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Original article

Impact of insects damaging seed cones of cypress, *Cupressus sempervirens*, in natural stands and plantations of southeastern Europe

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Abstract – A total of 18 stands of *Cupressus sempervirens* L. (Cupressaceae) were surveyed in the natural Greek range (plus one stand in Turkey) during 1994–1996 in order to identify the pests of seed cones and assess their impact on seed survival. Naturalised stands of mainland Greece, Albania and Malta were sampled for comparison. The cone entomofauna (seven insect and one mite species) did not differ between the native and introduced ranges of cypress. A tortricid, *Pseudococcyx tessulatana* (Lepidoptera:Tortricidae) and a mite, *Trisetacus juniperinus* (Acari:Nalepellidae), were the most damaging pests because they usually killed cones during the growth period. A more intensive survey of damage together with cone development in four Greek stands showed that only 11–37 % of the initial cones survived until maturity. The seed crop decreased by 78–95 %. Pests, predominantly tortricid larvae, mites and *Orsillus* seed bugs (Hemiptera:Lygaeidae), were responsible for 41–84 % of that decrease according to the stand. (© Inra/Elsevier, Paris.)

***Cupressus sempervirens* / insect pests/ cone/ seed / Greece**

Résumé – Impact des ravageurs des cônes et graines de cyprès, *Cupressus sempervirens*, dans des peuplements naturels et plantations du sud-est de l'Europe. Un total de 18 peuplements de *Cupressus sempervirens* L. (Cupressaceae) ont été échantillonnés en 1994–1995 dans l'aire naturelle grecque (plus un peuplement en Turquie) de l'essence en vue d'identifier les ravageurs des cônes et graines et estimer leur impact sur la survie des graines. Des peuplements naturalisés ont été étudiés pour comparaison en Grèce, en Albanie et dans l'île de Malte. Aucune différence d'entomofaune (sept espèces d'insectes et un acarien) n'a été constatée entre l'aire naturelle et les zones d'introduction du cyprès. La tordeuse *Pseudococcyx tessulatana* (Lepidoptera: Tortricidae) et l'acarien *Trisetacus juniperinus* (Acari : Nalepellidae) constituaient les ravageurs les plus importants, leur attaque induisant la disparition des cônes durant la période de croissance. Un inventaire de l'évolution des dégâts d'insectes au cours du développement des cônes dans quatre sites de Grèce a montré que seulement 11 à 37 % des cônes de départ atteignaient la maturité. La production de graines a diminué de 78 à 95 % par rapport au potentiel de départ. Les ravageurs, principalement les chenilles de tordeuses, les acariens, et les punaises *Orsillus* spp. (Hemiptera: Lygaeidae), ont été responsables de 41 à 84 % de cette diminution. (© Inra/Elsevier, Paris.)

***Cupressus sempervirens* / insectes ravageurs / cône/ graines / Grèce**

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1. INTRODUCTION

Cupressus sempervirens originates from the eastern part of the Mediterranean basin where its natural distribution covers northern Iran, Asia Minor, Crete and Cyprus [16]. However, this Cupressaceae species has been introduced on a large-scale basis throughout southern Europe and northern Africa for at least two millenniums, first by the Ancient Greeks, and then by the Romans [2]. Once established, these introduced specimens propagated along the Mediterranean coast, never forming extensive forest stands but rather small groves (hereafter referred to as naturalised stands) that generally consist of columnar, pyramidal trees (*C. sempervirens* var. *pyramidalis* Nyman). In the natural range, however, trees grow in pure stands (hereafter referred to as natural stands) consisting mostly of horizontally branched trees (*C. sempervirens* var. *horizontalis* [Mill.] Gord.). In Greece, natural stands of *C. sempervirens* are found in Crete and in some of the eastern Aegean islands (e.g. Kos, Samos, Rhodes and Simi) although forest fires are continuously decreasing their range. Only naturalised stands and plantations are found in mainland Greece and other countries of southeastern Europe.

Although the insect pests damaging cones and seeds of conifers have been extensively surveyed, most of the studies have focused almost exclusively on economically important species of the Pinaceae family, whereas the Cupressaceae has received little attention [15]. Most of the research on insects exploiting cones and seeds of *C. sempervirens* has been conducted in Italy [9, 10, 16], France [12], Greece [11], Morocco [7, 8], Algeria [4, 5] and Tunisia [3] where this tree species has been introduced. Research in the natural range, however, is much more limited [6, 13].

Thus, our objectives were to: *i)* identify the entomofauna attacking seed cones in the natural range of *C. sempervirens,* where the variety *horizontalis* is dominant; *ii)* assess insect impact on cones and seeds of *C. sempervirens* in these same areas; and *iii)* compare the composition and impact of the cone entomofauna between the natural and naturalised areas where the variety *pyramidalis* grows.

2. MATERIALS AND METHODS

2.1. Study sites

A total of 30 sites were surveyed in Greece, Turkey, Albania and Malta (*figure 1*). In the natural range of *C. sempervirens*, 19 stands distributed among three Greek islands of the eastern Aegean Sea, Crete and Turkey were surveyed to investigate the geographic variation in insect colonisation and damage. In Rhodes and Samos, several stands were sampled to assess damage variation within the island but only one stand could be sampled in Kos and in Turkey. In Crete, six stands ranging in altitude from sea level to > 1 000 m were surveyed. In the area where *C. sempervirens* was introduced, we surveyed three naturalised stands of northern Greece, two plantations near Athens, five plantations in Albania and one plantation in Malta.

