phoenicea* L. but not *J. thurifera*), and an exotic Port Orford cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.), whereas only very low densities of *O. maculatus* were found on *C. arizonica*, *C. atlantica*, and *C. lawsoniana*, and none were found on junipers (Table 1). The relative abundance of *O. maculatus* was insignificant at all sites except two plantations of *C. arizonica* in southeastern France (sites 32 and 34), where

this exotic species was mixed with *C. sempervirens* (Fig. 3). In contrast, *C. arizonica* was colonized by *O. depressus* only when isolated (site 4 in Morocco and site 9 in Spain). *Orsillus depressus* was especially abundant in a native stand of *C. atlantica* in Morocco, where it represented 92.2% of the sampled bugs (site 2), on *J. communis* (site 24) and *J. phoenicea* (site 48), and on the exotic species *C. lawsoniana* (sites 6–8). In southern Greece, a clear segregation was observed: at site 48, *O. depressus* was found only on *J. phoenicea* and *O. maculatus* was found only on *C. sempervirens*. *Orsillus depressus* was also sampled at a relatively high altitude in Morocco (site 2) and in France (site 30).

No *Orsillus* specimens were observed in the samples collected in California on native *C. macrocarpa* and in South Africa on introduced *C. sempervirens*, but a nymph of an unidentified *Orsillus* species was found in cones of native *Chamaecyparis funebris* (Endl.) Franco in China (Table 1).

On all Cupressaceae, seed bugs were found only on trees or shrubs bearing mature cones (*i.e.*, cones in their 2nd or 3rd year of development). For example, *O. maculatus* was sampled at Vitrolles (site 33, Table 1) on the few *C. sempervirens* trees carrying cones, but no adults were sampled on the immature, nonflowering trees of *C. atlantica*. At Marcillac (site 24, Table 1), a total of 266 adults of

Fig. 3. Comparison of the relative abundances (percentages of total number of seed bugs sampled) of *Orsillus maculatus* (open symbols) and *O. depressus* (solid symbols) on Cupressaceae species other than *Cupressus sempervirens* as longitudes increase from west to east of the Mediterranean Basin.



O. depressus were sampled on two *J. communis* shrubs bearing maturing berries in 2001. In contrast, no adults were found on the same trees in 2002 when those trees had no berries. A similar situation was observed on *C. atlantica* in a natural stand at Idni, Morocco (site 2, Table 1), where a large number of *O. depressus* and a few *O. maculatus* were sampled in 1998 on trees with 3-year-old cones but no bugs were found in 1999 when no mature cones were present.

Comparison of the life histories of *O. depressus* and *O. maculatus* under laboratory conditions

Mating and feeding

In both *Orsillus* species, mating occurs abdomen against abdomen, with the male shaking its abdomen from left to right in front of the female before mating. Under our laboratory conditions, females of *O. depressus* usually laid groups of eggs either in the emergence holes of *M. wachtli* or on the inner side of cone scales, as previously observed for *O. maculatus* (Battisti *et al.* 1997).

On *C. sempervirens*, *O. depressus* was observed to feed on seeds by inserting its rostrum between cone scales in a way similar to that of *O. maculatus*, leaving salivary tracks on scales. On *J. communis*, no salivary tracks were noticed

on or in the cones, but *O. depressus* was observed to feed directly on the foliage. Salivary tracks were also noticed on the bark of *J. communis*. Adults of *O. depressus* collected on *J. communis* and reared in the laboratory on *C. sempervirens* fed on cypress cones, on which they oviposited within a week of the onset of rearing. In contrast, adults of *O. maculatus* collected from *C. sempervirens* did not survive more than a week when placed on *J. communis* in the laboratory, and no traces of feeding were observed.

Egg development

The duration of egg development was approximately 19 days for both species (Table 2). Variation in egg coloration during embryo development was similar in *O. maculatus* and *O. depressus*. Eggs were light yellow immediately after oviposition but then became dark yellow, and red punctuations subsequently appeared within the embryo and at the site of future eyes. Just before hatching, embryos were yellowish red with many red punctuations. The head of embryos was always directed towards the aeromicropyles.

