Survey of Insect Impact on Seed Cones of Two Species of Cupressaceae, *Cupressus sempervirens* L. and *Tetraclinis articulata* Mast. in Tunisia

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Abstract

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Cone and seed pests of the Mediterranean cypress (*Cupressus sempervirens* L.) and of Berberian thuja (*Tetraclinis articulata* Mast.) were surveyed during 1995-96 in 3 stands in Tunisia, including the relict cypress stand of Makthar. A total of 6 insects and 1 mite species were observed in cypress cones, and 3 insects and a mite species in these of thuja. The species damaging thuja cones also attacked cypress cones. With respect to both the attack period and the type of damage, a cone tortricid, *Pseudococcyx tessulatana*, and a mite, *Trisetacus* sp., appeared to be the most damaging cone pests in both conifers. Tortricids were observed to attack up to 72.7 % of the 1-year cones in some cypress trees of 2 sites whilst mite damage could reach 63.3 %. Overall and specific damage was lower in thuja than in cypress. A survey of cone mortality along cypress cone development in Makthar showed that 10.7 % of the initial cones survived until maturation. Seed crop decreased by 94.0 %. Torticids and mites were responsible for 41.9 % and 22.2 % of the decrease in the potential seed yield, respectively, while grazing activity conducted by man accounted for 23.7% of the seed loss.

Key words: *Cupressacae*, insects, seed cones, Tunisia.

Introduction

In Tunisia, the Cupressaceae consist of the Mediterranean cypress (*Cupressus sempervirens* L.), the Berberian thuja (*Tetraclinis articulata* Mast.) and two junipers (*Juniperus phoenicea* L. and *J. oxycedrus* L.). *C. sempervirens* originates from the eastern part of the Mediterranean basin where its natural distribution covers North Iran, Asia Minor, Crete and Cyprus (14), but it is still not ascertained whether *C. sempervirens* var. *numidica* Trabut is native or was introduced by Ancient Romans to Tunisia. However, this species may be considered a relict, which has been largely decimated by man, and thus confined within isolated humid locations. In fact, only remains a cypress stand, located in the Makthar area, develop in a semi-arid climate with cool winter. Beside this stand, planted cypress trees are largely used as windbreaks and ornamentals throughout northern Tunisia. The Berberian thuja is endemic from north Africa with a few stands in Spain and Malta. The species constitute large pure stands near Cap Bon in Tunisia.

Although cone and seed insects are nowadays considered as the most important seed pests during the predispersal phase of seed development. The knowledge on the insects attacking cones of Cupressaceae is quite limited (13). Several recent studies refred to cone entomofauna of *Cupressus sempervirens* in southern Europe (1, 8, 11) but the entomofauna of *Tetraclinis* is quite unknown except for fragmentary studies (7, 12). Moreover, only few works dealt with cone and seed insects in Morocco (4, 5, 6, 7) and Algeria (2, 3) but none in Tunisia. Our objectives were, therefore: a) to identify the entomofauna attacking seed cones of *C. sempervirens* and *T. articulata* in Tunisia; b) to evaluate insect infestation impact on the potential of natural regeneration of such tree species in selected areas; and, c) to compare the composition and impact of cone entomofauna in Tunisia with those observed in other Mediterranean countries between the original natural and relatively recent introduction areas.

Materials and Methods

Location of the study sites

The major part of study on the cypress was carried out in the cypress stand of Bou Abdallah, near Makthar, in the Kessra mountains (35º50'65"N, 9º25'61"E, 795m altitude, Figure 1), 250 Km from Tunis the capital. In this area, annual rainfall is 500 mm/ year, and the average temperature is 14.6ºC (32.6 - 1.8ºC). The selected 20-ha stand consisted of sparse trees, which were several hundreds- year old. The trees developped on a degraded marly soil with some limestone outcrops. Tree shape wass irregular, often Y-shaped, branchless at the base, and the average tree height was less than 12 m. Tree roots were overdevelopped and outcropped where the slope was steep. The undercover was degraded and essentially consisted of xerophytic species (e.g., *Juniperus oxycedrus*, *Ampellosoma mauritanica*, *Callicotum villosum*, *Marubium* sp., ...). For comparison, a cypress windbreak was selected at Beni Ayeche, 40 km east of Tunis region. It consisted of tall, 20m-high cypress trees. A neighbour stand of *Tetraclinis articulata* was selected at Semeche (Figure 1), 50 Km from the capital. The Thuja stand was dense and consisted of two to five meter tall tress.