2.2. Species richness and damage assessment

Standardised cone collections were carried out in June 1994 and May 1995 in Greece. All of the islands and regions of Greece were sampled both years, except that of Kos where collections were made in 1994 only. Thirteen of the 23 Greek sites were sampled both years. In each stand, two 40-cm branches (one from the lower crown and another from the mid- or upper crown) were selected randomly from each of ten trees. First, the branches were beaten immediately over a net to collect the insects present on the cone surface. The cones on these branches were removed and counted according to the cone development categories defined by Roques and Battisti [13] in order to homogenise the observations of entomologists with these of tree physiologists – i.e. the 1st year of cone development corresponds to the initiation and differentiation of flowers, the 2nd year to the cone growth period and the beginning of seed maturation (green cones), the 3rd year to the achievement of seed maturation and the beginning of seed dispersal (ash-grey cones), since the cones are too mature during the 4th year of development. Characteristics of the stand (i.e. cone crop size, climate, exposure) and of the sampled trees (i.e. type of crown: horizontal versus pyramidal, cone crop size, tree height and diameter and tree position in the stand) were also noted at the time of sampling. In the other surveyed countries, more limited collections of full-grown cones in the 2nd year of development were realised during 1995 (Turkey) and 1996 (Albania, Malta). In each stand, 20 to 100 cones were collected at random but no other data were surveyed.

At the laboratory, cone morphology (length [*L*], width [*W*] and volume [$\pi l(3W^2+L^2)/24$]) was measured for full-grown cones in the 2nd and 3rd year of development. The cones which were just entering the growing process, as well as half of the full grown cones which were in the 3rd and 4th year of development, were dissected to look for internal insect damage and for the presence of larvae. For each dissected cone, the seeds were extracted, and each seed lot was individually irradiated with X-rays

