Preliminary results of European budworm
Choristoneura murinana (Hubner) impact on Greek fir radial growth at Mts Parnassus and Giona

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Abstract

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During the spring of 2020, *Choristoneura murinana* (Hubner) otherwise European Fir Budworm (EFB) was recorded on *Abies cephalonica* near the villages of Kaloskopi and Agoriani in Central Greece at the mountains Giona and Parnassus respectively. To our knowledge, the occurrence of the particular pest on the specific mountains has not been described yet. We found that EFB mostly prefers *Abies cephalonica* and less *Juniperus oxycedrous* in sunny areas and/or near the country roads. Across the study area, local severe infestations of scale 2, 3 and 5, were observed. In many cases the infestation was observed in adult fir individuals. Defoliations and severe outbreaks, which are presumably incurred by EFB were also observed by local people (beekeepers, foresters, herb collectors) in the past. Our laboratories field measurements and the analysis of the Singular Spectrum analysis trendlines revealed growth decline, not connected with climatic parameters but probably associated with observed defoliations by the EFB.

Keywords

Abies cephalonica, climate, Giona Mountain, infestation, monitoring, tree rings

Introduction

It is known that there is a link between the radial growth and water relations of trees or the prevailing temperature of the area. In general, it is a relatively straightforward matter to link growth variations to climate change scenarios or regional meteorological events. KOULELIS et. al. (2019) confirmed this assumption by finding statistical significance between mean relative annual periodic increment of volume and summer precipitation for beech and oak plots which wasn't proved in the case of fir. BARNA et al. (2010) mentioned that dendrochronological studies quantifying the influence of discrete events on the radial growth are based on the comparison of the tree ring widths before and after the beginning of presence of a specified controlled actor. The tree rings analysis, is commonly used by the literature to reflecting the connections between abiotic factors like selected climatic variables (air temperature, precipitation amount, soil water content) or biotic like insects or pests' infestations and annual ring indexes. DOBBERTIN (2005), in a review, focused on tree growth as indicator of tree vitality, that is responsive to environmental stress and concluded that ring width can be used for this purpose. Useful information regarding survival probabilities of trees and growth assessments on monitoring plots can be derived from ring width.

The radial growth is the realization of cambial activity, which is connected to carbon. The water availability causes the closing of stomata and is engaged with the C balance by reducing assimilation of CO_2, inhibits cell division and cell enlargement (ZWEIFEL et al., 2006, 2007). In spite that

several factors were implicated to this phenomenon no special mechanism has been suggested as of yet. Six factors are usually involved in radial growth: (i) carbon and C-based compounds, (ii) nutrients, temperature and water scarcities which are limiting factors, (iii) plant hormones, such as auxin, which regulate cambial activity, (iv) mechanical stress which affects cambial activity, and (v) the leaf area which is affected either by the location the leaf buds had in the previous season or the interaction between trees and biotic factors which reduce the functionality of leaves either by removing it or reducing the size of the leaves or needles. Many of the above factors and mostly 'leaf area' are a direct prediction of the pipe model theory (PMT) (CHIBA, 1998; PETIT and ANFODILLO, 2009; SHINOZAKI et al., 1964) which is used for the hydraulic explanation of the eventually observed patterns of reduced CO_2 assimilation. Among many biotic factors behind the reduction of leaf area is the bud and needle eating insect *Choristoneura murinana* (Lep., Tortricidae) (EFB, European Fir Budworm) which has been recorded at Kaloskopi, (Mt Giona area) in the recent 60 years.