Insect inventory and assessement of insect damage to seeds

Standardized collections of cypress cones were gathered out during July 1995 and July 1996 from Bou Abdallah, and July 1995 from Beni Ayeche. Two branches 40 cm long (1 from the lower crown and 1 from the mid- or upper crown) were randomly taken and tagged on 10 different trees. First, the branches were beaten over a net in order to collect the insects presented on cone surfaces. Then counts, all the 1- year cones (green cones which are in the growth process), 2- year cones (ash-grey cones which completed the growth period the year before, and are to release seeds during the following winter), and 3- year cones (overmature cones) on the examined branches were made, 2- and 3-year cones were then removed from the branch. Concurrent with cone collection, the characteristics of the sampled trees (i.e., type of crown, cone crop size, height, diameter, position in the stand,...) were noted. In the laboratory, cone morphology (length [l], width [w], and volume [$\pi l(3w^2+l^2)/24$]) was measured on 2-year cones. Half of the lots of 2- and 3- year cones were dissected in order to look for internal insect damage and the presence of any larvae. For each of the dissected cones, the seeds were extracted, and the seed lots were individually radiographed using a Faxitron-43855® apparatus (20 Kv, 3mA, 4mn) and

X-rays sensitive films (Kodak ® "Industrex M"). The total number of seeds per cone was worked out, and the seed quality (number and proportion of filled, empty, *and* insect-infested seeds*)* was determined depending on the radiographs. The remain of the cones were put into rearing boxes and stored in an outdoor insectary at Orléans, France. Adult insects were killed at emergence and identified to species. The emergence pattern of both sexes of seed chalcids was surveyed on a per-day basis during 1996 in order to investigate possible differences in the emergence period with insect sex and geographic origin of the infested seeds. The male sex-ratio was determined.

Cone collection of *Tetraclinis articulata* was carried out in Semeche during July 1995. A total of 276 cones was collected at random from 10 different trees. The sampling procedure, cone analysis, and insect rearing procedures were similar to those described for *C. sempervirens*.

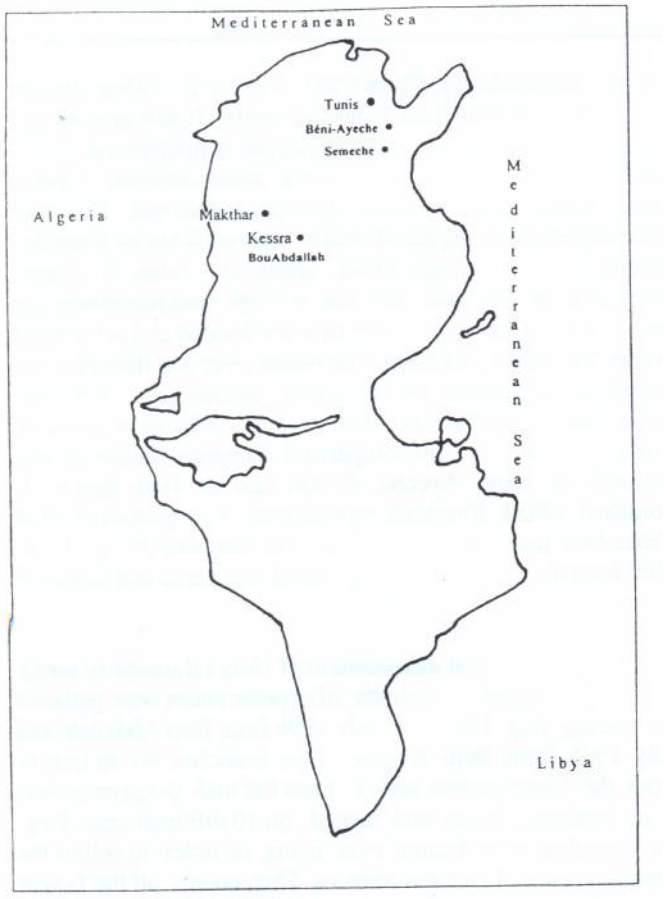

Figure 1. A Map showing the study sites in Tunisia.