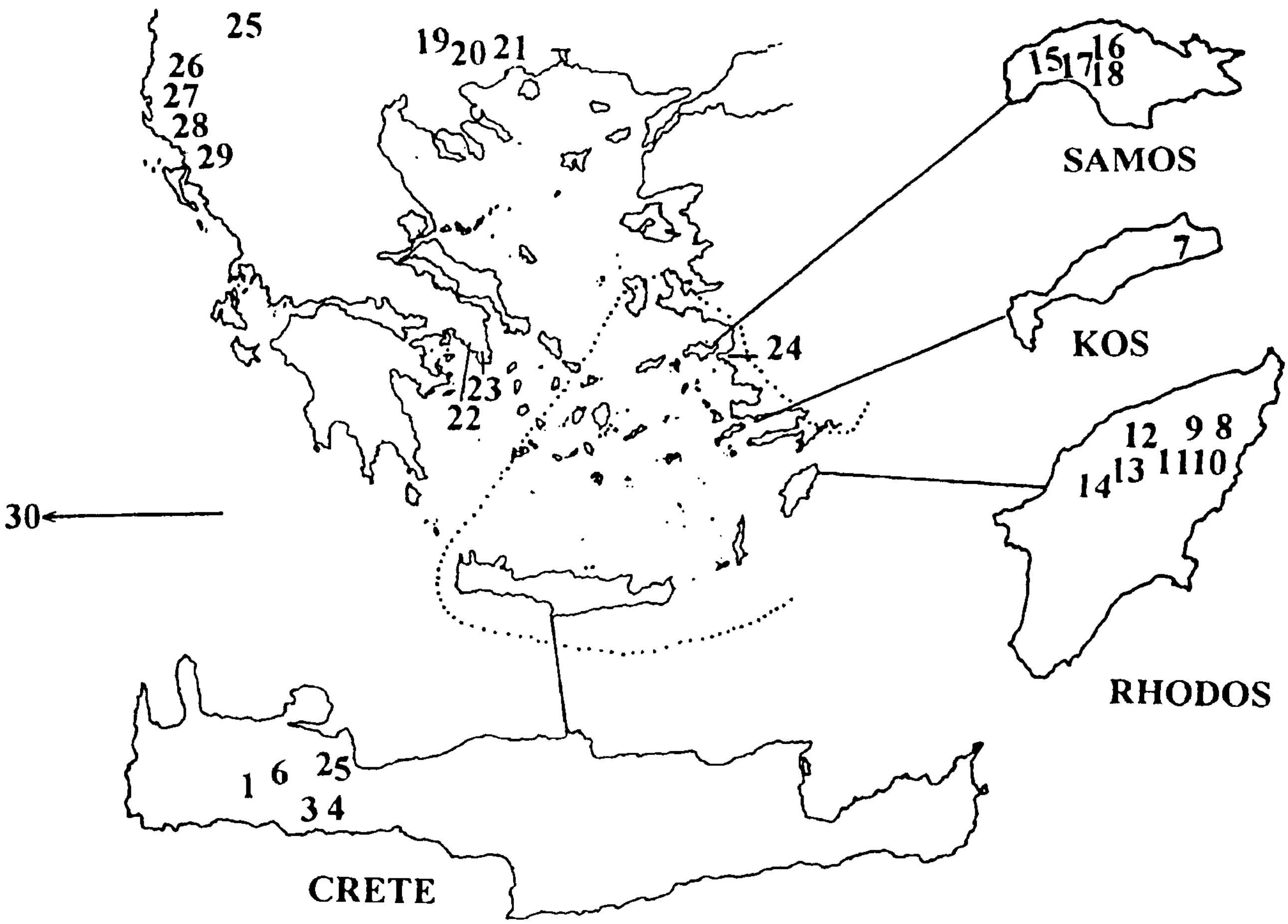

Figure 1. Location of the study sites. **Greece-Crete:** 1- Omalos, 2- Frès, 3- Aradena, 4- Anapolis, 5-Vrisses, 6- Zourva; **Greece-Kos:** 7- Aghios Dimitrios; **Greece-Rhodes:** 8- Alepohori, 9- Eleousa, 10- Plataria, 11- Appolonia, 12- Salakos I, 13- Salakos II, 14- Ebonas; **Greece-Samos:** 15- Metamorphosis, 16- Idrousa, 17- Kastania I, 18- Kastania II; **Greece-north:** 19- Nea Sichni, 20- Serres, 21- Kavalla; **Greece-Athens region:** 22- Voula, 23- Vari; **Turkey:** 24- Muğlar (Kuşadasi); **Albania:** 25- Tirana, 26- Vajkani, 27- Vjiftoht, 28- Dukati i ri, 29- Dhermin; **Malta:** 30- Msida (not represented).The dotted line represents the western limit of the natural range of *Cupressus sempervirens*.

using a Faxitron-43855® apparatus (20 Kv, 3 mA, 4 min) and X-ray sensitive films (Kodak® Industrex M). The total number of seeds per cone was counted, and seed quality (i.e. number and proportion of filled, empty and insect-infested seeds) was assessed from the radiographic images. The remainder of the cones were placed into rearing boxes stored in an outdoor insectary at Orléans, France. Adult insects were killed at emergence and identified to species.

2.3. Relationship between insect damage and cone development

A second experiment, aimed at surveying the development of insect damage along with that of the cone until seed dispersal, was initiated in June 1994 in three natural stands of the Greek islands (Crete–Aradena, Rhodes–Salakos, Samos–Metamorphosis), and in a naturalised stand of northern Greece (Nea Sichni). Only a few of the *C. sempervirens* from the Aradena stand, where trees were probably more than 300 years old, flowered that year. We chose five flowering trees, on each of which four branches bearing cones in the 2nd year of development were selected at random. In the other stands, we randomly chose two branches bearing cones in the 2nd year of development from each of 20 flowering trees selected at random. Each branch was tagged, and the position of the different categories of cones on the branch was mapped. The initial condition (e.g. healthy, damaged, dead) was recorded for each seed cone. Each branch was monitored again in May 1995. At that time, the number of surviving cones was determined and their condition was recorded once again. The cones in the 3rd year of development (i.e. the cones which were in the 2nd year of development in 1994 and survived through 1995) and the cones in the 4th year of development (i.e. the cones which were in the 3rd year of development in 1994 and survived through 1995) were collected in late May 1995, measured and individually dissected. The corresponding seeds were irradiated with X-rays to estimate the number of filled seeds.

2.4. Data treatment

We analysed the following variables per tree, stand and region of Greece: *i*) mean number of cones in the 2nd, 3rd and 4th year of development per branch; *ii*) mean length and volume of cones; *iii*) percentage of sound cones per branch; *iv*) percentage of cones of each age category damaged by each insect species per branch; *v*) mean number of seeds per cone; *vi*) mean number and percentage of filled, empty, *Orsillus*-damaged and *Megastigmus*-infested seeds per cone. In Albania, Malta and Turkey, we only analysed the percentage of full-grown cones damaged by each insect species, the mean number of seeds per cone and the mean number and percentage of filled, empty, *Orsillus*-damaged and *Megastigmus*-infested seeds per cone. In the Greek stands surveyed for the development and survival of seed cones until seed dispersal, the final number of filled seeds was compared to the potential yield, which for both stands was extrapolated from both the number of initial flowers and the percent of filled seeds per cone in that stand.

The percentages (p) were transformed by arcsin $\sqrt{p}$ to equalise variances before statistical analysis. The data were then submitted to analysis of variance (ANOVA) with the tree characteristics (crown type, height, diameter, position, cone crop size) as covariates. ANOVAs were followed by Tukey's test to look for differences between locations and regions. When the counts of cases per cell were unequal, the Tukey–Kramer adjustment was applied. The relationships between the cone's seed content and cone dimensions were tested by regression analysis. Computations were done using the SYSTAT statistical package (Systat Inc., Evanston, Illinois).

3. RESULTS AND DISCUSSION

3.1. Entomofauna of *C. sempervirens* seed cones

Approximately 6 000 cones were dissected or stored to rear insects and about 84 500 seeds were irradiated with X-rays. Seven insect and one mite species were observed to attack the cone and seeds of *Cupressus sempervirens* (*table I*). In Greece, the qualitative composi-

Table I. Mites and insects found in cones and seeds of *Cupressus sempervirens* collected in stands from the natural and introduced range in southeastern Europe.