Fir species is the dominant forest tree across many mountainous areas of Greece. Namely, in the northern parts of Greece the species *Abies alba* dominates and is replaced by *Abies borisii-regis*. In the central and southern Greece, Greek fir replaces the other two at a geographical latitude corresponding to Mt. Vardoussia, Mt. Giona, and Mt. Parnassus. However, in all three species the major defoliating insect is EFB. It was found to defoliate fir trees at Pertouli, Trikala (MARKALAS, 1992; MARKALAS and BOGENSCHÜTZ, 1995) while in central Greece near Athens it has been considered one of the major causes of fir tree mortality among scolytids, mistletoe *(Viscum album)* and decreased proportion of fine root biomass (RAFTOYANNIS et al., 2008; RAFTOYANNIS et al., 2015; TSOPELAS et al., 2001; TSOPELAS et al., 2004). However, on Mt. Giona the most important defoliator is the EFB although there is no reference in the last 60 years of severe attacks of scolytids as the commonly observed insects eating the wood of the Greek fir is *Phaenops knotecki* (Col., Buprestidae) and *Acathocinus reticulatus* (Col., Cerambycidae) (KAILIDIS and GEORGEVITS, 1972; MARKALAS, 1992). In this context, the main aim of this study is to separate the impact of climate factors (precipitation and temperature) on radial growth from the relevant caused by biotic factors (e.g. the main defoliator EFB). Our research focused mainly on field data originated from Mt. Giona, using published information (KOULELIS et al., 2022) regarding the impact of precipitation and air temperature on *A. cephalonica*, by evaluating radial growth data from tree ring analysis and the Singular Spectrum Analysis as well.

Materials and methods

Study area

Kaloskopi village is located in the middle of a dense fir forest at an altitude of 1,040m (Stand 1: 38°38'54.6504"N, 22°23'6.0216"E, alt: 988 m, Stand 2: 38°40'44.6808"N, 22°18'24.66"E, alt: 1,274 m) (KOULELIS et al., 2022). It is a mountainous area in northeastern Phocis, Greece, situated in the northeastern foothills of Giona Mt and belongs to Natura 2000 protected areas (Natura 2000 site code: GR2450002 and Mt Giona). The area includes Giona which is the highest mountain in Central Greece and the 5th highest in Greece (2,510 m). At lower altitude, we have the presence of evergreen broadleaf shrubs with the dominant plant species *Quercus coccifera* L. and *Juniperus oxycedrus* L. (only in GR2450007). *Quercus pubescens* (Willd) and *Ostrya carpinifolia* (Scop) have scattered occurrence. At higher altitudes, *Abies cephalonica* (Loudon) dominates, while along streams formations with *Platanus orientalis* L. and *Salix alba* L. (only in Natura 2000 site code: GR2450002) are found. At higher altitudes there are also meadows with *Juniperus communis* subsp. *alpina* (Fig. 1), (SOLOMOU, 2020). Also, Agoriani village is located on the north-western side of Parnassus at an altitudinal range of 830–950 m. The dense forest surrounding it consists mainly of *A. cephalonica, Prunus* sp., *Crataegus* sp. and *Juniperus* sp.

Climate data

The weather patterns follow the Greek weather patterns of cool and wet winters and hot and dry summers. Nonetheless, due to its mountainous location, Kaloskopi presents colder winters with heavier rainfalls and less dry summers, than it is generally observed in southeastern Greece (Fig. 2).

The available climate data, which were correlated with tree ring data, show that the mean annual precipitation of the area is 890 mm. Maximum annual precipitation was observed for 1963, with 1,866 mm, and the corresponding minimum for 1984, with 382.2 mm. The dry periods generally last from June to September, with an average cumulative precipitation of 115.31 mm (median 89.3 mm). The mean monthly precipitation for this period (June–September) is 28.83 mm, with a corresponding median of 18 mm. The temperature in the last 11 years varied between –19.4 °C in January to 32.8 °C in August (derived from Kaloskopi hydrometeorological station, coordinates: 38°41'21.552"N, 22°19'23.988"E, at 1,052.80 m, time series 1963–2019 and the Mavrolithari meteorological station, coordinates: 38°42'N, 22°18'E, at 1,220 m, time series 2009–2019).

Defoliation and Singular Spectrum Analysis

For the analysis of the FEB infestation in the area, measurements of the coverage of fir trees were performed and the mean value of defoliation was assessed in five categories (Table 1). In addition, two sites with different elevation of 988 m and 1,274 m near Kaloskopi (Giona Mt) were selected and 20 healthy looking dominant or codominant trees per site, separated by at least 5 m (KOULELIS et al., 2022), were sampled with a corer (Haglof, Sweden).

