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The interaction of pine scale with pines in Attica, Greece

Panos V. Petrakis · Vassilios Roussis ·
Constantinos Vagias · Maria Tsoukatou

Abstract The pine scale (*Marchalina hellenica*) has been introduced to Attica and reached unprecedented population densities. Using a randomised transect-based sampling design in eight sites, we sampled the insect in random 10 cm squares on the trunk and main branches of pine trees. The insect did not show any clumping other than a micro-site preference for fissures and cracks. Canonical discriminant analysis and generalised multinomial logit regression modelling were used to determine the most important ecological variables which were the following: the population density of the insects; crown transparency; mean distance between trees; and the quality of the habitat. The site and the distance from the line transect were insignificant. The interaction of crown transparency with the abundance of the insect, the mean distance between trees and the quality of each site were important in configuring the level of damage sustained by the pine tree.

Keywords *Marchalina hellenica* · *Pinus halepensis* · Generalised multinomial logistic regression · Crown transparency · Site index · Stomatal conductance

P. V. Petrakis (✉)
Laboratory of Entomology, Mediterranean Forest Research Institute, National Agricultural Research Foundation, Terma Alkmanos, 11528 Athens, Greece
e-mail: pvpetrakis@fria.gr

V. Roussis · C. Vagias · M. Tsoukatou
Department of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Products, University of Athens, Panepistemiopolis Zografou, 15771 Athens, Greece
e-mail: roussis@pharm.uoa.gr

Introduction

The archaeological record reveals that organised beekeeping has a long history in the Mediterranean (Anderson-Stojanovic and Jones 2002). Since then, to increase the yield of honey in beehives, many biotic cues have been exploited, the most important being the phenology of the flowering plants and the arthropod fauna associated with conifers. For example, in Greece, Turkey and possibly other Mediterranean countries such as Syria, Israel and Italy, beekeepers have contributed substantially to the colonisation of conifer forests by scale insects. In Greece, the Hellenic Ministry of Agriculture has financed the introduction of the scale into previously non-infested areas and pine species, even though it is known that in many other Mediterranean countries this has resulted in major problems for the local pine trees (Kunkel 1997; Bikos 2000).

The recent outbreak of the pine scale *Marchalina hellenica* Gennadius (Homoptera, Sternorrhyncha: Margarodidae) in central and southern Greece is related to such human introductions. The scale inhabits the eastern part of the Mediterranean basin where it is beneficial to beekeeping (Kunkel 1997; Erlinghagen 2001). *M. hellenica* naturally inhabits several pine species including *Pinus brutia*, *P. halepensis*, *P. nigra* and *P. sylvestris* and some populations of *P. pinea* (Mita et al. 2002). Conifer-inhabiting scales (Hemiptera, Sternorrhyncha, Coccoidea) excrete honeydew, which is used by bees to produce the bulk of honey in Greece (Kunkel 1997). The excreted honeydew attracts other insects, mainly ants, which protect and/or transport the scale, thereby aiding density regulation for optimal feeding and egg production (Holldöbler and Wilson 1990).

M. hellenica feeds on plant sap by means of a rostrum (stylets), which is unfolded and inserted into the cambial

zone of the trunk and main branches of the tree (Snodgrass 1935). Penetration is achieved by means of an enzymatic system that dissolves the cell walls of the wood, a common strategy among Coccoidea (Balch et al. 1964). When the feeding site is exhausted, the rostrum is cut with the aid of the *crumena* musculature and the insect then searches for another site appropriate for feeding.

On pine trees, *M. hellenica* has a tendency to occupy the fissures and cracks of the bark in order to reach the nutritious cambium and the sap of the tree with its rostrum (Balch et al. 1964; Mita et al. 2002; Bacandritsos 2004). It is unclear whether this is also an escape strategy from sunlight or natural enemies (Gullan and Kosztarab 1997). The significance of this preference for feeding site decreases with the distance from the ground because both bark fissures (a manifestation of bark thickness) and sap pressure decrease.

The first instar is a crawler, followed by two immature instars that are able to change feeding sites. The adult stage does not possess an extensive rostrum (Nicolopoulos 1965; Bacandritsos et al. 2004) and presumably does not feed. Eggs are produced, which remain in the body of the female, and an egg-sac is gradually formed, a feature common to many Margarodidae (Gullan and Kosztarab 1997). Immature instars and females with eggs are protected by means of waxes produced by special glands located on the terga. *M. hellenica* spreads relatively slowly, at a rate of around 40 m per year (Nicolopoulos 1965). The resulting uniformity of infestation pattern among pine trees at scales of 5–20 m coincides with the natural spread of *M. hellenica* from a point source within one generation.

