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Biotechnology of *Pinus brutia* and *Pinus halepensis* as Important Landscape Plants of the East Mediterranean

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ABSTRACT

Aleppo and Calabrian pines (*Pinus halepensis* and *P. brutia*) are members of the Mediterranean flora. They are thought to replace the evergreen sclerophylous species in cold periods and they are used as important indicators of adverse abiotic conditions. Although ancient people in the area used the pines in many different ways, they are presently used in reforestation projects in burnt areas, city parks and “Mediterranean gardens” as ornamental trees. The methods of biotechnology are now appearing to be applied on these trees. Genetic engineering, although recognised as important in selecting genotypes resistant to pests, faces strong reaction as severely impacting non-target organisms. The suggested precautions from experts applied to all genetically modified organisms in order to prevent the spread of genes from transgenic pines to other pines in natural populations do not seem at present to affect the opinion of many nature-passionate defenders against genetically engineered methods. The selection of specific genotypes having unattractive or even repellent terpenoid profiles is similarly ecologically problematic on the basis that the normal pine arthropod fauna would not recognise the altered pine profile. This is an especially unwanted result since it includes predators and parasitoids that keep in nature the population densities of pests at reasonable levels. Micropropagation techniques have already been developed for these two pine species with successful results. Semiochemical technology incorporating recent controlled release formulations involving supra-molecular models have been applied in many cases in pine formation with promising results. Many European and North American commercial firms have already developed and released products that are effective against insect pests of these pines species.

Keywords: genetic engineering, Halepenses, micropropagation, semiochemicals, terpenoids, transgenic plants
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **ABA**, abscisic acid; **AE**, von Arnold and Eriksson medium; **BA**, N^6-benzyladenine; **BAP**, 6-benzylaminopourine; **Btk**, *Bacillus thuringiensis*; **DCR**, Gupta and Durzan Douglas-fir cotyledon revised medium; **GD**, Gresshoff and Doy medium; **GE**, Genetic engineering; **GMO**, genetically modified organism; **IBA**, indole-3-butyric acid; **Kin**, kinetin; **LS**, Linsmaier and Skoog; **MCM**, Bornman’s medium for conifer morphogenesis; **MS**, Murashige and Skoog medium; **NAA**, α-naphthaleneacetic acid; **PEG**, polyethylene glycol; **SH**, Schenk and Hildebrandt medium; **TDZ**, Thidiazuron; **TS**, terpene synthase: **WSD**, water saturation deficit

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THE EAST MEDITERRANEAN LANDSCAPE

Both Aleppo and Calabrian pines are constitutive members of the Mediterranean pines in the sense of Klaus (1989). The first signs of the bio-climatic conditions collectively known as ‘mediterraneity’ appeared 2.2 mya (= million years ago) before the Pleistocene cold periods (Naveh and Vernet 1991). Besides this not all parts of the Mediterranean basin have the same historical and associated ecological background. In the eastern part of the Mediterranean basin the pines had to cope with ecological factors such as drought and anthropogenic transformation of the landscape. These factors acted in the area for more than one million years and in the same time interval humans populated the area in waves of immigration from the east being responsible many times for the transport of exotic plants. As a result, the prevailing economies were mobile in a landscape that was a mixture of remnant vegetation types, mainly pine forests (other tree species came later in a more humid period) and maquis shrublands (Beug 1975; Petrakis *et al*. 2000) and a degraded bushland (phrygana). This is in contrast to the western part where the landscape is well compartmentalized in parcels of properties belonging to members of more settled human societies (Barbero *et al*. 2000).

In this scenery of the eastern Mediterranean the pine species that belong to the ‘thermophilus’ group such as *P.*

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Invited Review

Fig. 1 *Pinus brutia* reach the spray zone on a south coast (West Crete). It can be noticed that the pines "avoid" the loose soil coming from the erosion of rocky slopes. These scenery is extremely rare now in the Greek Mediterranean coasts due to urbanization of coasts or the building of isolated tourist establishments.

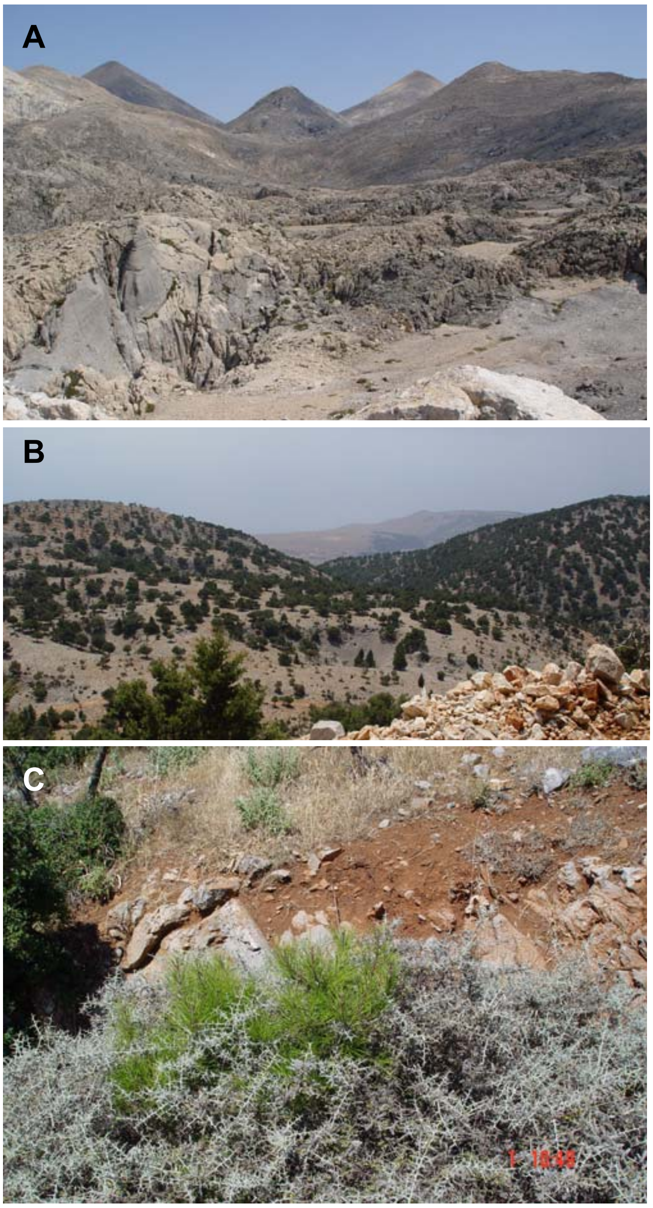

Fig. 2 The mountainous desert of western Crete as seen from the path leading to Pachnes summit (**A**). On the same mountain (Mt Lefka Ori) a sparse wood of *P. brutia* is formed, which becomes denser at lower altitudes (right part of the photo (**B**)). Anthropogenic factors and grazing are mistakenly invoked to explain the mountainous desertification. The Calabrian pine usually is growing with the aid of *Astragalus spinosus*, which is used as a nurse bush (**C**). The pine escapes grazing from goats within this spiny bush. The lack of pines from many places with low altitude is ought to the lack of nursing plants.

halepensis and *P. brutia* dominated the landscape (Barbero *et al*. 2000). This was especially prominent at cold epochs when pines replaced the mixed deciduous forests dominated by oaks, in many places (Bottema 1975). The retraction of pines from lowlands was the result of anthropogenic activities mainly the burning of forests to promote the growth of grasses creating thus grazing grounds for the flocks of domesticated animals; the creation of agricultural lands was another activity that used domesticated plants such as *Triticum dicocoides*, *Hordeum spontaneum*, and *Secale secale* and various legumes (Naveh and Vernet 1991). Only in inapproachable coasts could pinewoods extend up to the spray zone of the sea (**Fig. 1**). The mobile economy was a reaction of archaic man to the heterogeneous landscape after the abandonment of kinship bonds (= 'blood ties') where pines formed localized forests that harboured game or provided the proper vegetation for the improvement of some domesticated agricultural trees such as *Olea europaea* var. *oleaster* (Hort 1916, 1926; Levi-Strauss 1963; Coles and Higgs 1975). A similar landscape situation was created with the 'meso-Mediteranean' group of pines, which includes *P. canariensis*, *P. pinaster* and *P. pinea*. On Tenerife, Canary Islands *P. canariensis* colonises volcanic debris and forms a sparse forest belt at elevation between 400 and 2200 m (Barbero *et al*. 2000). In Greece the same situation is observed on large islands such as Crete where the Calabrian pine forms sparse forests that have disappeared from high elevations creating a mountainous desert (Rackham and Moody 1986) (**Fig. 2**). In mainland Greece *P. halepensis* prevails on mountain slopes up to 700 m but this pine as a rule forms dense woods, which are threatened only by fire and/or building.