The precipitation factor was analysed using the Standardized Precipitation Index (SPI). The SPI can be used to compare drought events over different timescales and among areas with different climatic conditions (BONACCORSO et al., 2003; KHAN et al., 2018) it can also be applied in drought warning systems and drought severity assessment. In this study, SPI was calculated with a 6-month time step considering the period of March to August, as this is the tree's annual growth period.

The effect of local precipitation as inferred by the SPI of the radial growth of Greek fir trees was investigated by means of a special statistical method known as Singular Spectrum Analysis (SSA). The main aim of using the SSA method could be helpful to uncover growth trends related to past infestations, to uncover hidden cycles or even to extract important long-term trends. The method is devoid of any assumption related to the

Fig. 1. Giona Mt vegetation a) *Quercus coccifera* (L), b) *Quercus pubescens* (W), c) *Ostrya carpinifolia* (S), d) *Abies cephalonica* (L.), e) *Platanus orientalis* (L.), f) *Salix alba* (L.), g) *Juniperus oxycedrus* (L.), h) *Juniperus communis* subsp. Alpine. Photos came from Solomou's personal archive.

Table 1. Categories of infestation / defoliation of fir trees by EFB

Category	% of twig infestation[a]
0	infestation signs were not observed
1	0–5 of twigs are infested
2	6–20 of twigs are infested
3	21–60 of twigs are infested
4	61–100 of twigs are infested
5	6–100 of twigs are infested together with the apical vertical twig

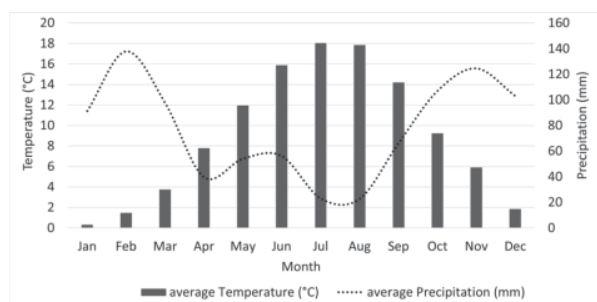[a] The twigs may be completely eaten, partly eaten needles, or needles exhibiting microphylly.

Fig. 2. Climadiagram showing rainfall distribution and temperature variation of the Kaloskopi area. (Time series 2009–2019).

original data such as normality or a special form of probability density. The other methods of time series analysis such as autoregressive moving average models, ARIMA models are parametric. In contrast, SSA needs no parameters and in fact outperforms these methods (Golyandina et al., 2018; Hassani and Mahmoudvand, 2018).

In addition, SSA can be run, at no cost, in the R procedural language (R Core Team, 2013) eventually using the library dpl (Bunn, 2010). However, in this work we used the library Rssa (Golyandina et al., 2013) together with the multivariate version of the routines listed in the book of Hassani and Mahmoudvand (Hassani and Mahmoudvand, 2018).

The multivariate form of SSA (MSSA) is used here since it performs better than univariate SSA in the correlation between time series, there is an inherent dependency among them (Rodrigues and Mahmoudvand, 2018). MSSA in this work is considered for the time series of the annual tree-ring width index (ATRWI), Koulelis et al., 2022, at both altitudes, namely 988 and 1,274 m asl and the Standardized Precipitation Index (SPI). The last time series is the representation of the local climate in the analysis.

Results

EFB infestation study area includes a monospecific Greek fir dominated forest from Arachova (Mt Parnassus) to Kaloskopi village (Mt Giona), central Greece. It comprises five stands as shown in Table 2.

In the study area, there were local infestations of scale 2, 3 and 5. In many cases, the infestation was found in adult trees. EFB mostly prefers *Abies cephalonica* and less *Juniperus oxycedrous* (Fig. 3) in sunny areas and/or near the country roads.