Survey of insect damage along cypress cone development

A second experiment was carried out at Makthar. It aimed at surveying the development of insect damage along with that of the cone, until seed dispersal. The experiment was initiated in July 1995 on the same trees and branches where cone collections was realized. The position of the 1-year cones on the branch was mapped. Then, the initial sanitary status was recorded for each cone. Evry selected branch was checked again in June, 1996. At that time, the surviving 2-year cones (i.e., the 1-year cones observed in 1995 that survived until 1996) were counted, then collected, measured and individually dissected. The corresponding seeds were X-rayed in order to estimate the number of filled seeds. Finally, the total number of filled seeds was compared to the potential yield which was extrapolated from both the number of initial flowers and the percent of filled seeds per cone in the considered stand.

Data treatment

In cypress, the following variables were analysed: 1) the mean number of 1-year, 2-year and 3- year cones per branch, tree, and stand; 2) the mean length and volume of cones; 3) the percentage of sound cones per branch; 4) the percentage of cones for every age category damaged by each insect species per branch; 5) the mean number of seeds per cone; 6) the mean number and percentage of filled, empty, bug-damaged, and chalcid- infested seeds per cone. In Thuja, the percentage of cones damaged by each insect species per tree, the mean number of seeds per cone and per tree, the mean number and percentage of filled, empty and insect-damaged seeds per cone, were only measured. To equalize variances before statistical analysis, the percentage data were transformed by arcsin. The data were then submitted to variance analysis with the tree characteristics (crown type, height, diameter, position, cone crop size) as covariates, followed by Tukey's test to look for differences between locations and regions. When the counts of cases per cell were not equal, Tukey-Kramer adjustment was applied. The relationships between cone seed content and cone dimensions were tested by regression analysis. The Computions were done using the SYSTAT statistical package (Systat, 1990).

Results and Discussion

Composition of the cone entomofauna of evergreen cypress and Berberian thuja in Tunisia

In cypress, a total of 6 insect species and 1 mite species were observed to attack the cone and seeds of *Cupressus sempervirens* in the surveyed stands (Table 1). No significant difference in the qualitative composition of the cone entomofauna was observed between the stands of Makthar and Beni Ayeche. All of the 7 pest species were found in other parts of the native and introduced range of evergreen cypress in the Mediterranean region (11). Of these species, only the seed chalcid attacked specifically cones of *Cupressus*, but the six others are specific of cones of Cupressaceae species (10). According to the feeding habits defined by Turgeon *et al* (13), two species are conophages (i.e., feed on cone tissues only), two species conospermatophages (i.e., feed on both cone tissues and seeds), and 3 species are spermatophages (i.e., feed on seeds only). With respect to both the attack period (during cone growth) and the type of damage (destruction of the whole cone or of a large part of the seeds, at least), a cone mite *Trisetacus* sp. (*juniperinus* Nalepa ?), that caused distortion and shriveled of the scales, and a cone tortricid, *Pseudococcyx tessulatana* (Staudinger) appeared the most noxious species. However, the seed bugs, *Orsillus maculatus* [Fieber] and *O. depressus* Dallas, which fed on seeds by inserting very long mouth stylets through the scales or through the emergence holes of the seed chalcid, might also have some importance for regeneration. Moreover, these species were recently found to disseminate spores of the fungus responsible of the cypress canker, *Seiridium cardinale* (1). By contrast, the cypress seed chalcid, *M. wachtli* (larva developed entirely within a seed) requires high population levels to produce significant damage as it is the latest to attack.