M. hellenica does not remove the photosynthetic tissue of the pine tree directly but damages the tracheids, resulting in blocked xylem flow (Vite and Rudinski 1959; Mendel and Liphschitz 1988). The gradual decline of the pine tree is rarely attributed to the scale alone. Other insects interfere and use the tree. Bark beetles, for instance, are able to detect tree stress through a drastic alteration in the volatile emission profile (Mita et al. 2002). Most bark beetles, such as *Pityogenes calcaratus* Eichh. and *Orthotomicus erosus* Woll. (Coleoptera: Scolytidae) (Georgevits 1974), prefer to attack weakened pine trees and are therefore considered secondary pests. These insects do not normally kill pine trees but in large numbers can become important factors in mortality (Mendel 2000). It has also been shown that insect infestation alone is rarely the sole determinant of tree decline and mortality. Host colonisation is complex and is related to water stress, nitrogen availability and several other nutrients (Pontius et al. 2006 and references therein) and their interactions.

In this report, we attempt to provide answers to the following questions: [1] Are *M. hellenica* densities on *Pinus halepensis* related significantly to damage, as defined by the needle colour and stomatal conductance of current year needles, expressed by three classes of decline; [2] what additional site and environmental variables are related to decline, such as site quality, mean inter-tree distance, crown transparency, site aspect and site particulars; and [3] what are the ecologically important variables that remain after removing these interactions?

Methods

The study was performed at eight sites (shown in "Appendix", Fig. 5) in Attica, Greece. In each location, approximately 25 circular tenth hectare plots were positioned randomly at random distance, on either side of a transect line forming a band with a width of 100 m. The locations were selected to represent the variety of the pine forests and their management regime in Attica whilst keeping the logistic costs at reasonable levels. Site description and important features are presented in Table 6 ("Appendix").

Site particulars

The pine forests of Mt Parnis and the Sounio National Park represented pine forests within protected areas. The pine forests on Mt Penteli and Thracomacedones represented areas under intense pressure to urbanise. The small coastal pinewood at Schinias is partly occupied (34%) by *P. pinea*. The forest on Mt Hymettus is heavily infested with the pine scale, while the site in Panepistemio is relatively undisturbed with no beehives. West Attica is represented by the pine forest near the village of Villia. The transect line in this pinewood is located in a valley with relatively high densities of the pine scale. This last site is located close to the Rackham line (1983), i.e. the boundary of *P. halepensis* in south Boeotia (northern Attica), north of which there are no native pine trees in mainland Greece. Rackham explained this pattern on the basis of historical human impact and rejected ecological explanations.

Predicting variables measured

Distance from the transect, aspect of the plot: Y, Asp

The distance from the transect line was stored as variable Y_i for each sampling point i. This was expected to affect the abundance of the insects, which are generally introduced along a transect line. The line itself was easily determined as paper envelopes full of egged *M. hellenica* can be observed, nailed to the trees. The aspect of each point was measured in compass degrees. A formula was used to linearise *Asp* because the analyses that follow rely on linear

variables. The formula of Beers et al. (1966) is $Asp = \cos(45° - azimuth) + 1$. The transformed aspect values ranged from 0 to 2; 0 corresponds to more south-western and dry and 2 to northern, humid, cooler slopes.

Density of pine scales—NI

The density of the pine scales was estimated in the second and third instars (September–February) when the insects were at feeding and oviposition sites. The phenology of the pine scale varies, and for this, we visited study areas fortnightly. For instance, in Panepistemio, many eggs we observed showed a second egg hatching in October (*personal observation*). The trunk, starting from the ground, and each one of the main four branches, starting from the trunk of the investigated pine trees, were divided in 2–4 consecutive sections (four sections on the trunk and two or three on each main branch). Within each section, 5–7 samples in the form of a 10 cm square were put in a 9.0-cm petri dish. The insects found in each sample were expressed as linear densities of pine scale. These values were then summed to represent the number of pine scale insects on the pine tree. The insect avoids the exposed parts of the trunk surface and enters the linear fissures and cracks of the trunk, so the sampling squares were standardised using the formula

$$n_i = l_i \cdot \sum_k n_{ik} \bigg/ \sum_k l_{ik} \qquad (1)$$

where n_i is the transformed number of insects in the ith part of the bole of the tree, l_i and the l_{ik} is the total length of the bark fissures of the trunk in the kth square of the ith part, n_{ik} is the number of insects in the kth square. Σn and Σl are the sums of insects and fissure lengths in the same part of the bole over all sampling squares. Densities were averaged for each tree, sampling plot and site.

