A VEGETATION INDICATOR TO ASSESS MEDITERRANEAN PINEWOOD AFFECTED BY BAST SCALE (*MATSUCOCCUS FEYTAUDI*)

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ABSTRACT

The bast scale (*Matsucoccus feytaudi*) is responsible for the destruction of most of the *Pinus pinaster* forests in the Mediterranean area, causing resination, defoliation and subsequent death of the tree.
Questions: is it possible to estimate pinewood health by analysing understory vegetation? Does the health status of pine forests changes during time? Do such variations involve understorey vegetation? What species in the understory vegetation better describe different conditions? What are the most relevant variables (defoliation and resination) influencing pine stands' condition. **Location:** The study area is located in the eastern part of *Liguria* (Italy) in pinewood affected by the bast scale *Matsucoccus feytaudi*. **Method**: we used: (1) hierarchical cluster analysis to discriminate pinewood health conditions (good and bad); (2) non-metric multidimensional scaling (NMS) to detect the most important variables influencing the pine stands' condition and understory vegetation; and (3) indicator species analysis (ISA) to determine indicator species corresponding to health conditions at the plot level. Our aim was to find a relationship between pines' health status and the variation in pinewood understory vegetation communities. **Results**: we found that understory vegetation composition depends on both pine stand health status and environment-related factors. Geographic variables (in particular latitude and altitude) and tree-related variables (percentage of resinated and defoliated trees) were associated with the main axes of variability of the understory vegetation. Three indicator species *(Erica arborea*, *Quercus ilex* and *Castanea sativa*), which were closely linked to pine stands health status, were significantly associated with different stages of pinewood dieback caused by *bast scale*. **Conclusion**: this study provides useful information and a good operational tool for technicians working in the forestry sector, and for public administrations and land managers to start good land-use planning.

KEY WORDS: maritime pine (*Pinus pinaster*), hierarchical cluster analysis, indicator species analysis, non-metric multidimensional scale, understory vegetation, bast scale (*Matsucoccus feytaudi*).

INTRODUCTION

Maritime pine (*Pinus pinaster* Aiton) is a conifer from the western Mediterranean Basin with a distribution exceeding 4 million hectares, under broad ranges of elevation, climate and soil (Alía et al., 1996) while at the Italian level, *Pinus pinaster* stands cover 60,000 ha (Gambi, 1983; Bernetti, 1995). In the study area pinewoods belong to the protected habitat (EU Habitat Directive 92/43, Annex I) named "Mediterranean pine forests with endemic Mesogean pines (cod. 9540)" (Mariotti, 2008). Currently *P. pinaster* woods are affected by a phytosanitary problem caused by the bast scale *Matsucoccus feytaudi* Duc. (Hemiptera: Coccoidea: Margarodidae). The bast scale is a specific pest of maritime pine, widespread in the western Mediterranean basin. While in the Iberian Peninsula and south-western France, the insect is endemic and its impact on the host tree is negligible, in south-eastern France, Corsica and Italy the pest has already destroyed thousands of hectares of maritime pine forest (Jactel et al., 1998; Riom, 1994). In the 1970s, the parasite reached Italy (Covassi & Binazzi, 1992), where it is still expanding south- and eastward. The symptoms of attack include yellowing and withering of the needles, peeling of the bark, waxy secretions and exudation of resin (Arzone & Vidano, 1981) as well as defoliation. Forest pests have considerable influence on the value and functionality of forest ecosystems, both directly (e.g. timber losses) and indirectly, as they may compromise the ability of the stand to provide ecosystem services (Gatto et al., 2009). Compromising pine stands' ecosystem services means risking a very important and protected Mediterranean habitat suitable for preventing soil erosion and reforesting highly degraded areas (Le Maitre, 1998), as well as managing watershed and storing carbon (Turcato et al., 2015).