Nymph development

Just after hatching, first-instar nymphs were pinkish white, with legs and antennae always folded down along the body, and then became

Table 2. Comparison of development of eggs and nymphs of *Orsillus depressus* and *O. maculatus* under laboratory conditions.

Orsillus species	Egg	Nymphal instar				
		L1	L2	L3	L4	L5
<i>O. depressus</i>	19.1±0.3 (39)	10.2±0.1 (135)	10.1±0.3 (80)	6.2±0.1 (94)	11.5±0.1 (81)	14.5±0.3 (100)
<i>O. maculatus</i>	18.6±0.1 (754)	7.3±0.3 (36)	9.1±0.4 (18)	9.4±0.6 (14)	8.8±0.3 (36)	19±0.7 (20)

Note: Values are the duration (mean ± SE) of each developmental stage, in days. The numbers in parentheses indicate the numbers of individuals measured per developmental stage.

dark brown. The presence of translucent, empty chorions allowed us to locate the position of eggs on the cones once nymphs emerged. First-instar nymphs of *O. maculatus* were observed using the emergence holes of *M. wachtli* to enter the cypress cones. Under these laboratory conditions, *O. depressus* had five nymphal stages, as previously reported for *O. maculatus* (Battisti *et al.* 1997). Although the mean duration of complete nymph development was similar in the two species (53.6 days in *O. maculatus* versus 52.5 days in *O. depressus*), the development of specific nymphal stages was either longer (third and fifth instars) or shorter (first and fourth instars) in *O. maculatus* than in *O. depressus* (Table 2). Following each moult, nymphs were pinkish white but quickly turned dark brown with red punctuations. Nymph coloration lightened before each moult. There was no difference in coloration during nymph development that allowed differentiation of *Orsillus* species.

Life history of *O. maculatus* under field conditions

All nymphal stages were observed in cones between May and October (Fig. 4). The mean number of nymphs in the first three instars of development was similar throughout the season (ANOVA: $F_{4,243} = 1.81$, $P = 0.12$), but there was a seasonal effect on the mean number of fourth- and fifth-instar nymphs in the cones ($F_{4,61} = 2.5$, $P = 0.04$) (Fig. 4). More than 50% of adults collected over the season were obtained in September, coincidentally with the main period of oviposition. Eggs were laid during all seasons except winter (Fig. 5). Early-instar nymphs and adults sheltered in cones to overwinter (Figs. 4, 5).

The life history of *O. depressus* could not be precisely established in the field. However, we found egg masses on the cone scales of an isolated *C. sempervirens* in central France (Orléans La Source) in May 2000 and in cones of *C. arizonica* in Morocco in May 2002 (Table 1). No egg masses were ever found on *C. lawsoniana* and *J. phoenicea*, but a few eggs were found on cone scales of *C. macrocarpa* and under the cuticle of old cones of *J. communis*. For *O. maculatus*, egg masses and salivary tracks were observed with certainty only on *C. sempervirens* (Table 3). On *C. arizonica* and *C. atlantica*, either no egg mass was present or *O. depressus* was sampled together with *O. maculatus* so that we could not

Fig. 4. Seasonal variation in the mean number of early-instar (L1L2L3: total of first, second, and third nymphal instars) and late-instar (L4L5: total of fourth and fifth nymphal instars) nymphs of *Orsillus maculatus* per cone of *Cupressus sempervirens* from May to October 1999 at Monte Morello, Italy.