All eastern Mediterranean pine formations are regenerated by seeds derived from the local soil seed bank. For this, since prehistory the various uses of pines never necessitated the artificial improvement of pines. Aleppo and Calabrian pines provided the raw material for many utilisations except seeds for which the stone pine *P. pinea* is more suitable. A list of uses of these two pines includes:

1. Construction material of houses and as resin source, a wood conservation agent, or resin-derived products substantially subsidized shipbuilding. The discovery of the copper made ripsaws by Minoans boosted the use of pinewoods since it is possible to convert logs into planks (le Maitre 2000).
2. The copper industry both in copper-mine props and smelting demanded a continuous and large quantity of fire- and construction wood. The natural durability of pinewood made it an ideal material for mine propping (Meiggs 1982).
3. In religion, pines played a major role and Greek mythology is full of legends and historical records about presumably these two species of pines. Many myths contain important ecological observations on pines and resin. For instance, the myth of *Pitys* who was the nymph living in pine trees had two lovers *Boreas* (= means the cold northern wind) and *Pan* (= the goat-footed god of forests and especially animals; *pan-is* = fauna). Because *Boreas* was jealous of *Pitys*, he threw her from a cliff on a rocky ledge. *Pitys* to cope with the adverse conditions became a pine and tears (resin) dropped from her eyes (Kerenyi 1966). The entire myth is impregnated with the ecological knowledge of the abiotic requirements of pines and the healing properties of the resin that was used – with the addition of *Hypericum perforatum* herb – as a healing agent for wounds inflicted in the battles.

4. In house building and the construction of kiln-fired pottery pines were broadly used and Aleppo and Calabrian pines were sufficiently abundant and particularly suitable.
5. Therefore it was a natural consequence to conserve some pine forests and even try to improve these pines. Because the reasons for conservation could not be other than religious ones, ancient Greeks and other near-East civilizations as well, devoted large fragments of pine forests to the gods and heroes. These forests were sacred and religious laws protected them from timbering, burning and hunting. Greeks called these forests *temenē* (sing. *temenos*), which actually means a land parcel (Petrakis *et al*. 2000).
6. Other minor uses include the construction of furniture, the marking of fields, arable land and properties in lowlands. In Roman and Byzantine times pines were used also for the construction of forts, army barracks or siege engines (Meiggs 1982).

For all these reasons the thermophilous Halepenses pines (*sectio* Halepenses) became extinct from many lowlands in Greece. Some sandy coasts were forested with *P. pinea* woods and within these forests some spots of *P. halepensis* survived (e.g. the pinewood at Schinias, **Fig. 3**, Petrakis 1991). It was noticed that some provenances were particularly resistant to insect attack or avoided cambio-phloeophagous insects and/or needle herbivores such as the pine processionary moth (*Thaumetopoea pityocampa*) and escaped from the damage conferred from the pine scale (*Marchalina hellenica*) (Yesil *et al*. 2005). The fact that stone pines have different varieties on morpho-anatomical and biochemical grounds created the need for improvement of the existing trees towards more drought, disease and insect resistance, quicker growth and robust shape. Although the conventional tree breeding techniques were successfully applied to stone pines they were not extended to Aleppo and Calabrian pines. Several reasons relate to this. *P. halepensis* and *P. brutia* are not used in parks and gardens in cities and in this respect they are economically unattractive in breeding programs.

Reforestation programs of burnt forests pines belonging to subsection Halepenses (Price *et al*. 2000) are also unattractive since their exploitation has a very low economic potential while their growth rate is very low. Halepenses pines are not considered economically valuable in spite of the fact that they are naturally very drought resistant (Radoglou 1987) and survive at acceptable levels even in very shallow soils. Brofas (1998) reports that in a rehabilitation program of quarry terraces in Drakia, Magnesia *P. halepensis* seedlings had a survival rate of 100% five years after the initial planting in soil having a depth of 40 cm while at 20 cm the survival was 66.7%. Pines belonging to subsection Halepenses are currently used for firewood, charcoal and resin extraction (Price *et al*. 2000) and only exceptionally is their timber used for furniture or construction (Roussodimos *et al*. 1987). However, the results from trials from many provenances around the globe suggested that *P. halepensis* and *P. brutia* – especially subspecies *eldarica* – have characteristics that make them suitable for reforestation programs not only in the Mediterranean region (Bariteau 1992; Diamantoglou and Banilas 1996) possibly among other properties for their ability to withstand drought. The latter is an important property as temperatures are expected to rise as a result of global warming, which can be so dramatic as to cause a change in the conservation priorities and the design of national parks (Scott *et al*. 2002).

Fig. 3 A coastal pinewood at Schinias. The pinewood is formed by *P. pinea* and *P. halepensis*. The stone pine forms woods on sandy soils, which are irrigated by fresh water. After the modification of the soil, Aleppo pine dominates. For that reason, the right part (northern) of the picture is dominated by Aleppo pine while the seaward (left) part is dominated by stone pines. The plants at the first place of the photo are *Juniperus phoenicea* and *Phlomis fruticosa*. These plants are typical of Mediterranean rocky slopes formations called macchia and in this case pseudo-macchia.

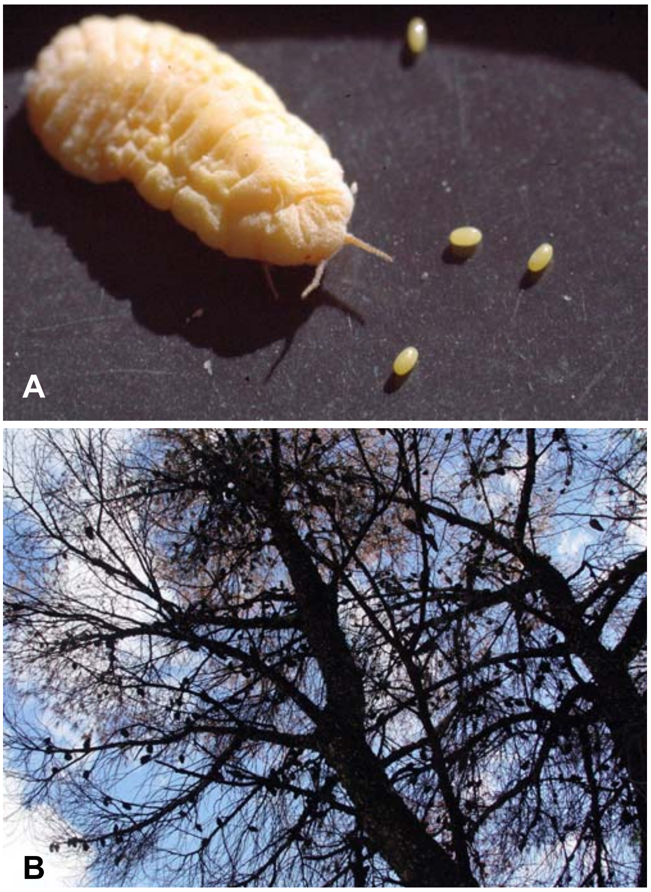

Fig. 4 (**A**) Female *Marchalina hellenica* (Hemiptera, Sternorrhyncha, Margarodidae) looking for oviposition places. In these places she will lay 250 eggs in average. Four such eggs are shown nearby. (**B**) Nevertheless, the adult stage does not feed in contrast to the immature instars that feed on the sap of the pine tree causing extensive die back.