The relationship between forest structure and understory vegetation in different ecosystems has been studied over the last decade in different habitats (Mazurek & Romane, 1986; Pitkanen, 1997; Hedman et al., 2000; Brosofske et al., 2001; Hiroaki et al., 2004; Osorio et al., 2009), but it has never been applied in *P. pinaster* forest affected by *M. feytaudi*. Studies on alterations of Mediterranean pine forests focus mainly on post-fire regeneration (Rego et al., 1991; Fernandes & Rigolot, 2007; Calvo et al., 2008) or on parasite attacks (Carle, 1974), but all of these studies focus their attention on trees while an analysis on the understory vegetation is still lacking. As the decrease in density of the tree crown results in an increase of sunlight that reaches the ground of the stands (Anderson et al., 1968; Federer & Tanner, 1966; Jennings et al., 1999). We hypothesise that the damage caused by bast scale may cause a significant alteration in the diversity and the composition of the understory vegetation in pinewoods.

Our goal in this study was to show how understory vegetation could represent a proper indicator to define pinewood health status and to define a more accurate measure of the forest health state. For this purpose, we used indicator species analysis (ISA) to compare performance of individual species across two groups of sample units. To test this hypothesis, we analysed a dataset of vegetation using two consequent analyses: 1) hierarchical cluster analysis and 2) non-metric multidimensional scaling (NMS).

MATERIALS AND METHODS

Study area

The study area is in the eastern part of Liguria region (northwest Italy) inside the Cinque Terre National Park (44°03”N, 9°37”E, figure 1), where pinewoods cover an important portion of the park (30%). Here, pinewoods belong to the protected habitat (Habitat Directive Annex I) named “Mediterranean pine forests with endemic Mesogean pines” (cod. 9540).

The considered pinewoods have a total extension of 764.5 hectares and an elevation range from 200 m to 400 m above sea level. The annual rainfall average and temperature are, respectively, 1430 mm and 15°C (Regione Liguria, 2005). The substrate is characterised by the presence of sandstone. According to the Rivas Martinez’s climate classification (1981) the coastal part of the “Parco Nazionale delle Cinque Terre” belongs to the Mediterranean-humid-temperate type, while at higher altitude tends to submediterranean.

Figure 1. Location of study area

Field sampling

We studied mixed and pure pinewood with maritime pine (*Pinus pinaster*) prevalent species. A total of 20 plots were randomly selected. In particular, we extracted 20 random where we installed a rectangular plot of 500 m2 (20 x 25 m), defining the borders of our plots using remote sensing (Landsat cartography) and field surveys (GPS technology) (Elzinga et al. 2001). In these plots sixty original phytosociological relevés were carried out in forestal plots during the spring–summer of 2010-2012 using a percentage scale method. Nomenclature of species follows Bartolucci et al. (2018).

Variables

We analysed all trees within each plot (a total of 382 pine trees). As the symptoms of bast scale attack include defoliation and exudation of resin, we counted trees that had visible resin on the stem and trees with a low crown density in order to describe the damage level of the trees. Moreover, we used resination and defoliation parameters because they are easy and fast to report and easily replicable. So, in each plot we surveyed this set of variables (Table 1):

1- Percentage of defoliated trees (%def) of the total number of trees in the plot

2- Percentage of resinated trees (%res) of the total number of trees in the plot

3- Latitude (lat)

4- Longitude (long)

5- Altitude (alt)

The degree of defoliation was visually assessed in June and July of 2010, 2011 and 2012 using damage classes (0-4) developed by the International Co-operative Programme (ICP Forests) (Fischer, 2010). Trees in classes 2, 3 and 4 (Eichorn et al., 1996) were were considered as ‘defoliated’in the subsequent statistical analysis. UTM WGS84 (EPSG 32362) coordinates and altitude (metres above sea level) were defined using GPS technology. Two matrices were considered when analysing data: (1) a matrix of sampling plots × understory vegetation species abundances; and (2) a matrix of sampling plots × environmental variables. The first matrix did not include data of *Pinus pinaster* coverage.

Table 1. Variables considered in this work. Values of %def and %res indicated in the table are average values.