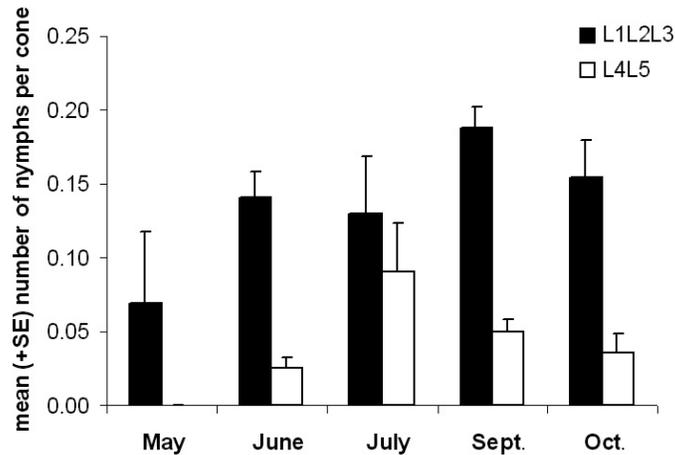
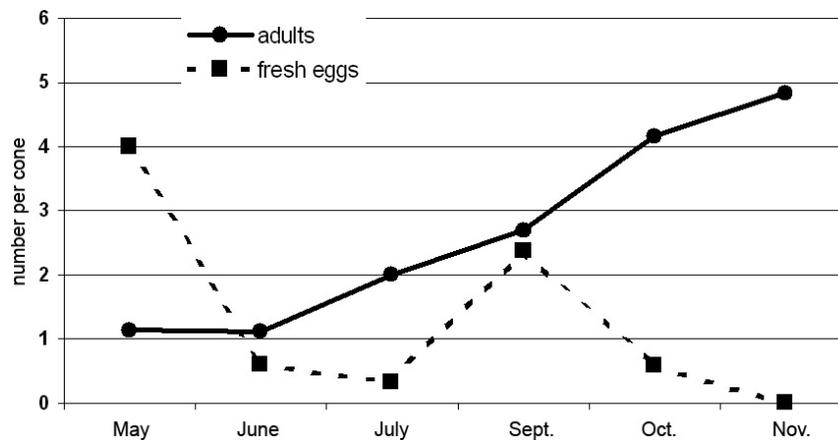


Fig. 5. Seasonal variation in the number of adults and fresh eggs of *Orsillus maculatus* per cone of *Cupressus sempervirens* from May to October 1999 at Monte Morello, Italy.



determine which species had laid the eggs (Table 3).

Comparative adult morphology of adults and nymphs of *Orsillus* seed bugs

Body length and length of the rostrum, as well as their ratio (R:Bd), were significantly lower in *O. depressus* than in *O. maculatus* for both males and females (Table 4). The length of the rostrum was significantly correlated with that of the body in adults of both species (*O. maculatus*: $r = 0.90$, $P < 0.05$, $n = 324$; *O. depressus*: $r = 0.77$, $P < 0.05$, $n = 524$). There was no significant difference in the R:Bd ratio between sexes in *O. maculatus* ($F_{1,324} = 1.75$, $P = 0.19$), whereas males showed a

significantly higher ratio than females in *O. depressus* ($F_{1,524} = 44.71$, $P < 0.01$; Table 4).

In *O. maculatus*, the rostrum length did not differ between females sampled in the native range of *C. sempervirens* in Greece and those sampled in the introduced range of the tree in Italy, France, and Corsica ($F_{3,135} = 2.6$, $P = 0.055$). In *O. depressus*, the rostrum length did not differ significantly between females sampled on *C. atlantica* and those found on *C. sempervirens* in Morocco ($F_{1,36} = 3.5$, $P = 0.07$). However, females of *O. depressus* living on *C. sempervirens* in France had a significantly longer rostrum than those found on *J. communis* and *C. macrocarpa* ($F_{2,168} = 50.25$,

Table 3. Summary of host utilization by *Orsillus maculatus* and *O. depressus* in Europe and the Mediterranean area.

Tree species	<i>O. maculatus</i>		<i>O. depressus</i>	
	Egg mass	Salivary sheath	Egg mass	Salivary sheath
<u><i>Cupressus arizonica</i></u>	—	+	+	+
<i>Cupressus atlantica</i>	+	+	+	+
<u><i>Cupressus lusitanica</i></u> *	—	—	+	+
<u><i>Cupressus macrocarpa</i></u>	—	—	+	+
<i>Cupressus sempervirens</i>	+	+	+	+
<u><i>Chamaecyparis lawsoniana</i></u>	?	?	?	?
<i>Juniperus communis</i>	—	—	+	+
<i>Juniperus excelsa</i> *	—	—	?	?
<i>Juniperus oxycedrus</i> *	—	—	?	?
<i>Juniperus phoenicea</i>	—	—	?	+
<i>Juniperus thurifera</i> *	—	—	?	?
<u><i>Thuja plicata</i></u> *	—	—	?	?
<u><i>Thuja orientalis</i></u> *	—	—	+	?