Species	Attack period[1] 2 yr-G	2 yr-FG	3 yr	Feeding habits	Occurrence[2] C	K	R	S	N	A	T	Al	M
Trisetacus juniperinus	+	+		Conospermatophage	+	+	+	+	+	+	+	+	–
Pseudococcyx tessulatana	+	+	+	Conospermatophage	+	+	+	+	+	+	+	+	+
Megastigmus wachtli		+		Spermatophage	+	+	+	+	+	+	+	+	+
Orsillus maculatus		+	+	Spermatophage	+	+	+	+	+	+	+	+	–
Orsillus depressus		+	+	Spermatophage	+	+	+	+	+	+	+	–	–
Brachyacma oxycedrella	+			Conophage	–	–	+	–	+	–	+	+	+
Ernobius cupressi	+			Conospermatophage	–	+	+	+	+	–	–	–	–
Nanodiscus transversus		+		Conophage	+	+	+	–	+	–	–	–	–

[1] 2 yr: cone in the 2nd year of development; 3 yr: cone in the 3rd year of development; G: cone during the growth period; FG: full-grown cone.
[2] C: Crete; K: Kos; R: Rhodes; S: Samos; N: northeastern Greece; A: Athens region; T: Turkey; Al: Albania; M: Malta.

tion of the cone entomofauna was quite similar among the stands located within the natural range of *C. sempervirens* and those surveyed in the introduced range. All eight pest species were found in Rhodes (native range) and northern Greece (introduced range), and five species were observed throughout the whole surveyed area in Greece. Differences in the distribution of three minor species might be due to our limited sample size. Because the sampling was too limited and considered only cones in the 3rd year of development, it is difficult to draw any conclusion from the more limited entomofauna observed in Malta.

According to the feeding habits defined by Turgeon et al. [15], two species are conophages (i.e. feed on cone tissues only), three are conospermatophages (i.e. feed on both cone tissues and seeds) and the remaining three species are spermatophages (i.e. feed on seeds only) (*table I*). The total number of species was higher than that recorded in previous studies carried out in other parts of the range where *C. sempervirens* was introduced (four in France [12], three in Morocco [8] and six in Italy [9]), although all of these species were already known to attack seed cones of *C. sempervirens*. However, evidence that two of these species were pests of seed cones was uncovered only recently in Italy by Guido et al. [9]. A mite, *Trisetacus juniperinus* Nalepa (Acari: Nalepellidae), causes distortion and shrivelling of cone scales, and a seed bug, *Orsillus maculatus* (Fieber) (Hemiptera:Lygaeidae), feeds on seeds by inserting its long stylets through the scales. In addition, we found another true bug, *Orsillus depressus* Dallas, not observed during the survey in Italy, but which had a behaviour similar to *O. maculatus*. These bugs appear to be closely related to cypress. They lay eggs into the cones using either cones precociously opened as a result of attack by the fungi responsible for cypress canker, *Seiridium cardinale* Wagener, or the emergence holes of a seed chalcid, *Megastigmus wachtli* Seitner (Hymenoptera: Torymidae).

T. juniperinus and a tortricid, *Pseudococcyx tessulatana* (Staudinger) (Lepidoptera:Tortricidae), appeared to be the most important pests both in terms of the attack period and the type of damage caused. Both species attacked cones during the growth period as well as full-grown cones during the process of seed maturation (*table I*). Attack during the growth period generally resulted in the destruction of the whole cone but full-grown cones usually survived the pest attack although a large portion of the seeds were destroyed. Two minor species, *Brachyacma oxycedrella* Millière (Lepidoptera: Gelechiidae) and *Ernobius cupressi* Chobaud (Coleoptera:Anobiidae), also attacked the cones during the growth period but they were scarce in most regions, although *E. cupressi* was frequent in Kos. The other species only attacked the full-grown cones once the seed maturation had begun (*table I*). We confirmed the synchronisation of adult emergence of seed chalcids, *M. wachtli*, with the onset of seed maturation as Guido et al. [10] observed in Italy.

3.2. Damage to cones

Pest damage varied with stand location, year and cone development stages. In 1994, more than 40 % of the cones were killed by pests during the 2nd year of development in ten of the twenty stands surveyed in Greece (*table II*). Damage resulted mostly from the feeding by *P. tessulatana* larvae which dominated the pest complex in 13 of the stands, and destroyed up to 98 % of the cones during the growth period in Eleousa (Rhodes). *T. juniperinus* was the most damaging pest of the complex in three stands, but never attacked more than 27 % of the cones during the growth period. No significant difference between regions was observed for the percentage of overall cone damage (ANOVA: $F_{5,13} = 1.930$, $P = 0.157$), the percentage of damage by *T. juniperinus* ($F_{5,13} = 0.658$, $P = 0.661$) and the percentage of damage by *P. tessulatana* larvae ($F_{5,13} = 2.638$, $P = 0.074$). Only cone damage by *E. cupressi* varied with the region (ANOVA: $F_{5,13} = 11.355$, $P < 0.001$), being significantly higher in Kos where it reached 19 %. Cone attack was not influenced by the size of the cone crop, cone size, distance to the branch apex, nor by any tree growth patterns such as crown shape.

Damage to cones in the 3rd year of development was also caused predominantly by *P. tessulatana* larvae, with *B. oxycedrella* never attacking more than 1 % of the seed cones. Cone damage did not differ significantly between regions (ANOVA: $F_{3,27} = 1.349$, $P = 0.279$), although nearly 100 % of the cones were attacked in some stands (e.g. Nea Sichni in northern Greece and Ebonas in Rhodes). Damage to cones in the 4th year of development was much lower than that to growing cones and cones in the 3rd year of development in all stands except in those from Ebonas (Rhodes) and Aradena (Crete). Both the percentage of sound cones (ANOVA: $F_{3,60} = 7.832$, $P < 0.001$) and the percentage of cones attacked by *P. tessulatana* ($F_{3,60} = 5.485$, $P = 0.002$) differed among the regions, mostly because cones were significantly more damaged by *P. tessulatana* in Samos and Rhodes than in other regions (Tukey test: $P < 0.05$). No significant difference in the percentage of cones attacked by *M. wachtli* was observed between regions (ANOVA: $F_{3,60} = 2.068$, $P = 0.114$).