PLOT NUMBER	CLUSTER CLASS	% DEF	% RES	LAT	LONG	ALT (m)
1	1	26	71	559557	4884560	414
64	2	60	32	557047	4886670	492
78	2	17	50	557630	4886128	475
79	2	20	91	556690	4887274	469
80	2	26	78	556305	4886832	388
81	2	18	35	555349	4888432	433
82	1	92	61	553720	4889157	559
83	2	18	53	562101	4882043	499
84	2	56	88	555219	4891249	536
85	2	70	86	554698	4890818	529
86	1	94	100	552819	4890555	586
87	2	13	43	552325	4889945	291
88	2	30	45	551840	4890248	336
90	1	17	80	560476	4883181	389
91	2	18	41	556486	4888665	390
92	2	23	36	557907	4885730	484
93	2	6	38	559076	4885466	407
94	2	25	35	558644	4885277	391
95	1	59	100	550757	4887912	262
96	1	94	100	550831	4889043	333

Hierarchical cluster analysis

We used the cluster analysis to place the twenty plots into groups based on differences in vegetation composition. The linkage method applied was Ward’s method (= hierarchical grouping = minimum variance method = Orloci’s method), based on minimising increases in the error sum of the square. This method finds minimum-variance spherical clusters (Ward, 1963;

Orloci, 1967; Wishart, 1969). Euclidean distance is superimposed in the analysis because Ward's method is incompatible with Sørensen distance. Ward's method gives results similar to Flexible-beta linkage (with beta value = 0.25). The analysis had a percentage of chaining of 2.04.

Non-metric Multidimensional Scaling (NMS)

We used NMS to detect the most important variables influencing the pine stands' condition and understory vegetation. McCune & Mefford (1999) recommend verifying the Beta diversity value in a dataset before choosing an ordination method. In our dataset, the Beta diversity value was 3.2, and for this reason we chose NMS (Mather, 1976; Kruskal, 1964) with Sørensen distance, an ordination method that is suited even when beta diversity is quite high. NMS was initially run in autopilot mode, comparing 1- to 6-dimensional solutions. We used random starting configurations. The best solution was a 3-dimensional configuration (maximised difference between the best of 40 runs of real data and 50 randomised runs, $p < 0.0196$ from Monte Carlo test; average stress = 12.146). After running in autopilot mode, we assessed an appropriate number of dimensions plotting final stress vs. the number of dimensions, then in order to run the final solution, we chose a number of axes (three) beyond which reductions in stress are small. In the final solution, we superimposed 400 iterations, with no step-down in dimensionality, one real run and no randomised runs. We assessed the stability of the solution plotting stress vs. iteration and observing the final instability value of the solution (0.00191). 11.95 was the final stress for the 3-dimensional solution. NMS finds a stable solution, interrupted until 80 iterations by low-level fluctuations in the stress.

Pearson correlation of quantitative predictor variables (Table 2) with ordination axes was used to interpret relationships of these variables to understory vegetation composition. Variables with $r = 0.007$ to 0.254 are described as weakly correlated, those with $r \geq 0.294$ are described as correlated. For comparison, a Pearson r of 0.254 with $N = 60$ is significant at $p = 0.05$.

Table 2. Correlation with second matrix.

Variables group	**Variables**	**Axis**								
		1 (r^2 0.248)			2 (r^2 0.408)			3 (r^2 0.218)		
		r	r^2	tau	r	r^2	tau	r	r^2	tau
Tree-related	%def	**- .479**	.229	- .360	**.538**	.290	.383	**.308**	.095	.141
	%res	**- .318**	.101	- .190	**.822**	.676	.514	.150	.022	.084
Geographic	Alt	**- .378**	.143	- .302	- .067	.005	- .057	- .201	.040	- .112
	Lat	.154	.024	.106	- .267	.071	- .169	**- .336**	.113	- .216
	Long	- .198	.039	- .202	.101	.010	.077	- .005	.000	- .041

Indicator species analysis (ISA)