Crown transparency—CrTr

Crown transparency effectively summarised the combined effect of the years since the first infestation with the pine scale, augmented by a population of bark beetles. We propose that crown transparency (*CrTr*) is an appropriate variable to account for tree vigour, together with the fact that it is easily assessed in the field (Hartmann et al. 2008). *CrTr* was measured on digital images of the crown taken from two opposite points at a distance 1.0 m from the bole. In the digital image of each crown, a total of 100,000 pixels were positioned and their colour (i.e. pixel depth) was measured automatically in the RGB (Red Green Blue) system using standard functions and a program written by us in Mathematica (Wolfram 2007). The measured pixels

were either in the RGB spectrum of pine needles or in the spectrum of the open sky, whilst we discarded all those pixels corresponding to woody structures, cones and dry matter. The percentage of the pixels that correspond to the open sky represents the crown transparency. This variable is related to the grams of the stemwood produced per square meter of leaf area (Waring et al. 1980; Mitchell and Preisler 1991). The other methods used for the estimation of tree vigour are more time-consuming (Hartmann et al. 2008).

Mean inter-tree distance—MDist

In the colonisation of trees by insects, there is always a spatial component with varying underlying mechanisms (Mitchell and Preisler 1991). In this study, the insect is unable to move distances greater then 40 m and usually it is intentionally introduced into forests (Bikos 2000). The variable *MDist* estimates the density of pine trees and the closeness of the canopy.

Site index—SI

We measured the quality of the forest locations at each sampling plot by estimating a site index in a circular area extending 50 m from the centre of the plot using the formula $SI = (h_0/1.1109969) \cdot \exp(-6.315464/d_0)$ where h_0 is the maximum tree height and d_0 is the diameter at breast height of dominant trees (Apatsides and Sifakis 1999) for the *P. halepensis* forest stands following well-established procedures and methods (Ares and Peinemann 1992). The *SI* formula integrates the soil condition, humidity and microhabitat factors at the scale of each plot. There is increasing evidence that site conditions affect the behaviour of many insects related to *M. hellenica* and many plants respond differently to various stressors (Pontius et al. 2006).

Biostatistical analysis

In selecting the significant independent variables for the analysis, we used the variable weighting procedure of canonical discriminant analysis (CDA) as judged by the F statistic (Johnson and Wichern 1998). The variables were selected to show which contributes most to the condition of the trees, on the basis of the category of damage incurred. The variables that contributed insignificantly to the separation of damage classes were removed.

We analysed the damage to the pine trees with generalized multinomial logit regression (GMLR) (Hosmer and Lemeshow 2000; Steinberg and Colla 1999; Petrakis et al. 2005). Three classes of damage were defined on the basis of the colour of the current year's pine needles (taken from a colour chart) and the stomatal conductance

(or inverse resistance) of the needles. Stomatal conductance was measured with a leaf porometer (Decagon Devices[®], Pullman, USA) in August (soil water deficit) at 1200–1600 hours in full sunshine and low wind velocity. This parameter indicates water and carbon dioxide passage to the photosynthetically active tissue (Seco et al. 2008). The type of insect ecological succession on the pine tree (Herms and Mattson 1992; Reid and Robb 1999; Stadler et al. 2005) and the percentage of apical branches exhibiting new growth (Pontius et al. 2006) were also considered. The observed damage category was stored in the categorical variable *Evbb* and presented in Table 1.

The model is used as in Eq. 2

$$\Pr(k,j|x_{k,j}) = \frac{x_{k,j}}{1 + e^{x_{k,j}}} \tag{2}$$

with a logit line presented thus

$$x_{k,j} = \ln\left(\frac{\pi_{k,j}}{1 - \pi_{k,j}}\right) = \alpha_{k,j} + \boldsymbol{\beta}^{\mathrm{T}} \cdot v_{k,j} + \text{error} \tag{3}$$

where $\Pr(k, j \mid x_{k,j})$ is the conditional probability that the kth pine tree damaged in the jth way (0, 1, 2) hosts *M. hellenica*, given the logit line $x_{k,j}$. The vector $\boldsymbol{\beta}^{\mathrm{T}}$ holds the parameters of the variables $v_{k,j}$. $\alpha_{k,j}$ is the constant of the regression line that here represents the common effect of the unmeasured variables, such as the various structural aspects of the habitat, microclimate and the terpene profile of pine trees (Mita et al. 2002). In this way, the logarithmic transformation of the odds ratio expresses the probability of each category of damage (Aukema et al. 2006).