Note: Exotic tree species imported from North America or Asia are underlined. ?, adult bugs recorded but no egg masses or salivary sheaths observed.

*Bibliographic data.

$P < 0.01$). Similarly, females sampled on *C. sempervirens* in Portugal had a longer rostrum than those found on *C. lawsoniana* ($F_{1,33} = 39.08$, $P < 0.01$).

All sampled populations of *O. maculatus* exhibited a mean R:Bd ratio higher than 0.85 for adult females, whereas this value was lower in populations of *O. depressus* (Fig. 6a). The interval 0.75–0.85 contained fewer than 5% of the sampled females of *O. maculatus*. Based on body and pronotum color, we identified as *O. maculatus* an individual on *C. atlantica* with an R:Bd ratio lower than 0.8, but the smallest female we found originated from Italy on *C. sempervirens* (site 45). For *O. depressus*, the interval 0.75–0.85 essentially corresponded to females that had *C. sempervirens* as host and originated from Spain, Morocco, and northern France (Fig. 6a).

The pattern was a bit different in males. Populations of *O. maculatus* showed a mean R:Bd ratio higher than 0.85 in approximately 93% of the cases, whereas 96% of the males of *O. depressus* presented a lower value, although values ranged from 0.62 to 0.89 (Fig. 6b). The interval 0.79–0.89 included mostly males of *O. depressus* originating from the same sites as the females cited above (Spain, Morocco, and northern France, with *C. sempervirens* as host).

First and second instars of *O. depressus* had longer bodies and rostrums than those of

O. maculatus (Table 4). The differences in body size disappeared from the third instar on, but the rostrum was significantly longer in *O. maculatus* during the fourth and fifth instars. The R:Bd ratio was significantly higher in *O. maculatus* than in *O. depressus* from the third nymphal instar (Table 4). The species can easily be differentiated from fourth and fifth instars (Fig. 7). From the fourth instar, 91% of the measured *O. maculatus* nymphs had an R:Bd ratio higher than 0.85. For 81% of the nymphs of *O. depressus* that we measured, the R:Bd ratio of fourth and fifth instars was lower than 0.85 (Fig. 7).

Discussion

Exotic tree species introduced into a new environment can be either accompanied by associated cohorts of insects from the original area or subjected to later colonization by indigenous insects that adapt to these new hosts (Krcmar-Nozic *et al.* 2000). The latter process generally implies the existence of preadaptations in the indigenous entomofauna (Frenzel *et al.* 2000). The human-mediated introduction of evergreen cypress, *C. sempervirens*, from Crete and the eastern Aegean Islands throughout the whole Mediterranean Basin probably began during the Roman period (Baumann 1982; Graniti 1998). Recent genetic studies showed that a seed

Table 4. Comparison of mean (\pm SE) body length (Bd), mean (\pm SE) rostrum length (R), and their ratio (R:Bd) among adults and nymphs of successive developmental stages of *Orsillus maculatus* and *O. depressus*.