We used ISA (Dufrêne & Legendre, 1997), to determine indicator species corresponding to pinewood conditions. This method combines information on species abundance with the reliability of occurrence of a species in a given ecological group. The method (1) calculates the proportional abundance of a species in a particular group relative to the abundance of that species in all groups; (2) calculates the proportional frequency of the species in each group; (3) combines the two proportions calculated in step 1 and 2 by multiplying them. The method requires two or more a priori groups of sample units, and data on species abundance or species presence in each of the sample units. We selected the groups highlighted by hierarchical cluster analysis. The statistical significance of the maximum observed for each species was tested by means of a Monte Carlo test, based on 1000 randomisations. We considered as a significant value, processed by the software, only those greater than 0.40 and with a p-value < 0.05. All analyses were performed with PCORD version 4.25 (McCune & Mefford, 1999).

RESULTS

Hierarchical cluster analysis

Two groups are clearly identifiable (Figure 2): the first (class 1) contains 6 plots; the second (class 2) contains 14 plots. Plots in class 1 are characterised by high percentages of defoliation (average 63.6%) and resination (average 85.3%). Plots in class 2 are characterised by low percentages of defoliation (28.5%) and resination (53.6%).

The two groups strongly separate in accordance with the percentage of trees with resination and defoliation (Table 2).

Non-metric Multidimensional Scaling

Geographic variables (in particular altitude and latitude) and tree-related variables (percentage of resinated and defoliated trees) were associated with the main axes of variability in the dataset (Figure 3). Cumulative Pearson r2 between distances in the original space and distances on the three ordination axes was 0.87. The axis with the lower r2 (0.218) was labelled Axis 3, followed by axis 1 (0.248) and axis 2 (0.408) (Figure 2). Axis numbers are arbitrary, so that the percentage of variance on a given axis does not necessarily form a descending series with increasing axis numbers.