The method has been applied already in an entomological context in several cases, such as polygyny frequency in termites (Brandl et al. 2001); specialist parasitoid persistence (van Nouhuys and Wee 2001); symbionts of bark beetles (Aukema et al. 2006); and behavioural data on *Thaumetopoea pityocampa* (Petrakis et al. 2005).

Results

The relationships between the independent variables are diverse. Figure 1 depicts the relationship between the crown transparency and the distance from the transect line. Although the pattern is not clear, it is evident from Fig. 1

Table 1 Damage classes

Category[a]	Health status	Description[b]
0	Perfect health, healthy with signs of earliest decline	70–100% new growth of apical branches
		>50 mmol m^{-2} s^{-1} stomatal conductance
		Vividly coloured needles
		<10% dieback of fine twigs
		Sparse bark beetles, invisible fungi on the trunk[c]
		No reduction in the width of annual growth rings[d]
1	Light–moderate decline	15–70% new growth of apical branches
		15–50 mmol m^{-2} s^{-1} stomatal conductance
		Discolouration and/or dullness of needles
		10–15% dieback of fine twigs
		moderate bark beetle attack, a few fungi on the trunk
		a highly variable and generally reduced width of annual growth rings
2	Severe decline or tree moribund	<15% new growth of apical branches
		<15 mmol m^{-2} s^{-1} stomatal conductance
		Advanced discolouration of the crown
		10–30% dieback of fine twigs
		Extensive bark beetle and/or fungi attack
		A highly variable and generally reduced width of annual growth rings

[a] Variable *Evbb* in the text, The variable is similar to the decline divisions of Pontius et al. (2006)

[b] All values are based on raw measured variables that are converted to the 0–2 scale

[c] In Attica region (central Greece), the most frequent bark beetles are *Orthotomicus erosus* and *Pityogenes calcaratus*, which are important components of the ecological succession on the pine bark. In high population densities the bark beetle attack results in the death of the pine tree (Mendel 2000)

[d] The width of annual growth rings is observed in the next growing period, and the values presented are associated with the width of previous years (Yesil et al. 2005)

that crown transparency decreases with the distance from the transect line ($r_{CrTr,Y} = 0.29$, $N = 238$, $P < 0.01$). There is no simple relationship between variables NI and Y ($r_{NI,Y} = -0.04$, $N = 238$, $P > 0.57$, NS) (Fig. 2).