Variable	Species	Adult female	Adult male	Stage				
				1st instar	2nd instar	3rd instar	4th instar	5th instar
				Rostrum length				
	<i>O. maculatus</i>	7.79 \pm 0.05	6.69 \pm 0.07	1.28 \pm 0.06	1.99 \pm 0.08	3.17 \pm 0.19	4.81 \pm 0.13	5.79 \pm 0.11
	<i>O. depressus</i>	5.01 \pm 0.04	4.64 \pm 0.03	1.53 \pm 0.06	2.37 \pm 0.12	3.22 \pm 0.09	4.23 \pm 0.09	4.34 \pm 0.07
<i>t</i> (df)		46.68 (331)	29.33 (328)	2.85 (17)	2.62 (19)	0.23 (28)	3.69 (15)	10.77 (21)
<i>P</i>		<i>0.000</i>	<i>0.000</i>	<i>0.011</i>	<i>0.017</i>	0.823	<i>0.002</i>	<i>0.000</i>
				Body length				
	<i>O. maculatus</i>	8.49 \pm 0.03	7.21 \pm 0.06	1.39 \pm 0.09	2.33 \pm 0.09	3.61 \pm 0.12	5.14 \pm 0.09	5.79 \pm 0.16
	<i>O. depressus</i>	7.08 \pm 0.05	6.28 \pm 0.04	1.76 \pm 0.10	2.84 \pm 0.10	3.97 \pm 0.13	5.00 \pm 0.05	5.96 \pm 0.11
<i>t</i> (df)		21.65 (331)	13.49 (328)	2.55 (17)	3.73 (19)	2.01 (28)	1.31 (15)	0.88 (21)
<i>P</i>		<i>0.000</i>	<i>0.000</i>	<i>0.02</i>	<i>0.001</i>	0.055	0.209	0.392
				Ratio R:Bd				
	<i>O. maculatus</i>	0.92 \pm 0.01	0.92 \pm 0.01	0.95 \pm 0.03	0.85 \pm 0.02	0.87 \pm 0.03	0.94 \pm 0.02	1.00 \pm 0.02
	<i>O. depressus</i>	0.71 \pm 0.01	0.74 \pm 0.01	0.87 \pm 0.03	0.83 \pm 0.02	0.81 \pm 0.01	0.85 \pm 0.02	0.73 \pm 0.01
<i>t</i> (df)		37.81 (331)	30.04 (328)	1.26 (17)	0.63 (19)	2.32 (28)	3.50 (15)	9.62 (21)
<i>P</i>		<i>0.000</i>	<i>0.000</i>	0.223	0.537	<i>0.03</i>	<i>0.003</i>	<i>0.000</i>

Note: Significant values of Student's *t* statistic ($P < 0.05$) are in italic.

Fig. 6. Variation in the ratio of rostrum length to body length (R:Bd) for females (a) and males (b) of *Orsillus maculatus* (open boxes) and *O. depressus* (shaded boxes). Dashed and solid horizontal lines indicate the intervals within which the R:Bd ratio is distributed for *O. maculatus* and *O. depressus*, respectively. Single individuals are indicated by a dash. Open circles represent individuals outside a normal distribution. Samples are ordered by site number (Table 1, Fig. 1). Host names are abbreviated as follows: Carz, *Cupressus arizonica*; Catl, *C. atlantica*; CM, *C. macrocarpa*; CS, *C. sempervirens*; Ch, *Chamaecyparis lawsoniana*; JC, *Juniperus communis*.