Very recently, Halepenses pines were attacked by beekeepers that included the honeydew-producing pine scale *Marchalina hellenica* (**Fig. 4**) in programs introducing the insect in pine forests and isolated trees (Bikos 2000), which have never been colonized by the insect. Bees are attracted by the honeydew produced by the insect (Crane and Walker 1985) in the dry season of the year thus producing honey in quantities almost twice as much as those produced in beehives in places with no pine scale (*pers. obs.*). However the insect causes a multitude of adverse effects on the pine hosts that weakens the tree and makes it more susceptible to bark beetles (Coleoptera, Scolytidae) (Byers *et al.* 1979). The bark beetles perceive the pines because of their altered profile and attack all trees colonised by *M. hellenica*. In the same way pine scale colonizes the pine trees because of their suitable profile. Indeed, some *P. halepensis* escape colonization by pine scale together with a form of *P. pinea* at Malaxa, Crete because they are judged as unsuitable feeding substrates (Mita *et al*. 2002).

On these grounds we tried to compose a mixture of terpenoids, which would cause a feeding disruption of the pine tree to the pine scale (Petrakis *et al*. 2006). Actually the mixture of terpenoids signals the fact that the substrate is not the proper one and for this the insect has to search in another place or in another host tree for the insertion of its feeding stylets. Because the insect can move only in the first crawling stage it relies largely on other attending insects for its spacing, feeding site and host selection. This has an adverse effect on the insect that diapauses because of food deprivation, dies of starvation or from the action of natural enemies including birds (Jantti *et al*. 2001). Further investigations showed another effect of the terpenoid mixture on the pine scale. It reduces the number of eggs in the ovarioles of the pine scale. The insect normally produces 180-250 eggs, sometimes 400. When it is treated with the invented mixture the number of eggs decreases substantially reaching 20-40 (Petrakis *et al*. 2006). This additional physiological effect on pine scale prevents the build up of an insect population after the action of a natural enemy or the repellence of the pine host.

It is hoped that this mixture, and other similar mixtures as well, will be the main agent in Greece for the reduction of pine scale in an ecologically meaningful way since it is very specific and does not cause problems to other arthropod and plant populations. Pine-dominated landscapes, urban parks, gardens and rows of trees in roads and avenues are expected to be replanted with Halepenses pines according to the pine species, which is native to the area. The increased demand for seedlings and saplings is expected to be satisfied with propagation and improvement techniques.

WHAT IS BIOTECHNOLOGY

According to a definition of biotechnology it includes "any technique that uses living organisms, or parts of organisms, to make or modify products, to improve plants or animals, or to develop micro-organisms for specific uses" (Persley 1990). If we restrict this definition to modern biotechnologies then all the techniques listed in Haines (1992) such as genetic engineering, micropropagation, cryopreservation, somaclonal variation, haploid cultures and others can be included. All these techniques are referring to the pine tree itself and not the biotechnology that can be used for the ecologically safe protection of Mediterranean pinewoods and city parks preserving also the rich fauna of these trees. With regard to the protection of the pine trees from arthropods, mainly insects, many approaches have been employed. The most important is the improvement of pine trees through reforestation with drought resistant provenances like the one in west Attica. Other methods include the breeding techniques towards insect resistant phenotypes, the natural enemy augmentation through controlled release formulations. These formulations involve supramolecular structures, usually made of β-cyclodextrine "cups". The attractant compound is inserted in these cups – or sandwiches – and released in a regular way causing, by attraction, the augmentation of the natural enemies of *T. pityocampa*, *M. hellenica* and bark beetles. The same controlled release technology is used for pheromone inclusion of pheromones of major pests or potential pests. We have used the technique (**Figs. 6-8**) in the case of two compounds comprising the pheromone of the pine processionary caterpillar *T. pityocampa* and the two compounds in the pheromone mixture of *Orthotomicus erosus* and *Pityogenes calcaratus*. Some slow release formulations have been also entered in the market.

PHEROMONES AND KAIROMONES

The use of pheromones and kairomones in forest trees and especially those species that are used in urban areas is mandatory. In a broader context it has been stated that it is possible a nationwide mass trapping of bark beetles by means of baited traps (Brockerhoff *et al*. 2006). The use of insecticides causes a multitude of side effects to non-targeted organisms (**Table 1**). Even in the case that bait trees or trap trees are used, the killing of non-targeted organisms is not minimised since many insects gather at places where the concentration of prey species attained high densities and in addition they are more susceptible to predation (**Fig. 5**). Unfortunately the insects that predate on easily captured

Table 1 Herbivore pest insects and non-target insects in traditional and intensive olive culture. These insects are commonly found on and are affected in various ways by attracticide formulations involving deltamethrin as a killing agent.

Order	Predators	Order	Herbivores
Dictyoptera		Diptera	
	Mantodea, Mantidae		Tephritidae
	Geomantis larvoides Pantel		*Bactrocera oleae* (Gmelin)
	Mantis religiosa L.		Muscidae
Neuroptera			*Musca domestica* L.
	Chrysopidae	Rhynchota, Sternorrhyncha	
	Chrysoperla carnea (Stephens)		Coccidae
	Raphidiidae		*Saisetia oleae* Olivier
	Raphidia attica Aspock & Aspock		Psyllidae
Mecoptera			*Euphylura olivina* (Costa)
	Panorpidae	Rhynchota, Auchenorrhyncha	
	Panorpa germanica graeca Lauterbach		Cicadellidae
Rhynchota, Heteroptera			*Synophropsis lauri* (Horvath)
	Anthocoridae		Issidae
	Orius laevigatus (Fieber)		*Quadrastylum basiniger* Dlabola
	Anthemis nemorum (L.)	Lepidoptera	
	Xylocoris galactinus (Fieber)		Ypomomeutidae
	Miridae		*Prays oleae* (Bernard)
	Deraecoris schach (Fabricius)		
	Calocoris trivialis Costa		
Hymenoptera			
	Encyrtidae		
	Ageniaspis fuscicollis Dalman		
Coleoptera			
	Staphylinidae		
	Staphylinidae sp.		
Diptera			
	Asilidae		
	Selidopogon diadema Fabricius		