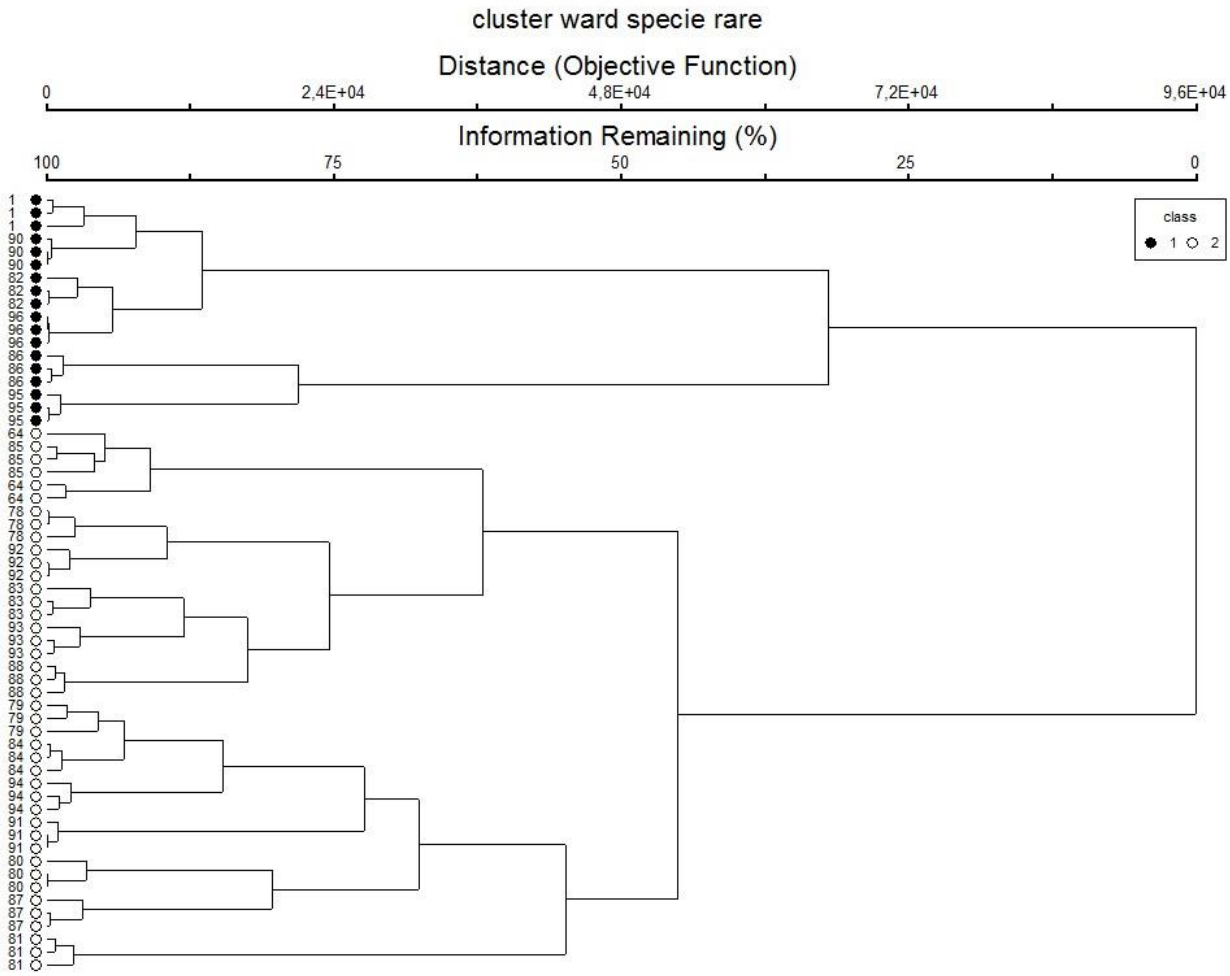

Figure 2. Hierarchical cluster analysis. On the left of the figure, plot numbers are listed. Plots are divided into class 1 (bad health conditions) and class 2 (good health conditions).

Tree-related variables (percentage of defoliated trees -%def-; percentage of resinated trees -%res) were those most strongly correlated with ordination axes. In particular, %def showed strong correlations with axes 1 and 2 and a low correlation with axis 3. In particular, positive values on axis 2 were associated with increasing %res. At the same time, negative values of %def on axis 1 were associated with increasing %res.

Geographic variables (altitude -alt-; latitude -lat-; longitude -long-) showed a different correlation to the axis: negative values of altitude (alt) were correlated with a negative value of tree-related variables on axis 1. In contrast, negative values of latitude (lat) were correlated with positive values in %def on axis 3. Longitude (long) showed weak or no correlations with all three axes.