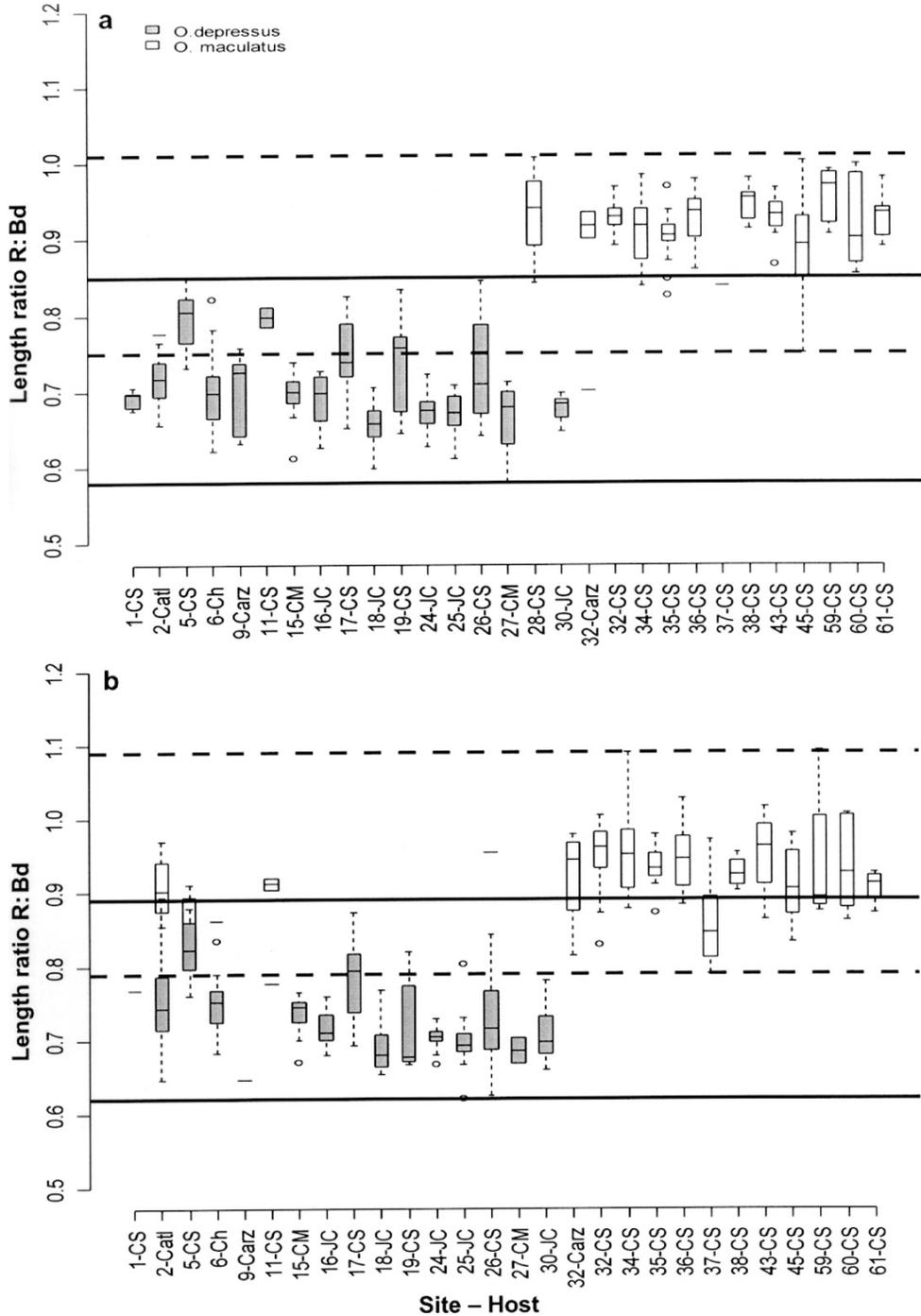
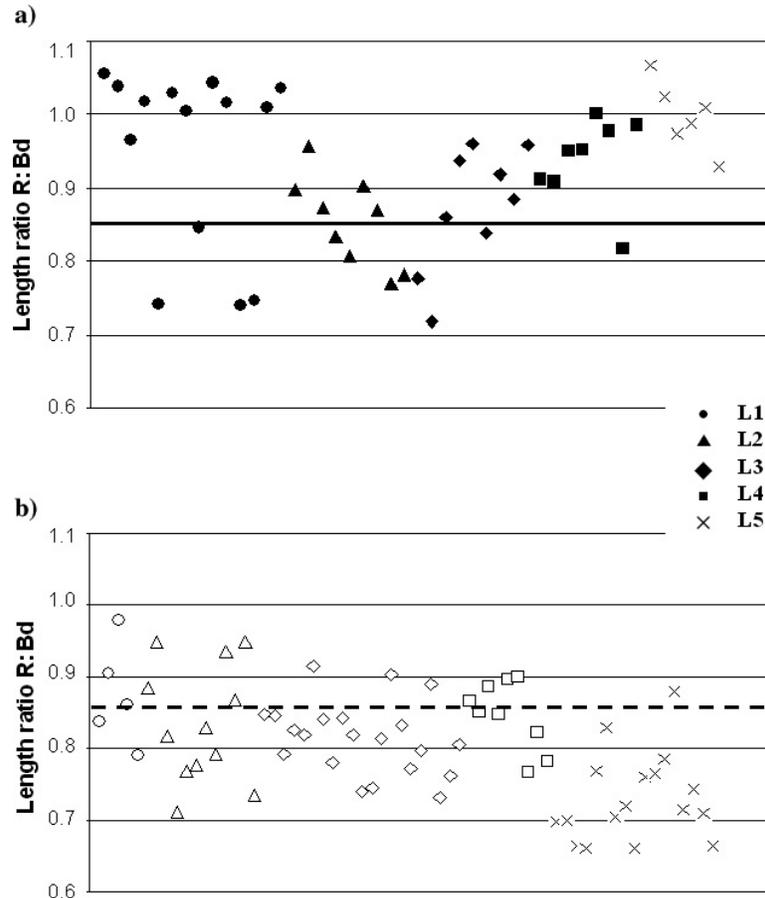