In general, fir trees above 1,100 m exhibited no signs of infestation by the EFB. Probably this is the result of human induced disturbances (roads, forest roads, forest paths, home building) which are practiced below 1,300 m. Field observations reveal that the EFB usually prefers sites that receive much solar radiation together with a certain amount of new fir needle tissue. The first is provided by forest openings typically associated with road and path construction. The second is ensured by the extension of needle unfolding phenology, which in broad sense configures the insect fauna of fir trees. The EFB shows a phenology that makes it easy to observe (biological cycle reviewed by Du Merle et al., 1992). The latter fact arises the question of why Greek researchers, did not monitor it at the first place but until it was quite late (Kailidis and Georgevits, 1971, 1972). Adults of the moth are seen in June–July and sometimes in early August. They lay egg clusters at the top branches of the fir tree in July–August and the L1 larvae that do not feed emerge and move to hibernacula where they molt to L2 and overwinter. Later in spring, they emerge and pierce the cap that covers the new emerged needles at the tips of twigs. At the same time, it spins a loose protective web at the apices of fir tree twigs within which L2 / L3 feed. They are unable to pierce and eat older needles and for this they move to them at later larval stages (L3–L6). At later larval stages, the EFB is capable to confer any degree of damage to the host tree. Since the biology of the EFB is closely connected to the phenology of the Greek fir, it is expected that favorable climatic conditions will positively affect both the width of the annual tree rings of the Greek fir and simultaneously the unfolding of new needles. A peculiar pattern of needle unfolding is exhibited by the Greek fir tree. Because of the high biological value of the new plant tissue the fir tree either protects the newly emerged needles with antifeedant chemicals or the tree shows a second unfolding of needles which are distinctly smaller than the needles of the first unfolding. This type of heterophylly seems to benefit the tree since the moth larvae are forced to consume more needle tissue and in this way they uptake more antifeedant compounds such as limonene which is found in the needles of Balkan firs (Koukos et al., 2001; Roussis et al., 2000;

Table 2. The sites where radial growth and infestations by EFB were estimated

Locality	Coverage percentage of fir tree (%)	Category of mean defoliation of trees[a]	Mountain range
Forest near the chapel of St Nikolaos[b] on the connecting road Arachova-Agoriani	65	2	Mt Parnassus
Agia Triadha, Agoriani[c] (site 1)	72	2	Mt Parnassus
Agia Triadha, Agoriani (site 2)	70	2	Mt Parnassus
51th Km Amfissa-Lamia[d]	75	2	Mt Giona
Krystallidhia on the road (51th – Kaloskopi village)	83	5	Mt Giona
Kaloskopi village[e]	84	3	Mt Giona

[a] Defoliation categories by EFB listed in Table 1.
Geographical coordinates: [b] 38°34'79.284''N, 22°32' 39.2856''E, [c] 38°34'57.3168''N, 22°29'22.1568''E, [d] 38°37'50.1024''N, 22°22'33.7656''E, [e] 38°41'22.6824''N, 22°19'22.8396''E.

Fig. 3. Observed infestations of EFB on *A. cephalonica* (a, b, c) and *J. oxycedrus* (d). Photos by P.V. Petrakis and P.P. Koulelis.

ZENELI et al., 2001). Tree ring growth depends on species, age, heredity, and climatic conditions of the study site (VIEIRA et al., 2009), and the complex phenomenon of tree growth could therefore also be a factor of competition with neighbor trees and lianas, ingrowth of the stand, or genotypic variation in a particular area (COOK, 1987; ZWEIFEL et al., 2007; KOULELIS et al, 2022). PAPADO-POULOS (2016), using quantification and analysis of the inter-annual variability of fir site chronologies, have shown that the principal factor affecting tree growth at a latitudinal scale is climate. According to the tree ring-to-climate relationships examined by this author, late spring and summer precipitation is the main climatic factor affecting the growth of Greek fir populations.