Table 1. Insects and Mites observed damaging cone and seeds in natural stands and plantations of *Cupressus sempervirens* and *Tetraclinis articulata* during 1995-96 in Tunisia.

Species	Family	Feeding habits	*Cupressus sempervirens.* Makthar	*Cupressus sempervirens.* B. Ayeche	*Tetraclinisar ticulata* Semeche
Pseudococcyx tessulatana (Staud.)	Tortricidae	Conospermatophage	+	+	+
Megastigmus wachtli Seitn.	Torymidae	Spermatophage	+	+	-
Orsillus maculatus (Fieb.)	Lygaeidae	Spermatophage	+	+	-
Orsillus depressus Dall.	Lygaeidae	Spermatophage	+	+	-
Brachyacma oxycedrella Mill.	Gelechiidae	Conophage	+	-	+
Nanodiscus transversus Aubé	Curculionidae	Conophage	-	-	-
Trisetacus sp.	Eriophyiidae	Conospermatophage	+	+	+

In *Tetraclinis*, 3 species attacked cypress cones (Table 1). *Pseudococcyx* was already observed in cones of *T. articulata* in Spain (12), Morocco (7), and Malta (Roques, unpublished observations). *Brachyacma* was also recorded from Morocco (7) and Algeria (10), but this mite was the first time to be observed in *Tetraclinis* cones. The external coulour of cones damaged by mites and tortricids progressively turned from green to dark blue, starting at the areas corresponding to the position of larval galleries.

Variations in insect damage to cones of cypress

Cone damage mostly resulted from the feeding on 1-year cones by *Pseudococcyx* larvae, that largely dominated the pest complex in both experimental stands. The cone tortricid destroyed a mean of 41.8% of the 1-year cones in Makthar in 1995, but the damage was lower in Beni Ayeche (25 %). The *Trisetacus* mites damaged a mean of 22.8 % of the 1-year cones in Makthar but only 16.5 % in Beni Ayeche. However, cone damage by both species greatly differed among the trees. The damage by *Pseudococcyx* larvae varied from 5.6 to 72.7 % of the 1-year cones whilest that of mites varied from 0 to 63.3 % according to tree in Makthar. Damage to 2-year cones also proceeded from the activity of *Pseudococcyx* larvae, but was more limited (10.8 % in Makthar). The minor other species were occasionnal, except the seed chalcid that was present into 20 % of the 2-year cones in Makthar. As a result of overall insect activity, sound cones only represented 17.8 % in Makthar and 52.4 % of total cones in Beni Ayeche (Figure 2). Similarly, large differences in the percentage of sound cones were observed among trees (87.5 - 19.0 %), thus suggesting that insects and mites selected the trees.

Variations in insect damage to cones of Berberian thuja

Damage increased as a result of the activity of *Pseudococcyx* larvae and *Trisetacus* mites. However, insect damage was rather limited than in cypress such two major pests attacked 18.4 % and 13.8 % of the cones, respectively,in Semeche (Figure 2). As in cypress, all surveyed trees were colonized by the tortricid (3.1 - 53.2 % of the cones) but not by the mite whose distribution seemed sparse (0 - 3 % of the cones). As a result, the percentage of sound cones tended to be more expensive and important than in the case of cypress, and ranged 47.5 - 89 % of the cones.

Variations in insect damage to cypress seeds

Cone morphology, seed number and seed quality significantly differed among trees but not among cones within the same tree. Table 2 presents the results of the variance analyses performed on these variables in the Makthar sample. The total number of seeds per mature cone also depended on cone length (r^2= 0.643, P=0.000) and cone volume (r^2= 0.651, P=0.000).The number of filled seeds per cone increased with cone dimensions (length: r^2= 0.500, P=0.003; volume: r^2= 0.516, P=0.002), but neither with the number of empty seeds (length: r^2= 0.293, P=0.097; volume: r^2= 0.101, P=0.084) nor the number of seeds damaged by *Megastigmus* (length: r^2= 0.03, P=0.881; volume: r^2= 0.02, P=0.987). The empty seeds represented from 10.3 to 81.3% of the mean seed content depending on trees (Table 2). These differences might be due to the variation in pollination rates and to variation in seed-bug damage but differentiation between bug-damaged seeds from other aborted seeds unfortunatly was not possible at the time of analysis. On the contrary, the number as well as the percentage of seeds infested with the cypress seed chalcid always remained limited although highly significant differences in the number and percentages of *Megastigmus*-infested seeds were observed between trees (Table 2).