CDA and variable selection

The values (F-ratios) that determine which variables to retain are shown in Table 2. It is important that the sampling site and the site of the transect line play no role in determining the decline of pines. The categories of damage are shown the scattergram (Fig. 3) in a discriminant space of the two canonical discriminant axes (*Wilks' λ* = 0.236, $F_{assoc.} = 7.24$, $df_1 = 14$, $df_2 = 96$, $P < 10^{-4}$). The scattergram accommodates almost all the variation in the original data (99.98%), while the greatest part of it is explained by the first discriminant axis (85.7%). The variables $CrTr$ and NI have high negative loads on the first standardised discriminant function (-0.871 and -0.865). Similarly, $MDist$ and Y have moderate loads on the second
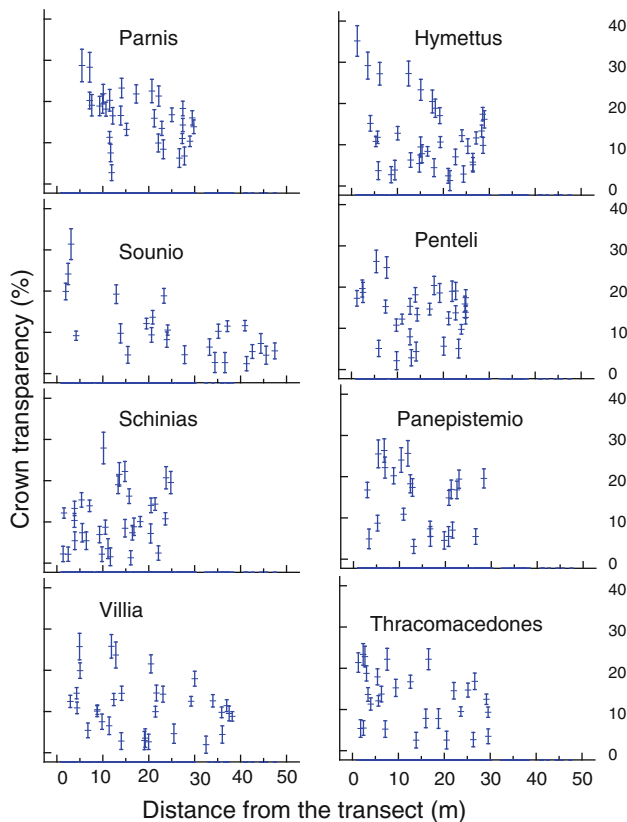
Fig. 1 Multiple diagrams showing the inverse relation between percentage crown transparency ($CrTr$, y-axis) and the distance from the transect line in metres (Y_i, x-axis). The locations of transect sites are shown in Appendix Fig. 5 [*error bar* = 1 SEM]
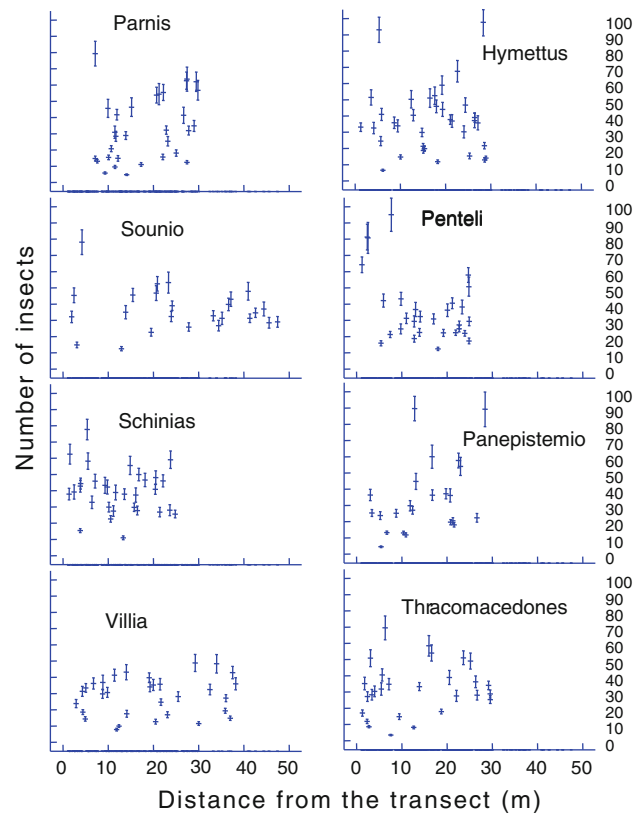
Fig. 2 Diagram that shows the relation between the number of insects (NI, y-axis) and the distance from the transect line in metres (Y_i, x-axis). The locations of transect sites are shown in Appendix Fig. 5 [*error bar* = 1 SEM]

Table 2 Variable selection procedure through F-value

| Variable	Variable symbol	F-value
Number of insects	NI	64.87
Crown transparency	$CrTr$	57.72
Mean inter-tree distance	$MDist$	14.29
Site quality	SI	12.09
Distance from the transect	Y	1.02[a]
Site	R	0.74[a]
Aspect	Asp	0.66[a]

The variables are written in decreasing F-values, and the horizontal line separates the significant (above) and the insignificant (below) variables