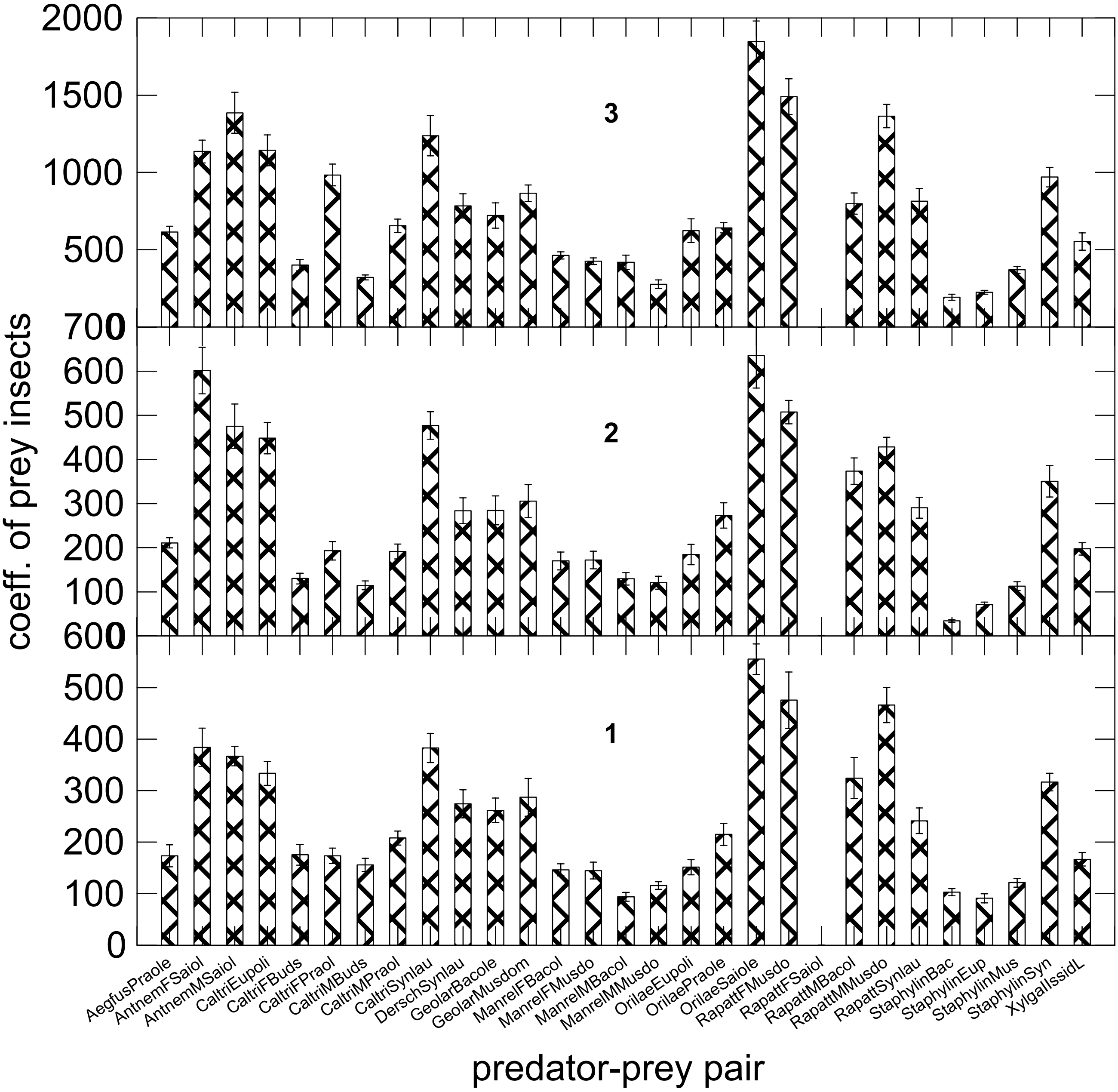

Fig. 5 Diagram showing the a_1 regression coefficient of the number of prey insects with the respective standard error in the three regressions i.e. 1: $T_{handle} = a_0 + a_1 N_{prey}$, T_{handle} is the handling time; 2: $T_{inabil} = a_0 + a_1 N_{prey}$, T_{inabil} is the time taken for the insect to be motionless but not dead; 3: $T_{death} = a_0 + a_1 N_{prey}$, T_{death} is the time to the death of an insect. Data are coming from an olive grove. The name of a specific regression and of a predator–prey pair is as follows. AegfusFPraole = *Ageniaspis fuscicollis* ♀– *Prays oleae*, AntnemFSaiole = *Anthemis nemorum* ♂ - *Saisetia oleae*, AntnemMSaiole = *Anthemis nemorum* ♀ - *Saisetia oleae* , CaltriEupoli = *Calocoris trivialis - Euphylura olivina* , CaltriSynlau = *Calocoris trivialis - Synophropsis lauri*, CaltriFBuds = *Calocoris trivialis* ♀ - Flower buds, CaltriFPraole = *Calocoris trivialis* ♀, CaltriMBuds = *Calocoris trivialis* ♂ - Flower buds, CaltriMPraole = *Calocoris trivialis* ♂ - *Prays oleae*, DerschSynlau = *Deraecoris schach - Synophropsis lauri*, GeolarBacole = *Geomantis larvoides - Bactrocera oleae*, GeolarMusdom = *Geomantis larvoides - Musca domestica*, ManrelFBacole = *Mantis religiosa - Bactrocera oleae*, ManrelFMusdom = *Mantis religiosa* ♀ - *Musca domestica*, ManrelMBacole = *Mantis religiosa* ♂ - *Bactrocera oleae*, ManrelMMusdom = *Mantis religiosa* ♂ - *Musca domestica*, OrilaeEupoli = *Orius laevigatus - Euphylura olivina*, OrilaePraole = *Orius laevigatus - Prays oleae*, OrilaeSaiole = *Orius laevigatus - Saisetia oleae*, RapattSynlau = *Raphidia attica - Synophropsis lauri*, RapattFMusdom = *Raphidia attica* ♀ - *Musca domestica*, RapattFSaiole = *Raphidia attica* ♀ - *Saisetia oleae*, RapattMBacole = *Raphidia attica* ♂ - *Bactrocera oleae*, RapattMMusdom = *Raphidia attica* ♂ - *Musca domestica*, StaphylinBac = Staphylinidae sp. - *Bactrocera oleae*, StaphylinEup = Staphylinidae sp. - *Euphylura olivina*, StaphylinMus = Staphylinidae sp. - *Musca domestica*, StaphylinSyn = Staphylinidae sp. - *Synophropsis lauri*, XylgalIssidL = *Xylocoris galactinus* – Issidae sp. larva (probably *Quadrastylum basiniger*).

prey, i.e. the prey on trap/bait trees, suffer many adverse effects such as reduced searching efficiency as a result of significant reductions in sensory abilities (Salerno *et al.* 2002; Petrakis, pers. obs.).

This technology was actually transferred from olive and apple culture without modifications. Possibly, this is due to the serious under-funding of forest entomological projects. Only very recently when it became a necessity to conserve the biotopes of many insects and associated hosts the problems created by the use of pesticides appeared as important (all the papers in Collins and Thomas 1991; Kogan 1993).

Insect conservation posed the necessity to find specialised methods for pests in order to suppress their populations, not to eradicate them (Kogan 1993). Many companies, especially small and medium enterprises employing academic entomologists as consultants, invented synthetic protocols and dispensers for pheromone and kairomone formulations that are used by many practitioners and researchers. A promising pheromone formulation and associated dispenser re-

Fig. 6 The cleaners of the wood belong to the family Scolytidae (Coleoptera). These insects are usually caught with Thyssen traps (**A**) (Trifolio-M GmbH, Germany) and can be either equipped with aggregation pheromones or not, acting thus as intercept traps. We used these traps extensively for monitoring scolytids. As a pheromone mixture we used either the Ortero™ blend for the attraction of *Orthtomicus erosus* hang inside the trap (shown outside in **B**). We have also used a different formulation involving β-cyclodextrine supramolecular cups in order to achieve a slow release of the pheromone blend. The slightly exposed drawer in the bottom of the trap is used for collecting the insects and it uses also a long lasting formulation of an insecticide.

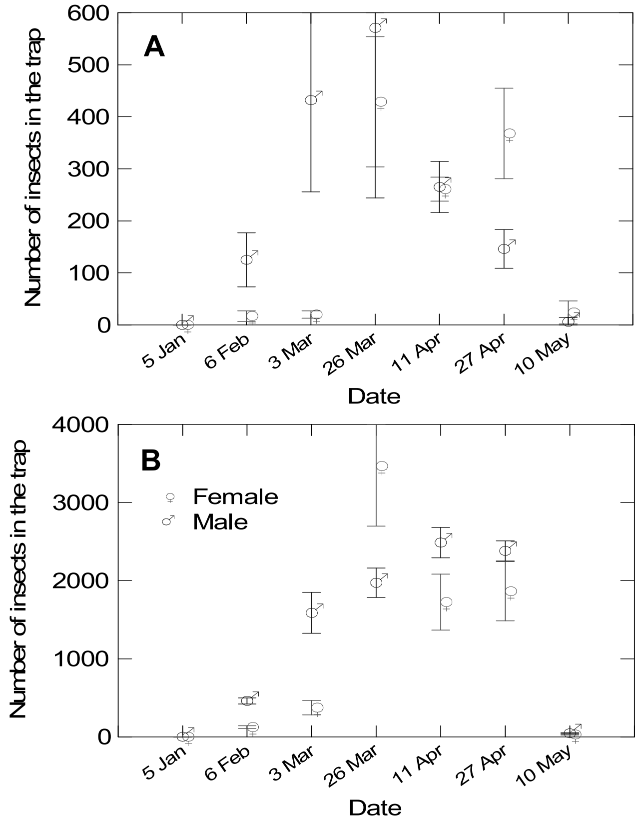

Fig. 7 Trap mean catches of *O. erosus* in Thyssen® traps without (**A**) and with (**B**) the pheromone of the insect. The dates are not equally spaced. The error bars correspond to one standard error of the mean (=1 SEM).

leased on the market for the enhancement of catches is the Thyssen® bark beetle trap. In these formulations the pheromones are encapsulated in microsomes and the entire mixture is put in a polyethylene bag ready to be put in the trap. The product is the Tripheron® formulation with controlled release dispensers Ortero® for *Orthotomicus erosus* and Ipstyp® for *Ips typographus* of Trifolio-M Gmbh, Germany with very good results for attracting *O. erosus* and *I. typographus*. We applied the technology in pinewoods seriously affected by pine scale, which caused aleppo pine trees to be weakened or sometimes die. These pines are the best substrates for *O. erosus*. We found that the catches of pheromone-equipped traps were significantly more numerous than the ones of simple Thyssen traps (**Fig. 6**). Indeed the difference in the mean seasonal catches of insects in all traps set was 989.5 and under the pooled variance model it was found that $t = 3.14$, d.f. = 26 (= number of traps under the pooled variance model), $P = 0.004$ (**Fig. 7**). The difference is pooled over all species caught in the trap since many species use the pheromone blend or some components as a kairomone and the trapping design was one trap (*c.* 30€) per tenth hectare set with ropes between two trees (**Fig. 6**).