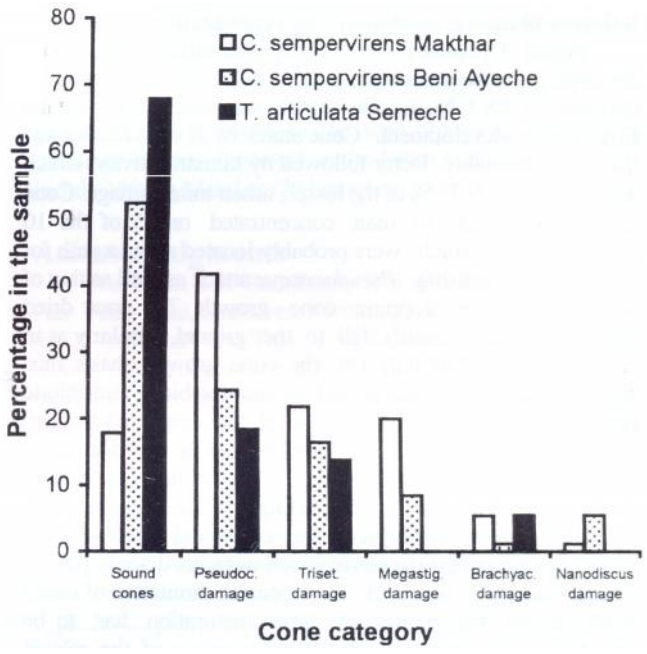

Figure 2. Insects and mite damage to 1-year cones of *Cupressus sempervirens* and *Tetraclinis articulata* observed in 1995 in 3 sites in Tunisia.

Emergence period and sex-ratio of seed chalcids

A total of 46 adults of *Megastigmus wachtli* emerged in 1996 from the seeds collected the same year. Emergence took place from late September to early October (Figure 3a). Although the data were only indicative because the weather conditions of north- central France (where the seeds were stored) largely differed from the Tunisian conditions, adults emergence appeared relatively delayed with regard to the same insects in Greece (July-August in the storage conditions, (11)).

Table 2. Cone dimensions, seed number and quality observed in the stand of *Cupressus sempervirens* of Makthar in 1995, and results of the variance analyses (ANOVA) performed with tree (df: 9, 23) and cone (df. 2, 30) as independant variables.

				Anova Tree		Anova Cone	
	Mean (se)	**Maximum**	**Minimum**	**F**	**P**	**F**	**P**
Cone length	2.5 (0.1)	3.1	1.8	2.722	0.025	1.960	0.158
Cone volume	7.2 (0.5)	14.1	2.4	3.893	0.004	1.935	0.162
Total seeds	108.8 (5.0)	168.0	59.0	4.234	0.002	1.300	0.287
Filled seeds	60.5 (4.1)	102.0	11.0	3.251	0.011	1.165	0.326
Empty seeds	47.6 (3.9)	95.0	11.0	3.506	0.007	0.993	0.382
Infested seeds	0.7 (0.4)	11.0	0.0	10.061	0.000	0.391	0.680
Percent filled seeds[1]	55.4 (3.2)	89.6	18.6	3.315	0.010	0.830	0.446
Percent empty seeds[1]	44.1(3.2)	81.4	10.4	3.073	0.014	0.942	0.401
Percent infested seeds[1]	0.5 (0.2)	6.9	0.0	10.772	0.000	0.298	0.745

[1] Anova performed on values transformed by asin x.
Significant values are figured in bold

No striking differences were observed between the emergence period of males or females. In contradiction with the other *Megastigmus* seed chalcids, no insects remained in prolonged diapause for more than 1 year (10). The sex-ratio largely varied with cones of the same trees (Figure 3b). The mean sex-ration (male/female) was 0.80. Females being dominant in 3 cases vs. 2 for males.In Algeria, Bouaziz (2) found a sex-ratio of 0.7. After those of Roques *et al.* (11) in Greece, these observations suggested that *M.wachtli* was capable of arrhenotokous parthenogenesis as did some other seed chalcids.