Table II. Cone crop abundance and pest damage to *Cupressus sempervirens* seed cones of different ages in natural and naturalised stands of Greece sampled in June 1994.

Region	Stand	Cones (2nd year)					Cones (3rd year)				Cones (4th year)			
				Damaged[3]					Damaged[3]				Damaged[3]	
		N[1]	Sound[2]	*Tj*	*Pt*	*Ec*	N[1]	Sound[2]	*Pt*	*Bo*	N[1]	Sound[2]	*Mw*	*Pt*
Crete	Omalos	3.4	54.6	25.2	20.2	0.0	1.1	100	0.0	0.0	3.0	100	0.0	0.0
	Fres	3.0	97.0	1.5	1.5	0.0	0.5	100	0.0	0.0	4.8	100	0.0	0:0
	Aradena	8.3	53.0	21.1	25.9	0.0	4.3	75.9	24.1	0.0	8.0	19.1	69.1	12.8
	Anapolis	2.8	98.0	1.0	1.0	0.0	0.9	100	0.0	0.0	1.6	100	0.0	0.0
Kos	Ag.Dimitrios	18.0	53.8	11.1	16.1	19.0	3.5	77.2	22.8	0.0	4.9	98.0	1.0	1.0
Rhodes	Alepohori	12.0	64.0	13.1	22.9	0.0	4.6	86.0	14.0	0.0	2.6	72.1	27.9	0.0
	Eleousa	2.0	1.0	1.0	98.0	0.0	0.0	*	*	*	0.0	*	*	*
	Plataria	13.0	61.0	5.0	28.0	0.0	6.0	89.0	11.0	0.0	2.2	80.0	0.0	20.0
	Appolonia	7.0	29.3	7.1	63.6	0.0	6.0	74.9	24.0	1.1	1.3	90.3	0.0	9.7
	Salakos I	6.8	51.7	15.1	33.2	4.0	2.0	0.63	0.38	0.0	0.0	*	*	*
	Ebonas	4.2	16.0	4.0	80.0	0.0	0.7	0.0	98.8	1.2	5.0	0.0	0.0	100
Samos	Metamorphosis	15.0	70.0	23.1	5.9	1.0	2.9	86.1	13.9	0.0	1.9	56.8	0.0	43.2
	Idrousa	4.8	73.8	1.0	25.1	0.0	0.0	*	*	*	3.3	62.4	31.1	7.5
	Kastania I	18.0	54.2	20.1	25.7	0.0	2.3	100	0.0	0.0	4.3	59.0	6.0	35.0
	Kastania II	18.0	71.1	26.1	2.8	0.0	9.5	74.1	26.9	0.0	0.0	*	*	*
Northern Greece														
	Nea Sichni	8.0	43.1	3.7	53.1	1.0	0.1	0.0	0.99	1.0	1.2	82.8	0.0	17.2
	Serres	16.0	49.0	1.0	50.0	0.0	0.0	*	*	*	0.0	*	*	*
	Kavalla	3.4	62.9	11.0	26.1	0.0	3.3	49.9	50.1	0.0	2.4	100	0.0	0.0
Athens region														
	Voula	12.0	66.8	1.2	33.0	0.0	5.0	73.0	27.0	0.0	1.0	100	0.0	0.0
	Vari	0.0	*	*	*	*	2.4	87.8	13.2	0.0	8.4	87.0	2.0	11.0

[1] N: mean number of seed cones per branch; [2] proportion of healthy seed cones; [3] proportion damaged by: *Tj*, *T. juniperinus; Pt*, *P. tessulatana*; *Ec*, *E. cupressi*; *Bo*, *B. oxycedrella*; *Mw*, *M. wachtli.*

Unlike 1994, the percentage of sound cones in the 2nd year of development significantly differed among Greek regions in 1995 (ANOVA: $F_{3,87} = 4.692$, $P = 0.004$) because the cones from northern Greece were significantly less damaged than those from the islands within the natural range of *C. sempervirens* (*table III*). Mite damage to cones during the growth period was more important than in 1994, attacking up to 53.9 % in Crete. Mite damage also varied among regions (ANOVA: $F_{3,87} = 3.410$, $P = 0.021$), being significantly more important in Rhodes than in northern Greece (Tukey test; $P = 0.012$). Damage by *P. tessulatana* to cones in the 2nd and 3rd year of development did not differ among regions (ANOVA: $F_{3,87} = 0.784$, $P = 0.506$ and $F_{3,60} = 2.383$, $P = 0.078$, respectively), although that to cones in the 3rd year of development did differ significantly between stands ($F_{13,50} = 5.070$, $P < 0.001$). This is likely because nearly all the cones from the Salakos-B stand (Rhodes region) were destroyed, whereas approximately 80 % of the cones from the all other stands were sound, except those from Ebonas in Rhodes and Nea Sichni in northern Greece where approximately 50 % of the cones were damaged. The number of overmature cones that remained on branches was too limited for a statistical analysis. More than 50 % of the cones from the Crete and Athens regions had exit holes of *Megastigmus wachtli.*

Only little damage by *P. tessulatana* was observed on cones in the 3rd year of development from Albania (1 to 12 % of damaged cones according to stands), Malta (1.2 %) and Turkey (17.6 %). *B. oxycedrella* was observed in 0.6 % of the cones in Malta and 4.3 % of the cones in Turkey.