In order to minimise the population densities of pests the method of mating disruption is usually applied. In this method two major techniques are tried to cause the disruption of the mating sequence in the targeted insect pest. The first is the disorientation of the pheromone searching sex (typically the male sex) by deploying many pheromone sources, which may be caged females or controlled release dispensers of pheromone compounds. The second technique corresponds to the disruption caused by the blocking of the

Fig. 8 Close up of a micro-delta trap (Oikos Ltd, UK). This type of trap is used for the mating disruption of *Thaumetopoea pityocampa* (Lepidopera, Thaumetopoeidae). The floor of the trap is covered with dust of wax. The trap is either equipped with the sex pheromone of the pine processionry moth or the pheromone can be inserted in the hole at the floor of the trap (slightly lifted in the photo to show the design of the trap). When the male approaches this pheromone source the dust covers its sensors on the antennae making the moth unable for further searching and mating with the female. In this way emerge two sources of population decrease. The first is the reduced reproductive output through the prevention of fertilisation of the eggs in the body of the female. The second mortality source is more sophisticate and is due to the increased searching time of the male combined with the concomitant augmentation of natural enemies because of the available food resource.

pheromone sensors of the searching sex either by filling the pinewood space with a pheromone compound released steadily from special dispensers or by acting directly on the ability of the searching sex to sense the pheromone emitted by the opposite sex. A commercial firm (Oikos, Ltd, UK) exploits this technology in the micro-delta traps (**Fig. 8**). These traps are sprinkled with a white powder around a pheromone dispenser located on the middle of the floor of the delta trap. The approaching male, which is the searching sex in *T. pityocampa*, receives the powder on the antennae, which are blocked as the particles of powder are probably inserted in or cover the pores on the surface of pheromone sensors. In this way the male is unable to locate emitting females and the mating behavioural sequence is thus disrupted. The white powder supplied with the traps by Oikos, Ltd (UK) is actually a plant wax derived from the surface of the leaves of a South American palm (Dr. Underwood, pers. comm.). Actually the powder can be any material that can block the sensors of the male moth.

Research must be done in this direction to test the applicability of the technique in other insect mating sequences. However, the method can be applied only in urban parks and small woods since the micro delta traps must be set at relatively short distances ranging from 25 to 50 meters. Also, as in all mating disruption methods, the population densities of the pest must be low – i.e. two or three medium-sized nests per pine tree having a height *c.*10 m and not more than 25 traps per tenth hectare – in order for the candidate mates to rely only on their olfaction to locate each other. We applied the method in a series of occasions with promising results (Petrakis, pers. obs.). In an application site on Mt. Hymettus, Attica the suppression of the population was 100%. However, the application terminated prematurely because of the outbreak of pine scale that is expected to jeopardise the results. We know of no other applications of the mating disruption method on pines eventually with other dispensers and protocols.

In Israel and many other East Mediterranean countries as well, the pine bast scale *Matsucoccus josephi* is oligophagous on the genus *Pinus* as a result of the parallel evolution of the two genera *Matsucoccus* and *Pinus*. In Israel the insect infests young pine trees and causes deformation and mortality to many plantations of *P. halepensis* (Mendel and Liphschitz 1988; Liphschitz and Mendel 1989). The pheromone of this insect is used by the main natural enemy *Elatophilus hebraicus* (Heteroptera, Anthocoridae) as a kairomone (Mendel *et al.* 1992, 1995). A possible attraction of *E. hebraicus* in plantations where the pine bast scale is still in low numbers should involve dispensers of the kairomone with long duration. However, the problem of all methods of natural enemy augmentation is the confusion of the attracted insects so they are unable to locate the prey – presumably a pest-insect.

BEHAVIOUR-MODIFYING CHEMICALS OTHER THAN PHEROMONES

It is very rare in Greece to manage insect populations on pines by involving semiochemical technology or even microbial insecticides although the ministry of agriculture has recently used *Bacillus thuringiensis* formulations for the management of *T. pityocampa* in west Attica forests. The usual recommendation is the spraying of an insecticide (Kailidis 1962; Georgevits 1974; Markalas 1987) and very rarely in the last decade forest entomologists suggest the use of a microbial, usually *B. thuringiensis* var. *kurstaki* (=Btk) for lepidopteran pine pests such as the pine processionary caterpillar (Rausell *et al.* 1999), which in most cases achieves a high degree of population suppression (Battisti *et al.* 1998) as a result of a series of toxins (Shevelev *et al.* 2001). In the first insecticide applications the problem of the effects on other insects inhabiting the pinewoods and on public health was not seriously taken into account. Recently, non-target organisms are starting to pose problems in many insecticide applications. For instance, the management of lepidopteran larvae with Btk is recommended in the late autumn/early winter months when most insects have completed their life cycle and no new larvae are expected for a particular year except for some Tortricidae moths, which overwinter as larvae. This generally restricts the use of Btk since it has best results on the first instars of lepidopteran pests such as *T. pityocampa* when many other non-target butterflies and moths are also in the larval instars. Nevertheless, Btk is applied in many cases presumably when no other insects eat foliage except for the target pest. Such applications are done in the Aleppo pine forests in west Attica. The effect on the west Attica arthropod fauna is currently being investigated though in many cases we know that the application of any kind of insecticides has a multitude of adverse effects on arthropod assemblages (Saarinen *et al.* 2005).

In nature, it is now evident that populations of pests can be regulated only by means of natural enemies. The techniques associated with this are directed towards to what is known as *natural enemy augmentation*. The attempts to augment the fauna of natural enemies are categorised as [1] leaving native vegetation to grow in special biotope fragments [2] attract natural enemies from nearby sites by using mixtures of compounds. The first method is appropriate for small parks and urban woods where the strips of native vegetation are expected to harbour several especially polyphagous insects (Tscharntke *et al.* 2002) that can support predatory and parasitoid species. The second method is more suitable for natural pinewoods and is used mainly to increase the population densities of predators and parasitoids in forest spots where they are needed. In this respect we increased the parasitism by egg parasitoids of *T. pityocampa* in Villia-Porto Germeno, west Attica, Greece in a set of forest spots where we applied L-tryptophane, a universal attractant of natural enemies (Petrakis *pers. obs.*). For long-lasting attraction we used supramolecular β-cyclodextrine cups where the attractant compound was enclosed and subsequently were released at small quantities.

Although the method can be potentially applied in many occasions nevertheless the entire pine forest must possess a substantial fauna of natural enemies. This was not the case of several Albanian small woods, e.g. near Pogradetz and Puka, where local pines belonging to *P. nigra* subsp. *austriaca* were heavily attacked by *T. pityocampa*. The trees were distantly planted in the area and short enough to harbour any substantial fauna of natural enemies. Moreover, their attraction from nearby sites is impossible since no pinewoods exist in nearby formations.

It is inherent in all conservation programs that old forests offer the most appropriate biotope for many important arthropod species (Simberloff 1998; Ranius 2002; Ranius *et al*. 2005). In small fragments of these old forests it is common practice to apply prescribed fires around old trees to reduce fuel biomass (Spies *et al*. 2005). If the old trees are Aleppo or Calabrian pines then it is very common that these trees are attacked by jewel beetles (Coleoptera, Buprestidae) and other insects. The attraction technique by means of long-lasting attractants of natural enemies can serve for the rapid augmentation of natural enemies that can potentially control pine pests.