The results of our analysis in the field revealed for the first time regarding the particular area, that there is a strong relationship between precipitation and fir growth (KOULELIS et al., 2022). The comparison of growth with the climatic data, using tree rings analysis and the Standardized Precipitation Index (SPI), which is based on the probability of precipitation for various time scales depending on the user's interest (MCKEE et al., 1993), following a period of 55 years showed extreme dry events followed by significant growth decline but also tree growth reduction not related with climate or meteorological events (KOULELIS et al., 2022). This observation has led us to the investigation of the EFB infestation and its implications.

In Figure 4 the trendlines of the three-time series, are shown. Average Tree Ring Width Index regarding both altitudes (ATRWI 988), (ATRWI 1274) and the Standardized Precipitation Index (SPI) were calculated. The first feature of the diagram is the constantly negative location of the SPI trendline. The second prominent pattern is the higher variability of the tree ring

index at lower altitudes, acting on growth, regarding the same period. In the higher altitude – i.e. 1,274– no EFB was ever observed. For this, the trendline is configured mainly by climate or minor biotic relations. The ring width peak 4 coincides with the extreme drought shown in SPI trendline and it is a boundary condition since it marks the onset of decrease of radial growth. The subtle peak 7 at the higher altitude (red line) coincides with a much prominent peak at the lower altitude (blue line) which may be the result of the previous SPI trough, which controlled the EFB population densities and permitted the observed radial growth. At the lower altitude, there are more than seven peaks and troughs created presumably by the eating action of the EFB in addition to other minor biotic interactions. The effect of climate, as it is expressed by SPI, seems to be in opposite directions with the trendline ATRWI at 1,274 m asl. It seems that precipitation governs the tree ring widths, a fact that is shown also in peak 4, in the year 1989, of the trendline ATRWI at 988 m asl. Importantly, in the year 1989, the SPI trough corresponds to two simultaneous peaks of 988 and 1,274 ATRWIs. An inconsistency can also be seen between the radial growth of the Greek firs and the peaks 2 and 4. Troughs 1, 3, and 5 can be attributed to the EFB together with the other smaller troughs on the trendline. The precipitation on the area as expressed by SPI shows an extreme drought between the years 1984 and 1992 but the overall trend shows a final increase. The increase in 2006 seems to be responsible for the troughs around peak 6.

Discussion

Sᴀʀɪᴋᴀʏᴀ et al. (2005) reviewed the distribution of the EFB in Europe and found that the insect occurred in Germany (Fʀᴀɴᴢ, 1940; Pᴀᴛᴏčᴋᴀ, 1960), in the former Czechoslovakia

(Bɪʀᴏᴠᴀ, 1966), Bulgaria, Romania, Yugoslavia and Poland (Dᴜ Mᴇʀʟᴇ et al., 1992), with infestations to silver fir, but is capable of causing significant damage to other conifers as well. Kᴀɪʟɪᴅɪs and Gᴇᴏʀɢᴇᴠɪᴛs (1971) found the insect on *A. cephalonica* and the hybrid form of *A. borissi regis* in Greece. Furthermore, the EFB was recorded on *Cedrus atlantica* Endl. (Dᴜ Mᴇʀʟᴇ and Cᴏʀɴɪᴄ, 1989, 1991) and *Picea abies* L. (Dᴜ Mᴇʀʟᴇ et al., 1990) in France. In Turkey, it is considered as a dangerous pest of *A. cilicica* in forests in the county of Isparta (Sᴀʀɪᴋᴀʏᴀ, 2004). On the other hand, to our knowledge, there are no studies across Europe, where the effects of historically documented outbreaks on stands structure on radial growth on host and nonhost species and its relation with climate are analyzed in depth. In this study, the Multivariate Singular Spectrum Analysis (MSSA) of field measurements revealed growth decline, not connected with climatic parameters. This approach could be important in interpreting further results using this or alternative methodologies. It is known, for example, that ^{13}C enrichment of the α-cellulose in the tree rings via the increased photosynthetic rate caused by needle eating insects can be used as a bioindicator of past insect infestations, as few studies report (Sɪᴍᴀʀᴅ et al., 2008). In addition, the MSSA is able to reveal such trends between time series associated with radial growth of trees and climate. The basis behind this enrichment of tree ring carbon through discrimination of the heavier carbon isotope has been extensively investigated by O'Lᴇᴀʀʏ (1981) and Fᴀʀǫᴜʜᴀʀ et al. (1982). However, there are also only a few studies that investigated past insect outbreaks through this approach. Also, a future line of research involves the implementation of linear aggregate models (Cᴏᴏᴋ, 1987) and ICA (Independent Component Analysis, Hᴜᴍʙᴇʀᴛ and Kɴᴇᴇsʜᴀᴡ, 2011). Since the MSSA is a combination of SSA and principal component analysis (PCA), it is able to find trends and oscillating pairs of series involved in the analysis. At this stage, it is mandatory to examine possible correlation of ATRWI at both altitudes. According to this study, the distance between the two plots is large enough to exclude any migration of the gravid female EFBs from low to high altitude trees. In effect, any interaction of fir's infestation at the two altitudes is biologically decoupled.