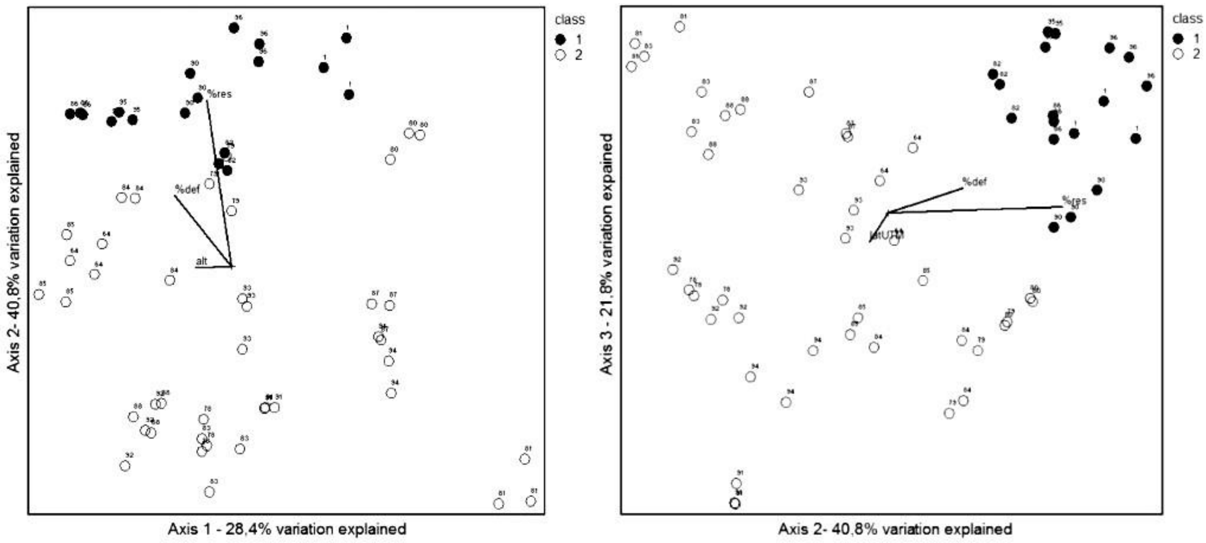

Figure 3. NMS ordination of plots based on understory vegetation. Lengths of arrows for predictive factors represent strength of correlations. Plots are divided into class 1 (bad health conditions) and class 2 (good health conditions).

Indicator species analysis (ISA)

Erica arborea has an observed indicator value (IV) of 80.4 and a p-value of 0.001, so this is the species that best describes pine stands in class 1 (bad health conditions). In this class, *Pteridium aquilinum* also has a relatively high IV (54.7) but also a high p-value (0.108), so this species was considered irrelevant. In class 2 (good health conditions) two species have strong IV and low p-value: *Quercus ilex* (IV 78.4 – p-value 0.001) and *Castanea sativa* (IV 76.3 – p-value 0.001). In this class, *Teucrium scorodonia* and *Rubia peregrina* also had high IV, associated, in the former species with a low p-value and in the latter species with a high p-value. Results related to the analysis are summarized in Table 3.

Table 3. Species with observed indicator values higher than 30. In grey, indicator species.

species	**Class**	**Observed indicator value (OIV)**	**Mean**	**S.Dev**	**p-value**
Erica arborea	1	80.4	50.3	4.49	0.001
Pteridium aquilinum	1	54.7	48.8	4.59	0.108
Brachypodium rupestre	1	48.6	44.5	5.1	0.197
Cistus salviifolius	1	43.2	35.9	5.83	0.123
Arbutus unedo	1	40.3	29.1	5.6	0.052
Limodorum abortivum	1	33.3	9.3	3.84	0.002
Quercus ilex	2	78.4	49.9	4.89	0.001
Castanea sativa	2	76.3	43.9	5.02	0.001
Teucrium scorodonia	2	63.3	46.9	5.87	0.011
Rubia peregrina	2	51.2	46.9	4.6	0.17
Rubus ulmifolius	2	40.8	48.5	5.52	0.991
Cytisus villosus	2	37	21.2	5.51	0.02

Indicator species selected by ISA have been plotted on the NMS ordination (Figures 4, 5, 6), showing strong correlation with axis 2 (*Erica arborea* r = 0.886; *Castanea sativa* r =-0.424; *Quercus ilex* r = -0.822).