A future direction of research on natural enemy attractant formulations and dispensers could be the investigation on alternatives to L-tryptophane such as the mixtures of waste products of fermenting bioreactors producing Btk. The product has an overall attraction to natural enemies but their duration is very short, just 3-5 days (Petrakis 1994). A supramolecular formulation seems to enhance the durability of the attraction although our initial experiments with β-cyclodextrin failed to show any consistent pattern of enclosure in a molecular simulation of the active compounds.

For honeydew-producing pests of pines exists the possibility that the honeydew can trigger an intense searching behaviour to hyperparasitoids (Buitenhuis *et al*. 2004). The isolation of chemicals that affect the foraging behaviour of parasitoids from the honeydew of healthy, i.e. non parasitized, pest insects is the next step to be undertaken in future research. These chemicals can be adequately used to attract natural enemies that are effective control agents of the honeydew-producing pine pests, e.g. *M. hellenica*.

A debate has begun on the ultimate effect of Btk on insects eating transgenic pines in the case that the toxins of Btk are inserted in the genome of Halepenses pines. Sayyed *et al*. (2003) expressed serious concern about the possibility of insect pests developing resistance to the inserted Btk toxin. More precisely they state that resistant insects can consume additional protein from the transgenic plant so they can benefit from it. Moreover, they list the cases of other insects such as the tree locust, *Anacridium melanohodon* that uses gallic acid (a phenol) to complete the sclerotization of its cuticle and oblique-banded leaf roller, *Choristoneura rosaceana* that use tannic acid for its faster development.

TERPENOIDS

Terpenoids occur in nature in more than 30,000 molecular forms (Bernays and Chapman 1994). In conifers they are said to have many roles ranging from the odour-associated fingerprinting of the host to a protective function through the terpenoid-based defence they offer to the plant against bark beetles, fungi and bacteria (Petrakis *et al*. 2001; Trapp and Croteau 2001; Phillips *et al*. 2006; Erbilgin *et al*. 2006). The pines that belong to the subsection Halepenses are not an exception to this. It was found that *P. halepensis* has several terpenoids, which differentiate it from *P. brutia* and both pines are differentiated on the basis of constitutive terpenoids of needles (Roussis *et al*. 1995) and in addition they constitute excellent phylogenetic markers (Petrakis *et al*. 2000).

Although the details of the early and late steps in the biosynthetic pathways of terpenoids have not been explored in conifers it is known that only a few enzymes catalyzing their biogenetic pathway − i.e. terpene synthases (TPS) − exist (see **Table 1** in the review of Keeling and Bohlmann 2006). The elucidation of their evolutionary history revealed that the TPS genes in all conifers originate from a single ancestral TPS gene. The variety of TPS genes results from gene duplication and changes. In many genomes each TPS gene produces a single terpenoid. Nevertheless, the presence of a few TPS genes produces a variety of compounds. Except from the main terpenoid compounds several other terpenoids are produced in minor though stable quantities (Huber *et al*. 2004). In addition to this diversity are the chemically-induced terpenoids by traumatic simulation of the conifer tissue involving the production and release of methyl jasmonate (Phillips *et al*. 2006). With this induction the conifer has sufficient anatomical and associated chemical defences to face the attack of an insect or pathogen (e.g. Christiansen *et al*. 1999; Fransceschi *et al*. 2005; Nicole *et al*. 2006; Zeneli *et al*. 2006). The same morpho-anatomical modifications were observed in pines colonised by the pine scale *M. hellenica* in a few days after the insect had inserted its stylets in the pine bark and cambial zone (Petrakis *et al*. 2005).

The above findings create the notion that it is very difficult for terpenoids to be engaged in a pine improvement project. Typically such a project would create a pine genotype that is plastic enough to be armed with the proper terpenoid armature against insects and pathogens. Added to this difficulty is the alteration of the terpenoid profile that can render the pine almost "invisible" to the arthropods that use green leaf volatiles as main or auxiliary compounds in the configuration of the profile (Pschorn-Walcher 1977). The alteration of the terpenoid profile of the pines may render them unpalatable or unattractive to insect enemies (Miller and Borden 2000). The same alteration can be achieved by means of dispensers with a long duration or special mixtures of terpenes that can be sprayed on the Aleppo and Calabrian pines to protect them; importantly the specific mixtures of terpenes are made with reference to the insect pest (e.g. for the stripped ambrosia beetle, *Trypodendron lineatum* [Dubbel 1992]; for four bark beetles species [Kohnle *et al*. 1992]). In a tree improvement genetic engineering project the alteration of the profile is rapid and drastic in contrast to the gradual improvement through the improvement of the pine trees by means of seed propagation. In this case the alteration of the profile will be slower than the genetically engineered one but much faster than the natural one involving natural selection, which presumably removes all the genotypes with a poor, or easily detectable, terpenoid armature.

Several terpenoid compounds function as inhibitors of the growth of insect larvae such as the diterpene acids kaurenoic and trachylobanoic (Elliger *et al*. 1976). These terpenoids can be found outside conifers. Terpenoids received from Gorgonian corals (Roussis *et al*. 2001) have an intense and taxonomically diverse antibacterial action not only against the microbes found on conifers but also on bacteria commonly found in the human environment. In general it is believed that marine organisms are free from gravitational forces and the need to maintain a shape by means of an internal endoskeleton structures, have evolved biochemical pathways in which various chemical structures can be produced. Researchers engaged in tree improvement projects believe that chemical engineering can provide a means to make the conifer genome capable in synthesizing novel terpenoids or other defences. Micropropagation is a necessary step before the massive production of commercially available material. At present the research on non-industrial wood producing conifer tree improvement proceeds at such a slow pace this task remains a distant prospect; the same is true for most transgenic methods.

Finally, it must be kept in mind that most tree improvement projects on Halepenses have the strategy of directional growth toward straight trunks and primary lateral branches, faster growth and resistance to drought. Drought resistance is already a trait of *P. halepensis*, which is able to refill the embolised from severe drought xylem with water and in this way recover (Borghetti *et al*. 1998). In this sense this species is superior to the 'water spenders' of Mediterranean macchia formations *Quercus* coccifers and *Quercus ilex* (Baquedano and Castillo 2006). The related Calabrian pine, *P. brutia* in certain high quality habitats shows less water loss for a given decrease of water potential. This means that in Calabrian pines there is an eco-physiological mechanism with which the trees avoid the high values of *water saturation deficit* (WSD) (Radoglou 1987). With reference to growth, very limited alteration of the rates can be done. Mainly because the Halepenses are pre-adapted to cope with alterations of the soil humidity and the associated availability of nutrients. Moreover, it was found that these pines are adapted to varying nutrient levels, which is expected to be restrictive in the present global climate change (Sardans *et al*. 2005).

MICROPROPAGATION OF HALEPENSES

Halepenses are important components of Mediterranean plant formations. Mediterranean type ecosystems worldwide are exposed to frequent fire (Naveh 1974, 1975; Fox and Fox 1987). Both pine species are adapted to recover from fire, and other disturbances usually induced by humans. The adaptation is realised by serotiny that is their ability to open their cones and release seeds which subsequently germinate (Neéman and Neéman 1993; Thanos and Daskalakou 2000).

These pines are easily propagated by grafting (whip, cleft and side grafting). Propagation by cuttings is extremely difficult due to low rooting rate (Thorpe and Biondi 1984).

During the past decades biotechnological methods appeared for the clonal micropropagation of selected pine provenances, with predefined characteristics, that they include *in vitro* culture techniques (e.g. the technique used for *Cistus creticus* for medicinal uses Zygomala *et al*. 2003). In general the tissues from mature conifers have proven extremely difficult to culture *in vitro* (Attree and Fowke 1991). For these reasons immature or mature embryos, cotyledons or hypocotyls segments, seedlings, young needles or shoots, apical buds or other similar plant organs and parts are used as explant types (Aitken-Christi *et al.* 1985; Becwar *et al.* 1988).

