

## Dendroclimatic analysis of *Quercus robur* infected with *Fusarium eumartii*

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**Summary.** The relationship between the growth increments of declining and healthy *Quercus robur* and monthly rainfall and temperature data was explored in a *Q. robur* stand situated near Fagarè (Padova) in north-eastern Italy. As part of the same study, *Q. robur* growth rings were examined for gum deposits associated with *Fusarium eumartii*, an anamorphic fungus previously found on declining *Q. robur* individuals from the same area. The growth rings showed that over the period 1961–1994, declining trees had an average growth increment of 2.24 mm, while for healthy trees the increment was 3.57 mm. The mean monthly temperatures recorded between 1961 and 1994 were normal for the Po-Veneto basin. Mean monthly precipitation in March and April, the months in which resumption of vegetative growth occurs in *Q. robur*, was 100 mm or less in 1961, 1966, 1968, 1969, 1973, 1976, 1982, 1987, 1988, 1993 and 1994. It is precisely during these months that the rainfall needs of *Q. robur* exceed 100 mm/month, however. The lower growth recorded in those years was therefore to be attributed to drought in the months of March and April. At the same time *F. eumartii* was always isolated from the growth rings corresponding to the years 1962, 1967, 1969, 1970, 1977, 1983, 1988, 1989 and 1994. Each of these years followed a year characterised by reduced growth. It is concluded that the decline of the *Q. robur* population occurring at Fagarè may be due to periods of drought acting as an inciting factor to reduce growth, followed one year later by activity of the pathogen *F. eumartii* and its metabolites, as a contributing factor.

**Key words:** oak decline, growth ring analysis, dendroclimatic parameters.

### Introduction

Since 1980, stands (40–50 years old) of *Quercus robur* L. on an area of about 200 ha at Fagarè (Padova) (45° 49' 10" N, 12° 00' 35" E) in the north-east of Italy, were found to be affected with extensive decline. At an early visual investigation the plants did not show the characteristic symptoms (epicormic shoots along the trunk and on the branches, bark cracks from 3–4 to 20–25 cm in

length, xylem necrosis, and bleeding) that declining oaks usually present in Europe, including Italy, but only crown transparency, apical shoot wilting and stunting.

In 1994 hundreds of trees were felled as part of a sanitation cut of the stand, providing an opportunity to study the decline more closely. From the felled trees, which were about 35–40 m tall, discs were cut at 1-m intervals from the collar up to a distance of 20–25 m. On these discs many of the wood rings were seen to have diffuse occlusions of the vessels caused by deposits of gum. From these occluded areas, *Fusarium eumartii* Carpenter, an anamorphic fungus already reported by Moricca and Ragazzi (1991), was consistently isolated.

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This microorganism, tested in a controlled environment, was found to have climatic requirements similar to those of *Q. robur* and to be pathogenic on 2-year-old seedlings of *Q. robur*, in which it produced metabolites of a high molecular weight (Ragazzi *et al.*, 1993).

*F. eumartii*, known to cause damage to many shrubs and trees like citrus, rubber, cacao, fig, coffee, apple and cassava (Storey, 1932; Petri, 1937; Bugnicourt, 1939; Wollenweber, 1943; Gerlach and Ershad, 1970), was thought to have a role, may be in combination with abiotic factors, in the decline of oak.

To explore this hypothesis a study was undertaken to analyse the effect of spring rainfall and temperature on the growth of declining *Q. robur* trees at Fagarè, and simultaneously to ascertain whether these trees were infected with *F. eumartii*. In Fagarè, the spring rainfall coincides with the period of vegetative growth resumption, when increase in growth is most rapid.

## Materials and methods

### Growth ring analysis and occurrence of *Fusarium eumartii*

Twenty declining and 5 healthy trees were used to study the dendroclimatic parameters and the occurrence of *F. eumartii*. The trunk of each tree was cut into three 3-cm-thick discs at 1, 2 and 3 m from the ground. The width of the growth rings was measured by a dendrochronograph with 0.01-mm gradations on 20 radii per section. Moreover, gum deposits (gum accumulation obstructing vessels over 0.5 mm diameter) in each growth ring were counted on the disc taken at 2 m from the ground.

From all areas with gum deposits, wood fragments 4 mm long, 2 mm wide and 2–4 mm deep were excised. These fragments were cultured in Petri dishes each containing 20 ml PDA (Difco, Detroit, MI, USA), and incubated at 22°C. They were examined after 8 days for the typical, hyaline, falciform macroconidia of *F. eumartii* measuring 4–6.1 × 27.6–52.1 µm. A disc taken at 2 m from the ground from three healthy trees was used as control.

To verify how many colonies were obtainable from gum deposits, each deposit was divided into four pieces, which were then seeded on PDA.