Fig. 7. Variation in the ratio of rostrum length to body length (R:Bd) among successive instars (L1 to L5) of nymphs of *Orsillus maculatus* (a) and *O. depressus* (b). Horizontal solid (a) and dashed lines (b) indicate the R:Bd ratio of 0.85 that separates nymphs of *O. maculatus* and *O. depressus* (see text).



chalcid closely associated with *C. sempervirens*, *M. wachtli*, expanded from these eastern Mediterranean areas towards the west together with the introduction of the host (Roques *et al.* 1999a; Rasplus *et al.* 2000). *Megastigmus wachtli* is now established everywhere that *C. sempervirens* has been introduced in Europe (Roques and Skrzypczynska 2003). Increasing abundance of *O. maculatus* in cones of evergreen cypress along a longitudinal gradient from east to west suggests that the same process probably occurred for this species. The exclusive occupancy of cypress cones by *O. maculatus* within and near the native range of *C. sempervirens*, as well as the observation of *O. depressus* in the same area only on junipers, suggests a long-term association between *C. sempervirens* and *O. maculatus* but not *O. depressus*. In contrast, the domination of *O. depressus* in the western part of the

Mediterranean Basin, which probably corresponds to a more recent introduction of evergreen cypress, indicates that the colonization of the introduction range of *C. sempervirens* by *O. maculatus* is not yet complete. It also suggests that competition between seed bug species for seed cone exploitation could perturb this process. However, our survey confirmed that *O. depressus*, but not *O. maculatus*, is closely associated with native junipers such as *J. communis* and *J. thurifera* (Dupuis 1965; Roques 1983; Blasco-Zumeta 2000) in places where evergreen cypress has not been introduced in western Europe. Thus, *O. depressus* may have shifted from native junipers to *C. sempervirens* and, more recently, to the other exotic Cupressaceae. This tended to be confirmed by our rearing experiments, where *O. depressus* originating from junipers developed on evergreen cypress but the converse

situation was not true for *O. maculatus*. However, given the large number of *O. depressus* individuals found in the native Moroccan range of Atlantic cypress, *C. atlantica*, we cannot exclude the possibility that *O. depressus* was first associated with this cypress and then moved east with junipers.

Both *Orsillus* species appear definitely linked to cones, since individuals were observed only on trees bearing maturing cones, although *O. depressus* can alternatively feed on foliage. The nearly identical durations of nymph development observed in the two species probably result from an adjustment to cone phenology, which is similar in most Cupressaceae, showing a 3-year reproductive cycle. This biological pattern may favor reciprocal shifts between Cupressaceae species.

Differences in bug morphology may also be important for cone exploitation because cone size is highly variable among Cupressaceae species. The significantly longer rostrum of adult *O. maculatus* may allow easier access to seeds within large cones such as those of *C. sempervirens*, whereas *O. depressus*, with a shorter rostrum, appears more closely associated with species with smaller cones such as *Juniperus* spp., *Chamaecyparis* spp., and *C. atlantica*. Such morphological adaptation to fruit size was observed for seed bugs feeding on seeds of the tree family Sapindaceae in the United States (Carroll and Loye 1987). Carroll and Boyd (1992) showed that the length of the buccal stylet in bug species of the genus *Jadera* Stal (Hemiptera: Rhopalidae) is correlated to the distance between the fruit surface and seed within the fruit. The bug species exploiting native Sapindaceae have a short buccal stylet, whereas those that have shifted to introduced Sapindaceae, which have bigger fruits, have a longer buccal stylet (Carroll *et al.* 1997). According to Carroll *et al.* (1997), the rapid differentiation of new seed bug species on the introduced hosts would have proceeded from both genetic and ecological processes. Adults of *O. depressus* found on *J. communis* in France had a shorter rostrum than those sampled on *C. sempervirens*, but rostrum length was not significantly different between the individuals sampled on junipers and those collected on an exotic cypress of Californian origin, *C. macrocarpa*, which presents the largest cones in the genus *Cupressus*. This suggests a more recent colonization of *C. macrocarpa*,

probably by bugs shifting directly from junipers.