Influence of insects on the cypress regeneration potential

Figure 4 presents the 1995-96 life table of the cones developing on the branches tagged in July 1995 at Makthar. A decrease, of 88.3 %, in cone number was observed during the first year of development. Cone attack by *P. tessulatana* was the major mortality factor followed by human activity, which accounted for 23.7 % of the losses, athen mite damage. Cone disappearance due to man concentrated on 5 of the 10 surveyed trees, which were probably located along a path for used for goat grazing. *Pseudococcyx* attack as well as that of mites resulted in stopping cone growth. The cone dried precociously, and usually fell to the ground. Similarly as in Greece (11) and in Italy (9), the cone growth phasis thus appeared the most critical period because the biotic and abiotic factors resulted in an overall loss of the cone seed content, even of seeds which had not directly preyed. In contrast, the 2-year cones were fewly damaged. The limited decrease in cone numbers resulted from the feeding of the larvae of *P. tessulatana.* Most of the damaged cones did not disappear from the branch, and the seeds which were not directly preyed reached maturity. However, the apparent limitation of insect attack during the phasis of cone maturation has to be considered with regard to the feeding habits of the related insects. At that time, most of the attacks were due to spermatophages such *Orsillus* bugs and seed chalcids, whose damage was only visible by X-raying of seeds. Finally, only 10.7 % of the initial number of cones survived. In similar experiments, 24 % of the initial cones survived in Italy (9), and 11 to 37 % in Greece. Cone damage thus appeared more important in Tunisia. In addition, abiotic factors, especially lack of pollination, and fungi damage played a larger role in cone mortality in Italy and Greece, whereas insect damage was a more important factor limiting cone survival in Tunisia.