Table III. Cone crop abundance and insect damage to *Cupressus sempervirens* seed cones of different ages in natural and naturalised stands of Greece sampled in May 1995.

Region	Stand	Cones (2nd year)				Cones (3rd year)			Cones (4th year)			
				Damaged[3]				Damaged[3]			Damaged[3]	
		N[1]	Sound[2]	*Tj*	*Pt*	N[1]	Sound[2]	*Pt*	N[1]	Sound[2]	*Pt*	*Mw*
Crete	Omalos	10.0	46.1	53.9	0.0	5.1	100	0.0	0.0	*	*	*
	Fres	5.5	64.4	1.8	33.8	2.6	72.9	0.27	0.4	0.0	0.0	100
	Aradena	5.3	66.3	33.7	0.0	1.6	77.1	0.23	0.7	80.0	0.0	20.0
	Vrisses	8.1	70.1	17.0	12.9	2.3	86.9	0.13	0.0	*	*	*
	Zourva	4.7	53.5	42.6	3.8	0.0	*	*	0.0	*	*	*
Rhodes	Alepohori	4.8	71.0	6.3	22.8	11.3	100	0.0	0.5	100	0.0	0.0
	Eleousa	10.0	63.3	36.7	0.0	0.3	100	0.0	0.0	*	*	*
	Plataria	5.7	61.9	33.3	0.0	3.0	93.8	0.06	0.0	*	*	*
	Appolonia	11.0	18.2	36.4	45.5	0.0	*	*	0.0	*	*	*
	Salakos I	33.0	45.5	51.5	3.0	0.0	*	*	0.0	*	*	*
	Salakos II	13.7	46.8	47.6	5.6	0.7	0.0	1.0	0.0	*	*	*
	Ebonas	6.0	50.2	37.3	12.5	1.0	50.0	50.0	0.0	*	*	*
Samos	Metamorphosis	10.8	55.9	29.9	14.2	9.0	98.0	2.0	0.0	*	*	*
	Idrousa	4.8	86.6	13.4	0.0	4.8	82.0	18.0	1.5	*	*	*
	Kastania I	14.3	93.1	6.9	0.0	11.3	100	0.0	0.0	*	*	*
Northern Greece	Nea Sichni	5.5	88.0	2.0	10.0	4.0	43.0	57.0	0.0	*	*	*
	Serres	4.0	85.0	5.0	10.0	4.2	94.0	6.0	2.2	*	*	*
Athens region	Voula	0.0	*	*	*	0.0	*	*	5.0	6.0	0.0	94.0
	Vari	0.0	*	*	*	0.0	*	*	7.5	62.0	0.0	38.0

[1] N: mean number of seed cones per branch; [2] proportion of healthy seed cones; [3] proportion damaged by: *Tj*, *T. juniperinus; Pt*, *P. tessulatana*; *Mw*, *M. wachtli*.

3.3. Damage to seeds

Cone morphology, seed number and seed quality were all highly variable. The total number of seeds per mature cone was related to cone length ($n = 313$, $r^2 = 0.926$, $P < 0.000$) and cone volume ($n = 313$, $r^2 = 0.855$, $P < 0.001$). Cone length did not vary among regions (ANOVA: $F_{7,305} = 2.037$, $P = 0.052$), whereas cone volume did ($F_{7,305} = 4.336$, $P < 0.001$), being significantly more important in Rhodes (9.9 ± 0.9 mm^3, mean ± SE) than in Samos (7.3 ± 0.5) and Turkey (7.1 ± 0.5). However, this difference in volume did not translate into a significant variation of the total number of seeds per cone among regions (ANOVA: $F_{7,305} = 1.445$, $P = 0.187$).

The number of filled seeds per cone was also dependant on cone dimensions ($n = 313$, $r^2 = 0.893$, $P > 0.001$). The percentage of filled seeds per cone varied from 0 to 94.2 % whilst that of empty seeds per cone varied between 5.8 to 100 % (Dukati, Albania). *Figure 2* presents the average percentage of filled and empty seeds observed per location. We attributed the observed differences to rates of pollination and to damage by *Orsillus* spp., but at the time of this analysis it was not yet possible to differentiate *Orsillus*-damaged seeds from other aborted seeds. A regional tendency was found (ANOVA: $F_{7,305} = 3.987$, $P < 0.001$), the percentage of empty seeds per cone being significantly higher in samples from Turkey (0.674 ± 0.048) and Malta (0.775 ± 0.050) than in those from natural Greek stands (Crete: 0.478 ± 0.035; Rhodes: 0.479 ± 0.020; Samos: 0.472 ± 0.020) and northern Greece (0.454 ± 0.035). However, it should be pointed out that the percentage of empty seeds in a number of natural stands of Greece (e.g. Omalos and Aradena in Crete, Eleousa, Plataria and Salakos in Rhodes and Idrousa in Samos) was much higher than 50 %. Significant differences in the percentage of seeds infested by *M. wachtli* were observed among regions (ANOVA: $F_{7,305} = 4.093$, $P < 0.001$), although the values remained very low in all cases (*fig-*

ure 2C). Infestation by *M. wachtli* was significantly lower in the Rhodes region than in those of Crete and Athens (Tukey test: $P < 0.05$), but the chalcid had an influence on the potential of regeneration only at Omalos, Voula and Malta where the sum of empty and insect-infested seeds represented more than 75 % of the total seeds produced.