In the introduced area of *C. sempervirens*, both *Orsillus* species may occur on the same tree, but morphological variability of both adults and nymphs often hinders accurate identification of the species. Based on frequency distribution, measurement of the length of the rostrum relative to that of the body does not seem to necessarily result in an accurate identification of males, depending on host and geographical location. However, this measure does seem to constitute a diagnostic character for specific identification of fourth- and fifth-instar nymphs. When the R:Bd ratio is above 0.85, the species can be identified as *O. maculatus*, whereas the species is likely *O. depressus* when the ratio is below this value. When the R:Bd ratio is between 0.75 and 0.85, it is necessary to take into account the host tree and the geographical location.

Another striking difference between the two *Orsillus* species resides in the relationship between seed chalcids and adult bugs. All over the native and introduced range of *C. sempervirens*, a close association exists between *O. maculatus* and *M. wachtli*, the female bug ovipositing primarily in exit holes made by chalcids on the cone surface (Battisti *et al.* 1999; G. Rouault, unpublished data). Such an association was not observed on *C. atlantica* in Morocco: neither *O. depressus* nor *O. maculatus* oviposited in the exit holes of an endemic chalcid, *Megastigmus atlanticus* Roques *et* Skrzypczynska (Roques and Skrzypczynska 2003). Unfortunately, no data exist about the closely related species *C. dupreziana* and its entomofauna in the native Algerian range. We did not observe any eggs of *O. depressus* in the exit holes of a juniper seed chalcid, *Megastigmus bipunctatus* Swederus, on cones of *J. communis* in France. It is thus likely that the ternary association between *O. maculatus*, *M. wachtli*, and *C. sempervirens* developed a long time ago in the native range of *C. sempervirens* and then expanded progressively into new areas with the introduction of cypress. This behavioral association seems unique between seed bugs and seed chalcids. The seed chalcid *Megastigmus spermotrophus* Wachtl and the seed bug *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) share seed exploitation in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco; Pinaceae) cones, but bug egg laying appears

independent of chalcid exit holes (Blatt and Borden 1998).

The introduction of North American species of Cupressaceae to Europe could have facilitated the geographical expansion of *Orsillus* spp. in a different way. Depending on the European country, such trees have been planted on a more or less large scale since 1900. *Orsillus maculatus* is diversifying in a limited way its host range and distribution area by colonizing plantations of *C. arizonica* and *C. atlantica*, but it has not yet shifted to species of other genera, with the exception of a unique individual found on *Chamaecyparis* sp. in Spain. In contrast, *O. depressus* has already colonized most of these introduced species all over western Europe and in the Mediterranean area. Although *O. depressus* seems more flexible in its choice of host for feeding, there is no clear evidence that it can effectively oviposit and completely develop on all of these exotic tree species. Colonization of exotic species could play an important part in the dissemination of the cypress canker pathogen, *S. cardinale*, as this fungus is considered to be primarily associated with the exotic species *C. macrocarpa*. Indeed, attack by fungi causes earlier opening of 3-year-old cones of *C. sempervirens*, allowing individuals of *O. depressus* (with short rostrum) to feed directly on seeds that are otherwise available only through cone penetration by the long rostrum of *O. maculatus*. Because adult bugs move from tree to tree to feed and lay eggs, the numerous man-made formations (plantations, ornamental trees, windbreak hedges) of exotic Cupressaceae, especially in urban and semi-urban areas, may act as relays for *Orsillus* adults, thus allowing a probably faster dissemination of the fungal spores. From this point of view, the plantations surrounding the natural ranges of endangered native cypress species such as *C. dupreziana* and *C. atlantica* must be especially surveyed with regard to the expansion of *Orsillus* bugs that may carry spores responsible for cypress canker.

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