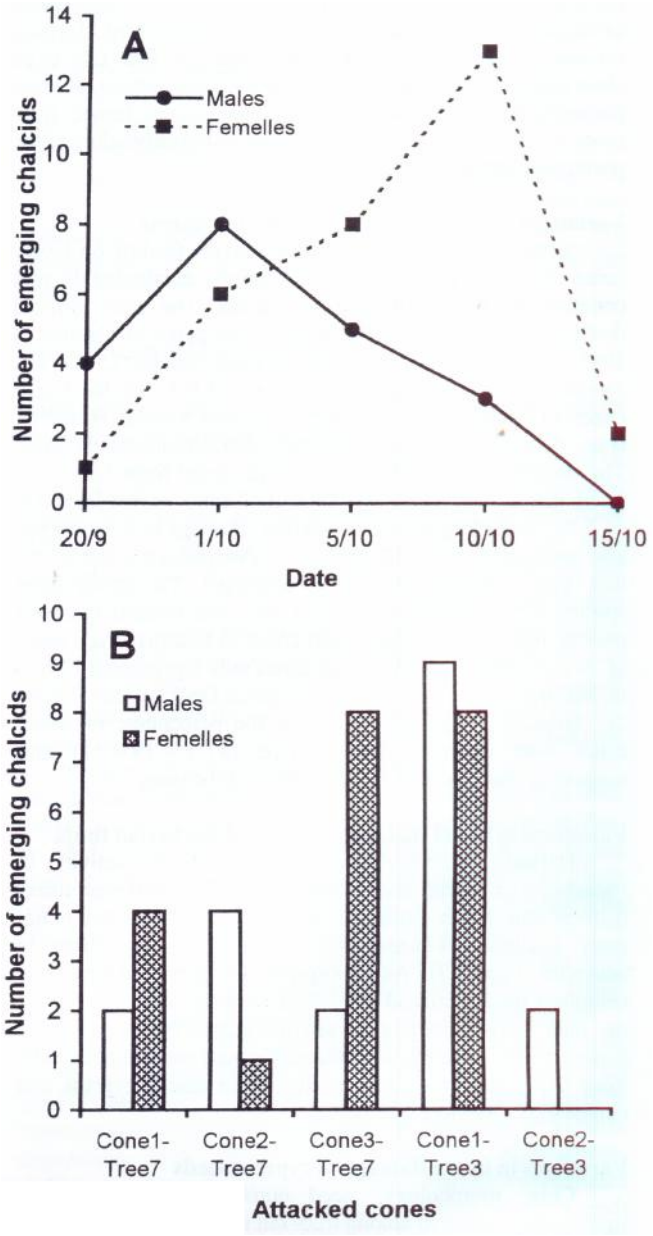

Figure 3. Emergence patterns of adult chalcids, Megastigmus wachtli, from cones collected in 1996 in Makthar. a) Period of emergence in autumn 1996; b) Sex-ratio (Meal/Female) of emerging insects. The seeds were stored at Orléans, France.

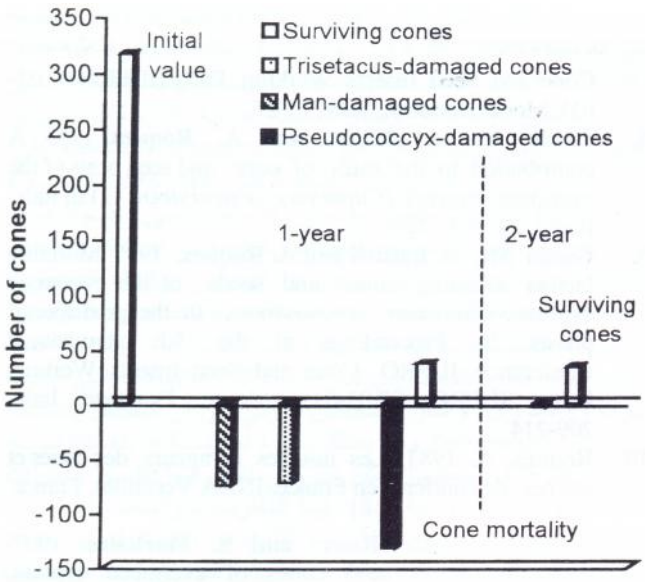

Figure 4. Development of the cone crop and impact of the mortality factors observed on the 1-year cypress cones tagged in July 1995 at Makthar (20 branches, 10 trees).

The relative importance of mortality factors in decreasing the potential seed crop is shown in Figure 5. The mean average of seeds per sound cone was 108.8 ± 5.0. The potential seed crop expected to result from the initial 1-year cone crop (316 cones) was therefore extrapolated to 34381 seeds (35961 - 32801). The 2-year cones damaged by tortricids still contained a mean of 54.6 seeds, i.e. tortricid decreased the seed content of attacked cones by 49,8%. However, the seed quality was largely reduced in such cones because the proportion of filled seeds significantly decreased to 25.2 % of the total seeds. Seeds showing *Megastigmus* larvae were 0.5 % only whilst they accounted for 2% and 0.7 % of the seed yield in Greece (11) and Italy (9), respectively. The number of empty seeds and seeds showing a shrivelled content (endosperm plus embryo), that was a characteristic damage of seed bugs, *Orsillus* spp. (1), was also limited, and accounted for only 3.6% of the total loss. Guido *et al.* (9) estimated 20.9 % damage of *Orsillus maculatus* to seed content of mature cypress cones in Italy.

Seed crop (mature sound seeds) represented 6 % of the potential value. This value was far less than that observed in Greece (5.3 to 22.3) (11). By extrapolating the data from Figure 4 (i.e., estimating the seed loss by the value [cone loss x mean number of seeds per cone], the losses in Tunisia appeared to result mostly from the activity of cone tortricid (41.9 % decrease in the potential seed yield), then man (23.7 %) and mites (22.8 %). It is concluded, therefore, that insects represent a real limiting factor for natural regeneration of the Makthar stand, in addition, of course, to the mortality factors that act on the subsequent phases of germination of the surviving seeds and on the growth of seedlings.