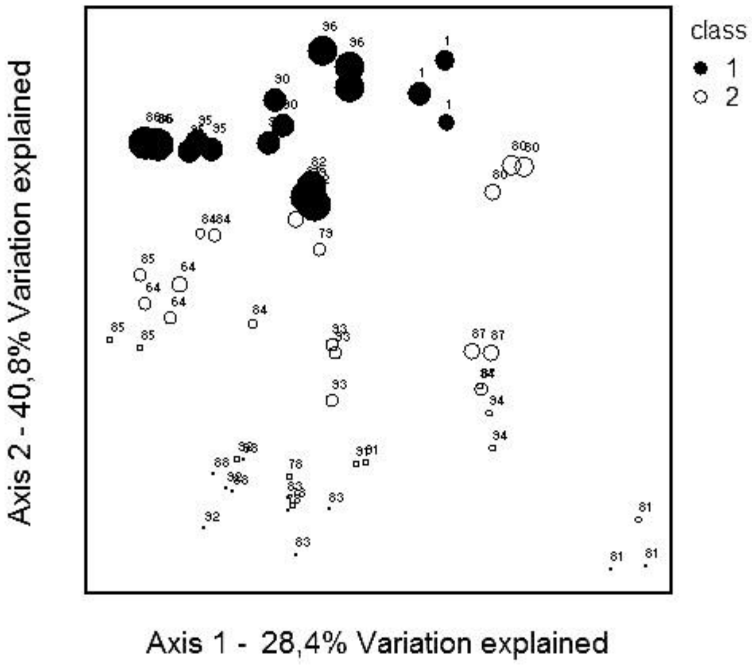

Figure 4. ISA indicator species (*Erica arborea*) in NMS analysis

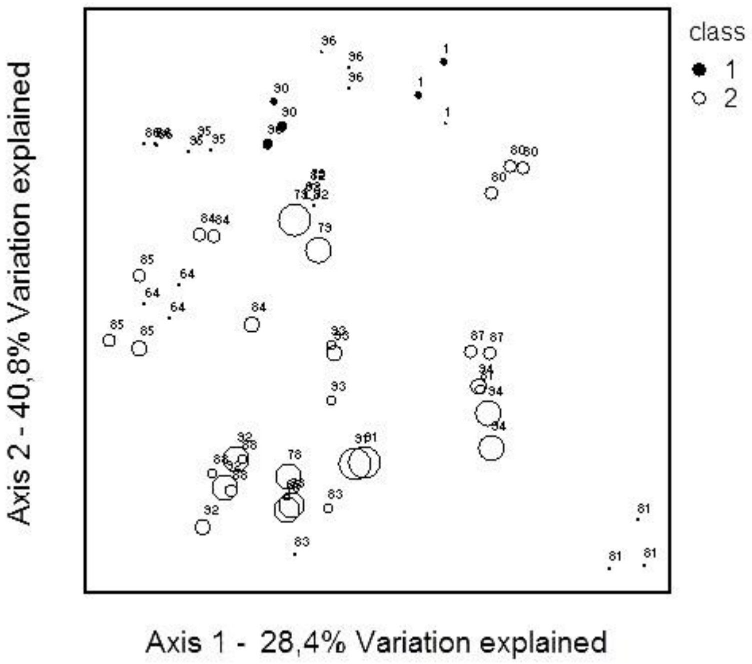

Figure 5. ISA indicator species (*Castanea sativa*) in NMS analysis

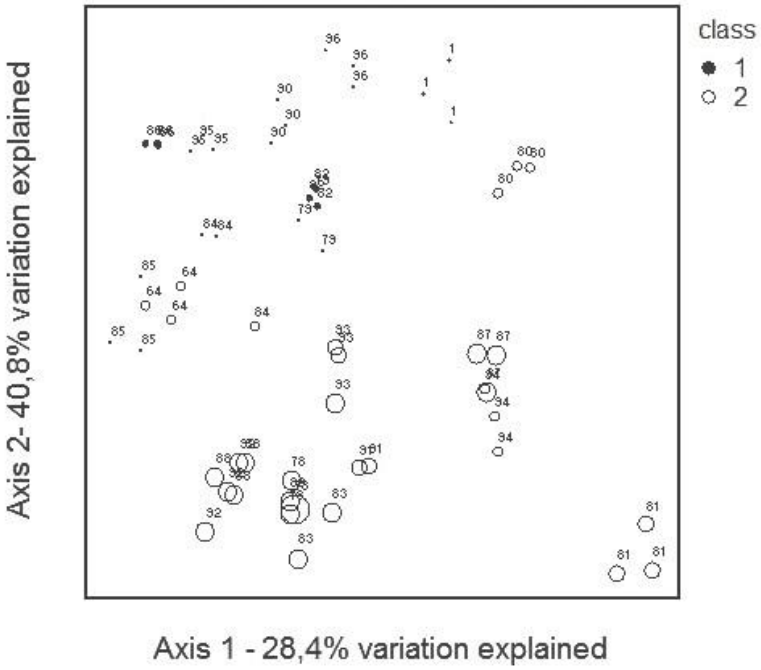

Figure 6. ISA indicator species (*Quercus ilex*) in NMS analysis

DISCUSSION

Our aim was to find a relationship between pine stands' health status and the variation in pinewood understory vegetation communities, through the analysis of several tree-related variables. Moreover, through the analysis of geographic variables, we wanted to know whether the variability in understory vegetation is consistent with the already known gradient source and movement of the parasite in the study area.