Shoot differentiation was obtained from embryonal explants of Aleppo pine when cultured on cytokinin-containing media (Lambardi *et al*. 1991). The concentration and duration of application of plant growth regulators alone or in combination, and the age of explants all significantly affected the bud-forming capacity. Lambardi *et al*. (1991) achieved shoot elongation on hormone-free medium and the elongated shoots were rooted. At least 90% of the shoots formed normal roots and the rooted shoots were readily hardened and transplanted to the greenhouse with 100% success. The same group (Lambardi *et al*. 1993) tested mature embryos and various explants of the embryo to optimize tissue culture conditions for micropropagation of Aleppo pine worked with two media (AE, von Arnold and Eriksson (1981) and MCM Bornman (1983)) at several concentrations. Over 90% of the embryo explants gave rise to adventitious buds within 4 weeks. Intact embryos were the most suitable explants for shoot bud induction. Both isolated cotyledons and hypocotyls produced adventitious buds, but these developed slowly and failed to elongate. Adventitious bud development was achieved on hormone-free AE medium and shoot elongation was optimum on three quarter-strength Bornman's MCM medium. Shoots were multiplied on MCM medium, containing 5 μM BA and induced adventitious roots by pulse treatment with 1 mM IBA for 6 h. After acclimatization for 3 to 4 weeks under mist, almost all the rooted shoots could be transplanted successfully to the greenhouse, where the plants exhibited normal growth habit.

P. halepensis embryos placed into MS medium showed hypocotyls and cotyledon development (Diamantoglou and Banilas 1996). At the root site a slimy brown callus was formed but upon the addition of NAA (at concentration of 0, 5, 1 and 2 mg/l) significant root growth was exhibited. Adventitious buds and some axillary buds were formed at the cotyledons and hypocotyls of embryos, which were cultured on AE medium supplemented with cytokinin (1 to 20 μM BAP). The concentration of BAP and the strength of the medium affected the bud formation with best results achieved at ½ x AE + 5 μM BAP combination. The development into shoots occurred after subculture in a medium without growth regulators and also on the induction medium supplemented with 5 μM BAP. The adventitious shoots were spontaneous rooted at a frequency of 5%. Somatic embryos at the early cotyledon stage were induced when Aleppo pine mature embryos were cultured on AE medium (half or full strength) supplemented with cytokinins, 2,4-D or NAA (10 μM) and auxin, BAP (5 μM). Best results on embryonic callus formation succeeded by the addition of 10 μM NAA and using the half strength medium. Somatic embryo maturation did not happen probably due to the addition of low ABA concentration (7 μM).

For bud regeneration, Tzfira *et al*. (1999) excised mature *P. halepensis* embryos which were cu1tured in an inverted position on MS medium (several strengths) supplemented with cytokinins and auxins, then p1aced in their norma1 position for further growth, and rooted. A1ternatively, the resu1ting adventitious buds served as a source for needles. Elongated needles were excised and placed horizonta1ly on the same medium with different concentrations of BA and/or ABA. In addition, 3 years old pine trees were pruned and sprayed with BA or TDZ to induce fascicular buds, and the resu1ting elongated shoots were then used for *in vitro* cu1ture and bud regeneration. For somatic embryogenesis, mature pine embryos were grown on a modified DCR medium (Gupta and Durzan 1985). The procedures for massive induction of adventitious shoots from mature pine embryos were thus refined, and a high rate of first and second "generation" adventitious buds was achieved. These buds were rooted and plantlets are being established.

For the Calabrian pine, Abdullah *et al*. (1985) reported the induction of adventitious buds when isolated whole embryos and excised cotyledons from treated seeds of *P. brutia* were cultured on three media (SH Schenk and Hildebrandt (1972), GD, Gresshoff and Doy (1972) and LS Linsmaier and Skoog (1965)) supplemented with several concentrations of cytokinins (BAP or NAA were applied either singly or in combination of concentrations of 1, 3, 5 and 7mg/1, and 0.01 mg/1 respectively. A higher concentration of l0 mg/l BAP was also tested with LS medium). The adventitious buds were formed directly from the cotyledons. The buds when separated and maintained individually on a full-strength medium without growth regulators developed into well-formed shoots.

Needles coming from a *P. brutia* seedling were cultured in SH medium solidified with agar and supplemented with cytokinins (BAP at the level of 10 mg/1, and BAP + Kin at the level of 1 mg/1 for each) for the induction of adventitious buds (Abdullah and Grace 1987). Irrespective of the mode of cytokinin application, 8 weeks was the time required to bring about bud formation. The induced buds grew into elongated shoots on a culture medium without cytokinins, but the inclusion of activated charcoal (1%) doubled the elongation rate. After 10 weeks, on the induction medium about 64% of the treated shoots induced adventitious roots using a combination of two auxins and a low level of cytokinin (NAA + IBA + BAP at a concentration of 1 + 2 + 0.05 mg/1, respectively). Subsequently, 86% of the rooted shoots survived transplanting into soil.

The effect of auxins (NAA at a concentration of 0.25, 0.50 and 1.00 mg/l and IBA at a concentration of 1.00 and 2.00 mg/l) and a cytokinin (BAP at a concentration of 0.05 and 0.50 mg/l) on induction of roots in cultured axillary shoots of *P. brutia* were tested by Abdullah and coworkers (Abdullah *et al*. 1989). Apical buds were excised from the cut shoot of eight-week seedlings and the segment were cultured on modified SH medium supplemented with several concentrations of plant growth regulators. Successive generations of axillary shoots (from buds) were achieved as described on previous works (Abdullah *et al*. 1984, 1985). Both auxin and cytokinin and the interactions between them affected the quantity and quality of the induced roots (see above for the applied concentrations).

Adventitious bud induction was achieved at the hypocotyls and cotyledons of *P. brutia* embryos, which were cultured on AE medium supplemented with BAP (Banilas 1995). The strength of the medium and the concentration of BAP affected the formation of buds with best results in ½ (=half strength) AE + 5μM BAP combination. The development of buds into shoots occurred after subculture to a medium without growth regulators or into the induction medium supplemented with ≤5μM BAP. *In vitro* rooting of shoots was not achieved. Embryonic callus was formed from mature embryos, which were cultured on AE medium (at several strengths x2, x1/2, x1/4, x1/8) supplemented with cytokinins (2,4-D or NAA at concentration of 4, 5, 10 μM for both) and auxin (BAP at concentration of 2.5-15.00 mg/l).

Embryogenic tissues of *P. brutia* were also initiated from immature precotyledonary zygotic embryos (Yildirim *et al*. 2006). DCR basal medium was used for the initiation and maintenance of the embryogenic tissues supplemented with 13.6 μM 2,4-D and 2.2 μM BAP. Overall the initiation frequency of embryogenic tissues in the study was 11.6%, initiation rates ranging between 4.7% and 24.1% per tree. Several treatments (addition of ABA, sucrose, maltose, PEG and gellan gum) were tested, and they found the most suitable combination for somatic embryo maturation (80 μM ABA, sucrose (3%) and maltose (3% and 6%), and 3.75% PEG combined with 1% gellan gum).

GENETIC ENGINEERING OF HALEPENSES

In this section we discuss some aspects of genetic engineering (GE) that were not discussed in previous sections. Also, due to the lack of any clear strategy for targeted traits

GE has not proceeded to the commercial phase even for purely ornamental pines planted in gardens and small city parks.

It is a widely held the opinion that the improvement of forest trees began in the last three centuries. Until the last thirty years breeding was done with traditional methods of selecting the tree with the desired properties and making it source of seed or other propagation material (Matthews and Cambell 2000). For Halepenses pines the same situation was not very different and usually the saplings are produced in nurseries from where they are used in reforestation projects. The need for the development of GE and other biotechnology techniques was very weak since for these pines the traditional aims of herbicide, drought and biotic stress resistance were rarely applied in Mediterranean pine ecosystems. Only recently the need for insect resistance and the predicted drought resistance in the face of global warming necessitated the need for the development of GE techniques. Especially, the rapid deforestation in countries around the Mediterranean and the simultaneous need for protection from drought-induced soil erosion have focused the interest, both scientific and commercial, to the improvement of forest trees. The advancement of GE techniques and methodologies made these improvement projects possible. These projects are now in the design phase of laboratory protocols and many times incorporate the achievements in other pine species, usually having financial interest, such as the loblolly pine, *P. taeda* (Gill *et al*. 2003).