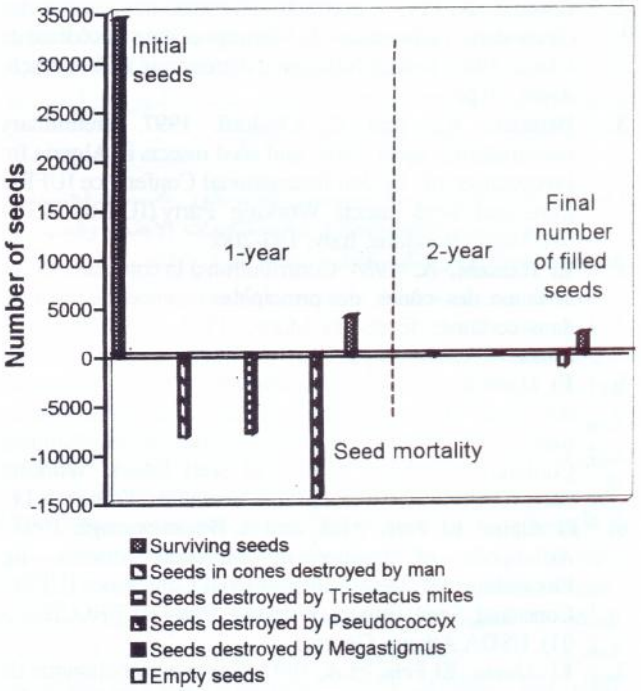

Figure 5. Development of the seed crop and impact of the mortality factors observed on the 1-year cypress cones tagged in July 1995 at Makthar (20 branches, 10 trees).

Acknowledgments

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الملخص

بن جامع، محمد الحبيب وألان روك. 1999. حصر ودراسة تأثير الآفات الحشرية في المخاريط الثمرية لأشجار السرو المتوسطي (*Cupressus sempervirens* L.) و العفصية البربرية (*Tetraclinis articulata* Mast.) وبذورهما بتونس. مجلة وقاية النبات العربية. 17(2): 107-112.

تم حصر ودراسة الآفات الحشرية والحلم التي تصيب المخاريط الثمرية وبذور أشجار السرو المتوسطي (*Cupressus sempervirens* L.) والعفصية البربرية (*Tetraclinis articulata* Mast.) بتونس، خلال السنوات 1995-1996، على كل من بقايا الأشجار بمنطقتي مكثر وبني عياش بالنسبة للسرو وغابة العفصية البربرية بسماش. وأمكن حصر 6 آفات حشرية ونوع واحد من الحلم على المخاريط الثمرية السرو و3 آفات حشرية والنوع ذاته من الحلم على ثمار العفصية. وكانت الآفات الحشرية لثمار العفصية تلك التي تصيب ثمار السرو. ولوحظ ان فاتلة الثمار *Pseudococcyx tessulatana* والحلم *Trisetacus* sp. هما الأكثر ضرراً بهاتين الصنوبريتين كونهما تتلفا ثمار العام الأول كلية. وبلغت نسبة الفقد في ثمار العام الأول 72.7% على أشجار السرو بينما بلغت نسبة الفقد في الثمار بسبب الحلم *Trisetacus* حوالي 63.3% وكانت نسبة الضرر على أشجار العفصية أقل نسبياً مقارنة بتلك على أشجار السرو. تبيّن، إثر مراقبة نمو أشجار السرو بمنطقة مكثر، تلف العديد منها حيث لم يصل منها إلى مرحلة النضج سوى 10.7%. كما نقص المحصول البذري عند الجني بنسبة 94%. وأرجع الفقد في الإنتاج لتأثير الفاتلات (41.9%) والحلم (22.2%) إضافة الى 23.7% بسبب الرعي الجائر (النشاط البشري).

كلمات مفتاحية: السرو المتوسطي، العفصية البربرية، آفات حشرية، ثمار، تونس.

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