According to the results, the specific composition and species abundance in understory vegetation are directly related to pine stands' health status as expressed by the percentage of defoliation and resination of the trees. Three species in understory vegetation are strongly associated with different conditions in pine stands. Resinated and defoliated trees are the best predictors for understory vegetation composition in the survey area.

Detecting geographic gradient

Low values of altitude (alt) were correlated with negative values of tree-related variables on axis 1. We expected the altitude to be negatively related to high values of %def and %res because the bast scale spreads mainly through the wind (Binazzi, 2005). Usually wind is stronger where the altitude is higher (in proximity to the ridges and at the top of the mountains), so we expected that pine stands at high altitudes would be those in the worse condition of health. The results do support this hypothesis.

On the other hand, low values of latitude (lat) were correlated with positive values in %def on axis 3. We expected that stands in bad conditions would be situated in prevalence in the north-west side of the Cinque Terre National park, which is consistent with the observed direction of bast scale infection at the Mediterranean basin scale (Covassi & Binazzi, 1992). The results are not in accord with the bast scale origin and with its movement through the Italian peninsula.

The effect on plant composition

Percentage of resinated trees is the best predictor to discriminate health conditions in pine stands. This result can be explained by the fact that exudation of resin is one of the first symptoms of bast scale presence (Arzone & Vidano, 1981) and it remains visible for a long time (also after pine death). Percentage of defoliated trees is the second-best predictor to discriminate health conditions in the survey area. The percentage of defoliation is the variable that we expected to be most relevant in influencing understory vegetation because varying the tree crown density varies light impact on understory vegetation. As can be seen in the analysis, on the contrary, it is not as relevant as the percentage of resinated trees; this may be due to the fact that there are many more resinated trees rather than defoliated ones, which is because resination is a symptom that occurs prior to defoliation (Arzone & Vidano, 1981). It is therefore possible to define defoliation and resination as good indicators of state.

Three indicator species (*Erica arborea, Quercus ilex and Castanea sativa*), closely linked to pine stand health status, were found in the understory vegetation. *Erica arborea* shows a positive correlation with axis 2 because in accordance with the ISA results, it is an indicator of bad pine stand conditions. *Castanea sativa* and *Quercus ilex*, in contrast, show a negative correlation with the same axis, so these species are indicators of good pine stand conditions. To determine if there were significant changes in understorey vegetation composition over the three years, we calculated the ISA for each year of sampling. The results showed no substantial change in indicator species. In the course of time, the pine cover decreases due to defoliation and so understory composition alters, moving towards more heliophilous species. Pine stands in bad condition, on the other, already have a limited canopy coverage, which worsens at a slower rate; their undergrowth is dominated by heliophilous species so the change in population is less pronounced.

CONCLUSION

This study offers a model to understand a pine stand's health status related to understorey vegetation composition and abundance. The results of the study show a clear correlation between trees affected by bast scale and a variation in the of understory vegetation emphasising the importance of considering and collecting data about understory vegetation composition and abundance. Unfortunately, in the study area, there are no previous comparable data on the floristic composition of the undergrowth in order to verify if there is a correlation between the undergrowth vegetation composition and the level of bast scale infestation.

Pinewood health is important not only for functionality of forest ecosystems (e.g. preventing soil erosion in watershed management and in carbon storage) but also in order to reduce the effects of wildfires in Mediterranean forests. Pinewood affected by bast scale produce a large quantity of highly flammable materials (resin and dead wood), increasing the risk of wildfire. Monitoring and mapping the level of damage of pinewood is important in order to define predictive models against wildfire.

This study provides useful information and a good operational tool for technicians working in the forestry sector, and for public administrations and land managers to start good land-use planning. As an example, pinewood state of health may be a significant factor in the development of predictive models against wildfire. The stronger the habitat calorific value, the greater the virulence that the fire can attain, and the greater, too, the difficulty of extinguishing it (Núñez-Regueira et al., 1996).

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