Although attack of the mature cones by *P. tessulatana* larvae did not usually result in an entire consumption of the cone, larval feeding significantly reduced the total number of seeds from 133.3 ± 3.2 (mean ± SE) in undamaged cones to 52.2 ± 6.5 in infested cones – a reduction of 60.5 % (ANOVA: $F_{1,311} = 51.3$, $P < 0.0010$). The average percentage of filled seeds decreased from 51.6 % in healthy cones to 21.7 % in attacked cones, whereas that of empty seeds increased from 47.9 to 78.3 %, respectively. Thus, the ratio of filled/empty seeds significantly decreased from 1.820 ± 0.116 to 0.311 ± 0.075 (ANOVA: $F_{1,311} = 6.674$, $P = 0.010$), indicating that *P. tessulatana* larvae fed mostly on filled seeds.

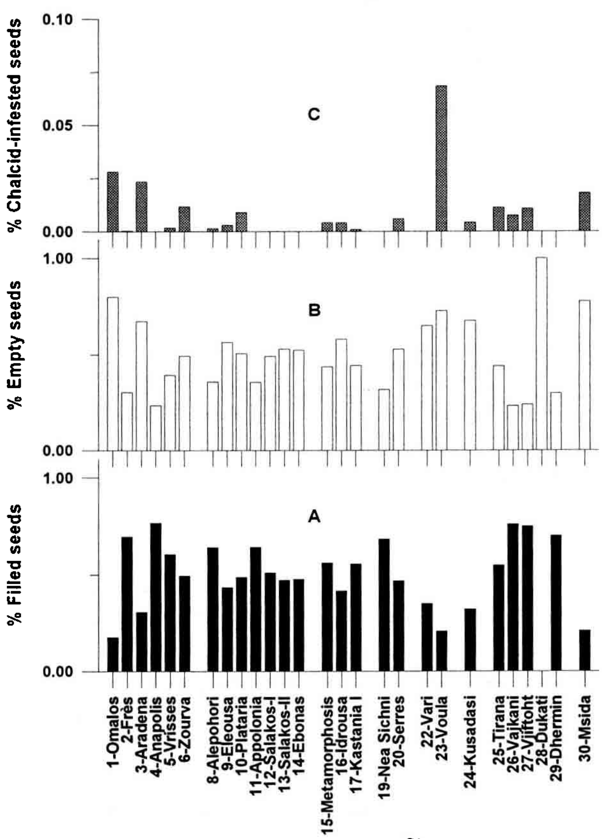

Figure 2. Percentage of (A) filled, (B) empty and *Orsillus*-damaged, and (C) *M. wachtli*-infested seeds per cone, in the stands of *Cupressus sempervirens* sampled in 1995 in Greece and Turkey, and in 1996 in Albania and Malta. Site numbers correspond to those of *figure 1*.

3.4. Influence of insects on the potential of regeneration of C. sempervirens

The 1994–1995 life table of several cohorts of cones tagged in four Greek stands revealed a significant decrease (by 47–86 % of the initial cone crop) during the 2nd year of development (*table IV*). The relative importance of mortality factors differed with the stand. Cone abortion, which was probably due to a lack of pollination, was the major mortality factor at Aradena. *P. tessulatana* was the most damaging at Nea Sichni and Metamorphosis whilst *T. juniperinus* killed ca. 50 % of the cones at Salakos. Attack by *T. juniperinus* as well as by larvae of the first generation of *P. tessulatana*, stopped the growth of seed cones as the cones dried up prematurely and usually dropped to the ground. The cone growth phase thus appeared to be the most critical period because the action of mortality factors resulted in an overall loss of the cones' entire seed content, even of seeds that had not been damaged directly. On the other hand, few seed cones were damaged during the remainder of the 2nd year of development in both years and locations. The limited decrease in the number of cones of that age resulted mainly from feeding by *P. tessulatana* larvae of the second and third generation. Unlike what occurred to cones during the growth period, most of the cones damaged during the summer and autumn of the 2nd year of development did not disappear from the branch, and seeds that were not damaged directly were able to reach maturity. However, the apparent limited impact of insect attack during the cone maturation phase is misleading because damage by spermatophagous insects such as *Orsillus* spp. and *M. wachtli*, which can be detected only by irradiating seeds with X-rays, has not yet been taken into consideration. Only 11–37 % of the initial numbers of cones survived according to the location (*table IV*). Pests accounted for 40.6 % of the total cone loss at Aradena (13 cones attacked by pests versus 19 cones aborted or disappeared), 72.2 % at Nea Sichni (52 versus 20), 72.3 % at Metamorphosis (48 versus 18) and 84.8 % at Salakos (84 versus 15). In a similar experiment conducted in Italy, 24 % of the cones reached maturity [10]. In that study, the greatest losses were due predominantly to abortion and fungi, insects and mites being responsible for only 5 % of the cone losses.

The mean number of seeds per sound cone (112.6 ± 14.8 [mean ± SE] at Aradena, 159.9 ± 12.5 at

Table IV. Life table of cohorts of seed cones of *Cupressus sempervirens* in the 2nd and 3rd years of development tagged in June 1994 in the natural stands of Aradena (Crete), Salakos (Rhodes), and Metamorphosis (Samos), and in the naturalised stand of Nea Sichni (northern Greece).