Conclusions

It seems that the EFB causes sporadically severe defoliation to *A. cephalonica* at Mts Giona and Parnassus. Moreover, an important periodical decline on radial growth of fir was measured for the first time at Mt. Giona. Our results indicated that the relation between climate and tree ring width is disturbed because of the EFB attack at Mt. Giona. The infestation of *A. cephalonica* by the EFB not only decreases radial growth but also moves the fir tree to competitive inferiority against lianas and ground vegetation. In addition, fir's decline is a combination of climate, water availability, seed germination suppression and the scarcity of the EFB's natural enemies. Nevertheless, these observations require further detailed investigation. Future research will unavoidably involve carbon ^{13}C and oxygen ^{18}O stable isotopes, under the literature based hypothesis. This study, underlined serious concerns about the health and the sustainability of fir ecosystem at Mts Giona and Parnassus. We recorded severe attack of the defoliator *C. murinana* and decline in growth, across many stands. In general, the need for research to improve
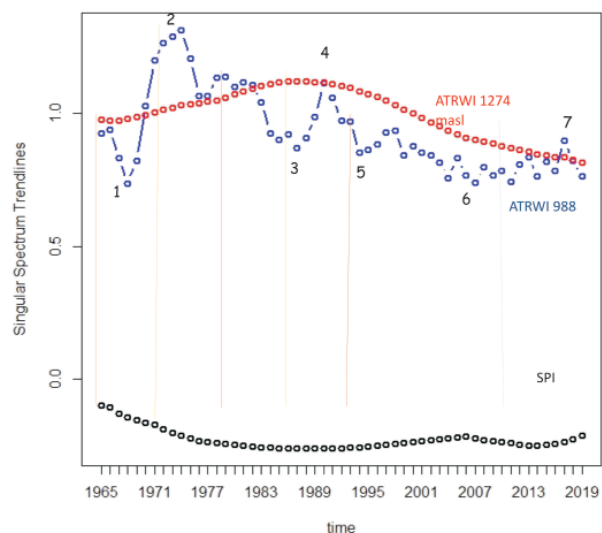

Fig. 4. Diagram showing the SSA (Singular Spectrum Analysis) trendlines of all three time series ATRW 1274 (red trendline), ATRWI 988 (blue trendline), and SPI (Standardized Precipitation Index) (black trendline). The values on y-axis are the SSA values, values on x-axis are the years over which the tree-ring width is measured. The Arabic numerals denote discussed in the text peaks and troughs while vertical lines indicate proximity of the ATRWI lines and/or higher values of the index at lower altitude (explanations in the text).

prove decision making in a way that addresses the present skepticism in projections of future climate and its impacts (positive or negative) on forest ecosystems is crucial. Our labs future research aiming to strengthen our knowledge of biotic and abiotic factors impacts on tree species, especially for the economically important species such as fir. The projections of forest dynamics across the 21st century at national level, considering climate change consequences, which will probably occur in the long-term perspective, are crucial in terms of supporting sustainable forest management and improving science based decision making in forest resource management.

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