However, GE of forest trees faces very strong concerns in Greece and in Mediterranean Europe in general, summarized by Matthews and Campbell (2000). In what follows is given their classification together with some additional points raised by us:

1. The negative effects may not be apparent immediately, and in the complex forest ecosystems many processes may be disrupted. To face this, all genetically modified organisms (GMOs) must be scrutinized against possible risks. This is the most common concern against GMO, not restricted to forest trees. Usually in the case of transgenic agricultural crops and their effects on forest trees the case of monarch butterfly *Danaus plexippus* is cited (Losey *et al*. 1999). Although this example is not a natural one and the GMO is not a forest tree, it describes a case where a distant non-target insect is affected by transgenic maize. A gene of *B. thuringiensis* responsible for an insecticidal toxin was expressed in the pollen of maize, which is found on the surface of milkweed, which is the monarch butterfly food plant. In this way the larvae of the butterfly are killed. Actually, the pollen is able to travel over large distances and the above case is indicative of the type of experimentation needed to test similar cases (Pullin 2000).
2. In the context of plantation forestry as opposed to seed and ramet (vegetative) forestry, the concern of the public and the political authorities is expected to grow in the commercial applications of genetically engineered Halepenses. The concern is on the possibility to have one or a few private companies that drive the genetic material of almost all the pines in the Mediterranean basin.
3. The transfer of genetic material, i.e. DNA, among taxonomically distant organisms is based on the belief that the genetic makeup of an organism is inherently wrong and the scientists improve it. Although the phenomenon of lateral DNA transfer has already been observed in nature the problem lies in its the rapidity and the artificial selection of the organisms. This is a definitive problem that is usually proposed as a general method to avoid the problem of the escape of the genetic material associated with sexual propagation is the GE of sterility in transgenic forest trees (Strauss *et al*. 1995). This is a promising method to avoid the spread of genes to non-target organisms since all commercial firms are interested to produce sterile trees since they avoid the stealing and crude reproduction of their technological achievement. More importantly, they prevent the creation of weeds since this is the most common threat if genetically engineered genes escape to other plants (Matthews and Campbell 2000).
4. The proponents of GE state that the end products of a GMO are actually those that have been produced by natural selection or by traditional breeding techniques, though in a much smaller time scale. However, the practice has proven that many GE improved forest trees changed their competitive ability towards higher replacement levels of other organisms probably as a result of their improved resistance against insects and diseases and depending on the adopted strategy for GE research, their ability to tolerate abiotic factors such as drought and salinity (Tabashnik 1994; Soil Association 1998).
5. The problem of time scales is the point on which the opponents of GMO refute their intervention on the complex autecological and ecological systems. For instance, the diversification of pine terpenoids was based on the duplication and change of one terpene synthase (TS). However, the time scale among GE introduction of TS and the naturally evolved TS's is very different. Because the evolution of new TS's and the associated terpenoids is done in a natural context of an evolutionary landscape. The immediate consequences of the creation of GMOs are that they lose the traits acquired from many evolutionary processes like the 'arms race' of an adaptation and counter-adaptation (Brooks and McLennan 1991).
6. The increasing demand for paper, wood and fibres is unavoidable and one way to solve the associated problems is through the development of GE techniques and the production of GMOs. Although the focus of GE research is on agricultural crops, the paper and wood demand imposes GE techniques on forest trees. However, Halepenses are not among the pines that are used for production purposes.
7. Many contradicting management strategies suggest different traits for pines of Mediterranean regions. One such strategy is the insect conservation that demand for natural genetic constitution of pines and substantial vegetative growth usually through seed propagation. The contradicting strategy is the prevention of desertification by soil erosion that suggests a substantial vegetation cover for the soils of pine areas, which can be achieved rapidly by GE.
8. Since it is impossible to exclude all the risks that can arise in the future from the GMOs, many countries especially in Europe have incorporated in their legal system the '*precautionary principle*' (Loefstedt *et al*. 2002). According to several authors this is an important legal milestone and a fundamental element of risk management. It has been incorporated in the UNCED Rio meeting and the Cartagena Biosafety Protocol and spread quickly to many international agreements.

The techniques to be employed in the GE of Aleppo and Calabrian pines are usually expected to be of the *Agrobacterium*-mediated transfer of DNA/genes type. This bacterium is a soil pathogen that infects dicots and conifers but not monocots. It is found and used as species *A. rhizogenes* and *A. tumefaciens*. These bacteria possess large plasmids, which are termed *Ri* and *Ti*. The bacterial plasmids are incorporated in the DNA of the pine host to which the bacterium is inserted through a discontinuity of the cuticle, usually in the root. However, works on other pine species, e.g. *P. radiata*, showed that the genotypes of hosts were vary variable in the susceptibility of infection with the bacterium, independently of the bacterial strain X host genotype effects (Haines 1992).

The traits that have been discussed for some time are numerous ranging from Btk to suppress feeding by the pine processionary caterpillar to herbicide tolerance that facilitates the weeding in nurseries saving much labour time. Another trait that has been in discussion over the last few years for Aleppo pine is cold tolerance. The idea lies in the fact that a small quantity of antifreeze proteins is capable of increasing the freeze tolerance of pine. In this way pine trees not only can sustain freezing temperatures but also escape from several folivorous pests either by occupying colder ranges (greater altitudes and latitudes) where the pests do not occur. Another trait to which GE strategies can be directed is the genes responsible for the production of proteinase inhibitors. Transgenic pines in this way can block the action of arthropod proteinases that are a significant part of the digestive enzymes. The problems associated with this GE are the same as with the Btk transgenic pines. Some minor arthropod pests will disappear and several insects monophagous on Halepenses will extinct to let aside the fact that the fauna of pollinators will be severely reduced together with the parasite fauna of bark beetles (Pschorn-Walcher 1977; Mendel and Halperin 1981; Mendel 2000).

It is widely known that wood properties are not among the features expected by the industry from Halepenses. The production and the special synthesis of lignin, although important for some pine species, are not among the commercially important traits of Aleppo and Calabrian pines. However, in the prospect of ecologically safe treatment of some sap-sucking pests such as pine scale *M. hellenica* the wood and the phloem can possess such properties that make it difficult to be enzymatically penetrable. As the penetration of the stylets is vital for the insect a failure to insert them into pine is expected for the pine scale to starve to death. Such impenetrable transgenic pines are possible by GE since the various steps of the biosynthetic pathway of lignin are well known and much research is done on various regulating enzymes in many laboratories. In this usage of GE technology only a few pine scales can survive, namely the scales of which the stylets found their way to the pine cambium and the associated sap. The insect certainly does not become extinct and the (Halepenses) pines do not die.

CONCLUSION

Halepenses pines − *P. halepensis* and *P. brutia* − are constituent members of the Mediterranean flora. Bottema (1975) in his coastal/interior−glacial/interglacial model, correlates the abundance of pines with colder Quaternary periods. Although these two pines possess traits that are considered as adaptations to the dry, warm and disturbed Mediterranean ecosystem they are not used for reforestation projects or for parks and gardens in cities. Nevertheless, many new biotechnological methods can be applied on these species in order to improve their resistance to insect pests and improve their vegetative characteristics such as their drought resistance and their ability to grow faster in nutrient poor soils. The advent of new biotechnological methods made possible the shortening of generation times necessary for the application of GE on these trees. The traits associated with certain genes and the carriers that are expected to transfer these genes to the pine genome are now clearly defined but unfortunately not yet stabilised. The pine scale is a good example of such a situation. The insect was a member of the pine fauna but became a pest only after the extensive introductions in pinewoods where it never existed and certainly not in high population densities. However, this situation is occasional and a classical tree improvement program cannot be set to face the resistance to the pine scale. However, GE is capable in handling single traits and in combination with the micropropagation techniques it can be very fruitful in improving the resistance of the pines without eradicating the pine scale or any other not targeted pine arthropod. The example of the pine scale is a good case to depict what conservationists believe. 'GE technology is here to stay and could be of great benefit to agriculture and other industries… Our task is to ensure that the technology is used appropriately… Much of this will involve insects and related invertebrates and the opportunities for research should be there for all to see' (Pullin 2000). With the global warming and climate change, and the loss of freshwater resources these pines are expected to become important reforestation products and valuable garden trees for cities.

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