[a] The variable does not produce statistically significant results

discriminant function (0.697 and 0.510), while the quality of the site (SI) has a moderate load (-0.413). In conjunction with Figs. 3 and 4, damage $Evbb = 1$ seems to correlate well with $MDist$, Y and SI, while the advanced damage of pines (type 2) is strongly correlated with $CrTr$ and NI.
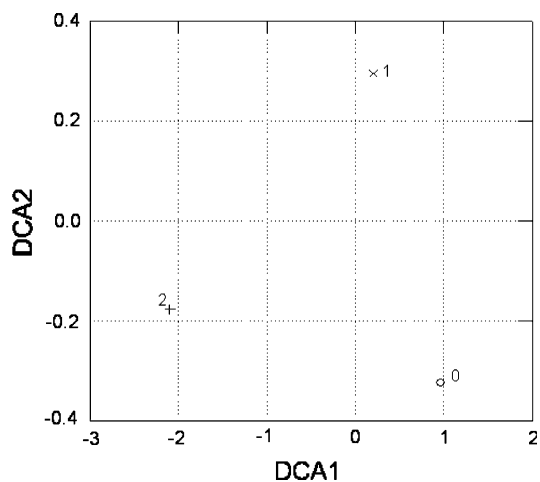
Fig. 3 Canonical discriminant diagram that shows the position of the three decline types. Axis DCA1 explains 85.70% and axis DCA2 14.28% of the existing variation in the original data
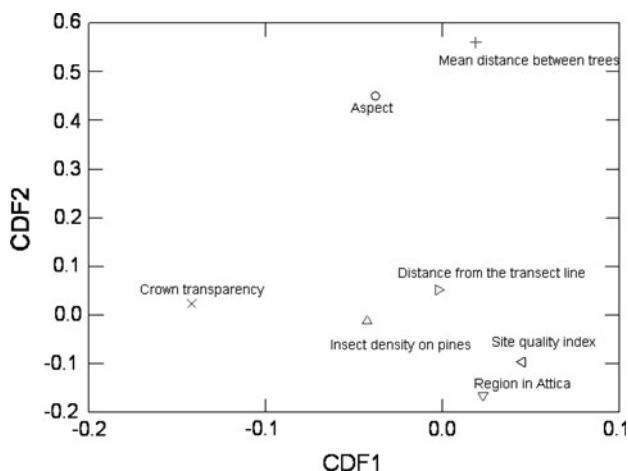
Fig. 4 The relative positions of the measured variables in the space of the two canonical discriminant functions explaining the bulk of existing variation (99.98%)

Results of the GMLR model

Significance of variables

The results of GMLR are given in Table 3, while Table 4 shows the importance of each measured variable in affecting the probability of each type of damage occurring. The ability of the GMLR analysis to predict the damage outcome from the measured significant variables is given in the classification table of the model (Table 5). Fitting the observed data to the model was very successful (likelihood ratio $G = 301.38$, $P < 10^{-4}$), and McFadden's $\rho^2 = 0.61$ was a particularly high value (Kleinbaum et al. 1982; Steinberg and Colla 1999).

Table 3 Coefficient estimates of the GMLR and associated significance levels

Variable	Damage type group	Coefficient in the GMLR model	P level
	0 vs. 2		
Constant		28.790	0.04
NI		−0.101	0.01
CrTr		−0.330	0.02
CrTr * NI		−0.027	10^{-4}
SI		−1.097	0.02
MDist * SI		0.119	0.02
	1 vs. 2		
Constant		29.668	0.02
NI		−0.335	$<10^{-4}$
CrTr		−0.906	$<10^{-4}$
CrTr * NI		0.013	$<3.0 \times 10^{-4}$
SI		−0.813	0.03
MDist * SI		0.079	0.03

Variable	Overall damage groups Wald statistic	P level of the respective χ^2
Constant	5.46	0.01
NI	35.02	$<10^{-4}$
CrTr	36.69	$<10^{-4}$
CrTr * NI	46.78	$<10^{-4}$
SI	14.25	0.05
MDist * SI	18.27	0.04

The variables linked with an asterisk (*) denote interaction

Table 4 Derivative values ($\times 100$) of all significant variables in Table 2 (explanations in the text)

Variable	Damage group		
	0	1	2
Constant	4.4	5.6	−10.0
NI	1.8	−3.3	1.5
CrTr	4.4	−8.5	4.1
CrTr * NI	−0.3	0.4	−0.1
SI	−2.7	−1.3	4.0
MDist * SI	0.4	0.0	−0.4

In the GMLR results (Table 3), only the significant variables (and their interactions) for each comparison are shown. The interaction terms of CrTr * NI and MDist * SI are highly relevant to *M. hellenica*. By the same token, it is not surprising that the variable MDist is not significant (although it is significant in CDA) and only its interaction with the site index was retained.

Table 5 Classification table of the GMLR model (explanations in the text)

		GMLR 0	Predicted 1	Affiliation 2
Observed	0	*63*	14	1
Group	1	13	*92*	8
Affiliation	2	1	8	*32*
Sum of predicted pines		77	114	47
Correctly predicted %		80.8	81.4	80.8
Index of success		48.0	33.9	61.1
Overall correct predictions %		81.09%		

All individuals are pine trees

Values in italics indicate correctly predicted group affiliation

The three panels in Table 3 resulted from the consideration of the severely damaged pines ($Evbb = 2$) as a reference class. For this, the first two panels contain the estimates of the coefficients and their significance in terms of comparison with the severely damaged group of pines. The last panel ('Overall damage groups') contains the significance of the variables for all models and comparisons. All variables are statistically significant, although *SI* and its interaction with *MDist* have higher levels of probability (0.05 and 0.04).