Cohort	Period	Mortality factors	Aradena			Salakos			Metamorphosis			Nea Sichni		
			x[1]	q_x[2]	S_x[3]	x	q_x	S_x	x	q_x	S_x	x	q_x	S_x
2nd year	June 1994		36			115			86			117		
		Abortion		0.53			0.05			0.12			0.17	
		Trisetacus		0.14			0.48			0.16			0.04	
		Pseudococcyx		0.19			0.12			0.19			0.35	
		Ernobius		0.00			0.03			0.00			0.00	
	May 1995		5		0.14	36		0.31	46		0.53	51		0.44
		Pseudococcyx		0.20			0.31			0.20			0.20	
		Unknown		0.00			0.25			0.11			0.00	
Final number	late May 1995		4		0.80	16		0.44	32		0.69	41		0.80
Final versus initial nb of cones					**0.11**			**0.14**			**0.37**			**0.37**
3rd year	June 1994													
			15			24			16			15		
		Pseudococcyx		0.07			0.67			0.06			0.33	
	May 1995		14		0.93	8		0.33	15		0.94	10		0.67
		Unknown		0.07			0.25			0.26			0.10	
Final number	late May 1995		13		0.93	6		0.75	11		0.74	9		0.90
Final versus initial nb of cones					**0.86**			**0.25**			**0.69**			**0.60**

[1] Number of cones alive; [2] mortality quotient; [3] survival rate.

Table V. Impact of mortality factors on the seed yield of seed cones of *Cupressus sempervirens* in the 2nd year of development tagged in June 1994 in the natural stands of Aradena (Crete), Salakos (Rhodes), and Metamorphosis (Samos), and in the naturalised stand of Nea Sichni (Northern Greece).

	Aradena		Salakos		Metamorphosis		Nea Sichni	
	No. of seeds	%	No. of seeds	%	No. of seeds	%	No. of seeds	%
Potential number of seeds (1994)	4057		18722		9907		18708	
Resulting seeds (1995)	451	11.1[1]	2605	13.9[1]	3686	37.2[1]	6556	35.0[1]
Empty seeds	57	12.6[2]	409	15.7[2]	494	13.4[2]	1331	20.3[2]
Megastigmus- infested seeds	10	2.2[2]	5	0.2[2]	15	0.4[2]	51	0.8[2]
Orsillus- damaged seeds	169	37.5[2]	917	35.2[2]	1109	30.1[2]	1006	15.3[2]
Filled seeds	215	47.7[2]	1274	48.9[2]	2068	56.1[2]	4168	63.6[2]
Filled seeds versus potential seeds		5.3[1]		6.8[1]		20.9[1]		22.3[1]

[1] Numbers divided by the potential number of seeds; [2] numbers divided by the resulting number of seeds.

Nea Sichni, 115.2 ± 3.8 at Metamorphosis and 162.8 ± 14.7 at Salakos) was multiplied by the initial number of cones in each site (36, 117, 86 and 115 cones, respectively; *table IV*) to extrapolate the potential seed crop of the surveyed branches at the four study sites. Analysis of the radiographic images of the overall seed yield of the cones collected at maturity revealed that 13–20 % of the seeds were empty (*table V*). A comparison between the seed content of surviving mature cones, either sound or attacked by *P. tessulatana,* revealed that damaged cones still contained an average of 52.1 (Aradena) to 60.3 seeds (Nea Sichni), indicating that the tortricid decreased the cones' seed content by 53.7 to 62.3 %. The seed quality of these damaged cones was also greatly affected as the percentage of filled seeds significantly decreased to less than 30 % of the total (22.2 % at Aradena, 30.4 % at Nea Sichni). Only 2 % or less of the seeds contained a *M. wachtli* larva (*table V*). Guido et al. [10] reported similar results in Italy, where *M. wachtli* infested only 0.7 % of the seeds. On the other hand, the images revealed a large number of seeds with a shrivelled endosperm and embryo, which is characteristic of damage by *Orsillus* spp. [1]. In our study, *Orsillus* damage was estimated at approximately 30–37 % in the natural stands, but at only 15.3 % in the naturalised stand of Nea Sichni, respectively (*table V*). In Italy, *O. maculatus* was responsible for damaging 20.9 % of the seed content of mature *C. sempervirens* cones [10].

Overall, the seed crop was decreased by 93.2 and 94.7 % of its original potential value at Salakos and Aradena, respectively. The decrease was lower at Metamorphosis (79.1 %) and Nea Sichni (77.7 %). By extrapolating the results from table IV (i.e. estimating the seed loss by the value 'cone loss x mean number of seeds per cone'), the difference seemed to result from a larger cone abortion at Aradena (52.8 % of decrease in the potential seed yield versus less than 20 % in other stands), and from a larger impact of insects and mites in Salakos (81.9 % of decrease in potential seed yield versus 40.5 % at Aradena). Pest impact was intermediate in Metamorphosis (65.8 %) and Nea Sichni (53.5 %). In any case, pests drastically reduced the number of viable seeds susceptible to germinate and face additional mortality factors which occur once the seeds fall on the ground (e.g. predation by animals, plant competition, soil quality, etc.). Although this study involved a limited number of samples, we can hypothesise that pests of cones and seeds may represent serious limiting factors for natural regeneration of natural and naturalised stands of cypress in Greece.

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