Variable derivatives

Quantitatively the importance of each covariate for each pine tree damage category can be deduced from the derivative values in Table 4. In this table, the row totals of the matrix are zero because an increase in one unit of each covariate variable means an increase/decrease in the probability of the respective type of damage occurring. Covariates *NI* and *CrTr* are inversely related in moderate damage categories 0 and 1, whilst their interaction *CrTr * NI* is just the opposite. The interaction term *MDist * SI* signifies a zero probability of occurrence of the moderate damage group ($Evbb = 1$) and is positively associated with light damage ($Evbb = 0$). In contrast, *SI* is positively associated with heavy damage ($Evbb = 2$), which is superficially biologically decoupled, since heavy damage results from high site quality.

GMLR-based classification: The model is very successful in classifying the observed data for all plots in Attica (Table 5). It was found that 81.09% of the sampled pine trees were classified correctly. The 'index of success' measures the gain over a completely random model (Steinberg and Colla 1999) and is highest in heavily damaged pines. Moderate damage has the lowest index of success in the GMLR model probably stemming from the fact that it cannot be accurately identified.

Discussion

The probabilistic GMLR method (Table 3) successfully modelled pine damage, although potentially many factors are responsible for the decline of pine trees (Pontius et al. 2006). Instead of finding a simple analytical relationship among quantitative variables of pine health and development, we defined three categories of damage in pines, based on eco-physiological parameters. The damage to *P. halepensis* is not reflected in the annual incremental rates but was shown in pine increment cores measured by Yesil et al. (2005) on *P. brutia* in Turkey.

The most pronounced property of the GMLR analytical model is its ability to classify damaged pines using the most parsimonious variable set (81.09% in Table 5). The model employed overcomes the differences in population density of scale resulting from contradictory factors. For instance, a high site index might prevent a pine tree from sustaining serious damage, even if it has a high density of scale, because the inter-tree distance is high and the scale is not easily protected from the sunlight. High *MDist* lowers the competition for nutrients between the pine trees, decreasing their crown transparency (*CrTr*). Moreover, in small *MDist*, a temporary increase in scale density may attract efficient predators such as the short-toed tree creeper (*Certhia brachydactyla*, a particularly shy bird species) (Jantti et al. 2001), although in some cases the attraction of predators is inefficient despite forest density, due to scarcity of food resources and competition from ants attending pine scales (e.g. Aho et al. 1997).

The number of insects (*NI*) does not vary consistently with the distance from the transect (Fig. 2), indicating that the introduction of the pine scale to the trees near the central transect line is not reflected in the variable *NI* after the spread of the scale. Crown transparency, unlike *NI*, exhibits a consistent pattern, most possibly because of repeated releases of scale more than 5 years earlier. The pine host condition expressed by *CrTr* is attained over several years, while the number of insects is the result of the previous year. In addition, in many trees, the insect does not inhabit pines with large *CrTr*, possibly because it relies on sap stream pressure in the phloem sieve tubes for feeding, rather than sucking the sap using its own power (van Hook et al. 1980); in effect, it avoids reduced cambial activity, commonly associated with large crown transparency. The mechanism for this is simple. The insect (or its conveyor) prefers the low radiation biotopes and selects them by assessing solar radiation, which coincides with decreased *CrTr*. The same mechanism works in the occupation of the adaxial (usually shaded) side of the primary branches. This mechanism also has an adaptive significance as the same method is used to avoid avian predators (MacArhtur 1958).

The quality of the sites does not seem to play a major role in the transparency of the crown of pine trees (Table 4), although it has a subtle combined effect that mitigates the effect of *MDist* (Table 4). This is because *P. halepensis* occupies infertile soils not used for agriculture since classical times (e.g. Meiggs 1982). In this way, increased *MDist* does not offer any competitive advantage to pines in high quality sites. Overall, the biological load is carried by the interaction terms. The interaction *CrTr * NI* shows the highest abundance of insects that tolerate current crown transparency, and the interaction *MDist * SI* is the smallest distance between pines that can be sustained under the observed site quality.

Not all variables were significant for configuring the types of damage to pine trees, as shown in the *F*-values analysis in CDA. Importantly, the inclusion of the excluded variables in GMLR produced insignificant coefficients (not shown here). Three variables were removed from GMLR model, namely the site variable *R*, the aspect *Asp* and the distance from the central transect line. The last variable was included initially as it presumably relates to the introduction of the scale. The exclusion of variables ensures that the adverse effect of *M. hellenica* on pines can be extrapolated and applied to pine forests across Attica.

The model employed accounts for almost all of the variation in the type of damage occurring in these pine trees (99.8%, Fig. 3) so it is not a result of covariates such as terpene concentration and width of increment cores (not included in the model). This is in contrast with the findings of other studies. For example, in a study on the reasons for the susceptibility of *Abies fraseri* trees to attacks by the balsam woolly aphid (*Adelges piceae*, Hemiptera, Adelgidae), it was found that terpene profiles are important (Sutton et al. 1997).

Conclusion

The impact of pine scale (*M. hellenica*) on pine trees occurs on multiple levels and eventually results in the death of many pine trees. This study investigated the relative importance of several eco-physiological variables, through the selection procedure of canonical discriminant analysis (CDA). The selected variables were analysed, and their effect on the type of damage sustained by the trees was estimated through generalised multinomial logit regression (GMLR) model. It was found that the most important variables were the population density of the insects; crown transparency; mean distance between trees; and the quality of the habitat in each plot. The aspect of the site and the distance from the line transect (presumably the route followed by pine scale introducers) were found to be unimportant. The density of the pine scale was associated with heavy damage to pine trees, indicating the effect of increased scale population density. The efficiency of the measured variables was very high.

Appendix

Table 6 and Fig. 5.

Table 6 Characteristics of various sites in Attica where the transects were positioned. The anthropogenic impact is rated in the interval [0–9] where 0 means no signs of human impact and 9 denotes that the site is next to agricultural land, a suburban area or other human establishment

	1. Parnis	2. Sounio	3. Schinias	4. Villia	5. Hymettus	6. Penteli	7. Panepistemio	8. Thracomacedones
Distance from the sea (km)	24	3	0	7	9	12	8	13.5
Years from the last forest fire[a]	>50	6	>55	≈35	≈40	≈28	>40	>50
Precipitation (mm in a year)[b]	566[h]	376	441	416	444	496	416	566[h]
Mean inter-tree distance (m)[c]	10.9 ± 1.9	10.4 ± 3.4	10.6 ± 1.6	9.8 ± 2.1	9.7 ± 2.4	13.1 ± 4.3	8.9 ± 2.1	18.6 ± 6.1
Coexisting *Pinus* species[d]	*P. nigra*		*P. pinea*					
Understory dominant plants[e]	vt4	vt5	vt5	vt5	vt3	vt3,5	vt2	vt1
Anthropogenic impact I the range [0…9][f]	2	5	1	8	6	7	5	8

Table 6 continued

	1. Parnis	2. Sounio	3. Schinias	4. Villia	5. Hymettus	6. Penteli	7. Panepistemio	8. Thracomacedones
Aspect range [min…Max][g]	[0.4–1.8]	[0.0–2.0]	[1.1–2.0]	[0.0–1.5]	[0.0–1.9]	[0.2–2.0]	[0.2–1.8]	[0.2–2.0]
Site index (SI)[c]	15.8 ± 2.1	16.3 ± 2.1	16.2 ± 3.6	22.3 ± 4.1	13.0 ± 1.4	11.9 ± 0.6	10.0 ± 1.2	12.0 ± 1.2
Altitude (m.a.s.l.)	500	200	0	300	400	250	200	250

[a] As a rule, the forest fire did not pass over all the transect length and locally did not burn all trees. For this, a great variation exists in stand age

[b] *Source*: Hellenic National Meteorological Service, Dr G. Baloutsos, Petrakis (1990)

[c] ±1 standard deviation

[d] Coexistence is declared even in the case that only one other *Pinus* species exists with *P. halepensis* in the transect area

[e] One or more of the following plant species per vegetation type (vt); vt1: none; vt2: *Quercus coccifera*, *Thymus capitatus*, *Brachypodium pinnatum*, *B. ramosum*; vt3: *Q. coccifera*, *Gistus* spp.; vt4: *Q. ilex*, *Philyrea media* and sparse various species of *Fabaceae* and *Poaceae* families; vt5: *Q. coccifera*, *Pistacia lentiscus*, *Asparagus aphyllus*, *B. distahyon*, perennial *Poaceae*

[f] 0 denotes no impact, and 9 means that the area is scattered in agricultural landscape; in this column, the effect exerted by beekeepers that spread the pine scale in various places is not taken into account. In Schnias, the impact is the mean annual impact since there is a great variation in the impact between summer and winter visitors

[g] The linearised transformed values are entered

[h] Sites 1 and 8 have the same height of precipitation due to the proximity to the same meteorological station

Fig. 5 Satellite map of Attica Greece showing the locations of the study

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