### Climate data

The Weather Centre at Teolo (Province of Padova), located 1 Km from Fagarè and at the same altitude, forming part of the Azienda Regionale per la Prevenzione e Protezione ambientale del Veneto, supplied mean monthly temperature and precipitation data for March and April from 1961 to 1994.

### Statistical analysis

The correlation between growth increments and climatic data was determined by calculating the simple linear regression of growth increments for the 20 declined trees (average of the month) as a dependent variable and the following climate data: 1) the overall mean growth increment of March and April in 1961–1994 (respectively 3.289 mm and 4.576 mm); 2) the rainfall of March–April for the same period (101.9 mm and 133.2 mm).

Significantly correlated parameters were compared in a correlation matrix according to the Hocking model (1976). Pairs containing parameters significantly correlated to each other were examined, and within each pair the parameter less correlated with the growth increment was eliminated (Tainter *et al.*, 1984).

The remaining parameters were used as independent variables.

## Results

### Growth ring analysis

The disks examined exhibited up to 55 growth rings, including false and double rings. Fig. 1 shows average radial growth for healthy and declining trees for 1961–1994, the years for which satisfactory weather data were available. Average annual growth from 1961 to 1994 was 3.57 mm in healthy trees, but only 2.24 mm in declining trees.

In declining trees annual growth of less than 1 mm was recorded in: 1961 (0.85 mm); 1966 (0.43); 1968 (0.11); 1969 (0.21); 1976 (0.88); 1982 (0.11); 1987 (0.65); 1988 (0.11); 1993 (0.11); and 1994 (0.99).

On healthy trees, on the contrary, only in 1993 was annual growth close to 1 mm, whereas in all other years it exceeded 2 mm.

Precipitation data from 1961 to 1994 for March–April are shown in Fig. 2. Marked fluctuations occurred from year to year. The mean precipitation in

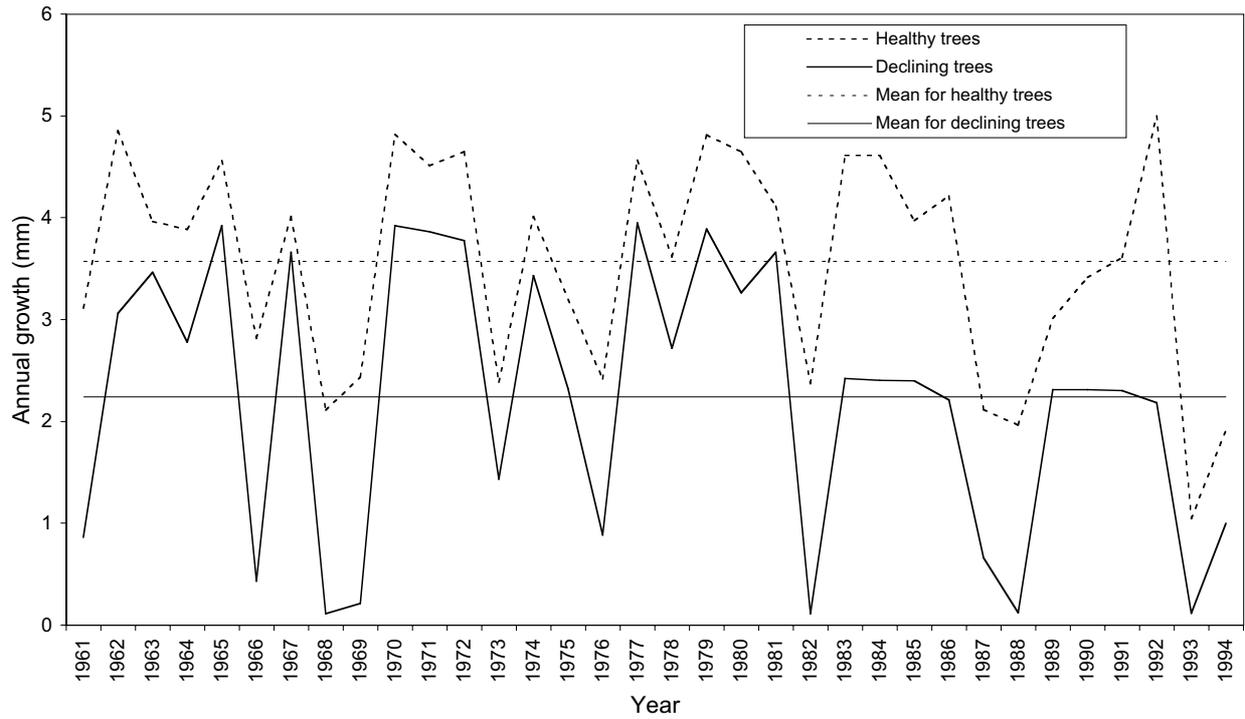


Fig. 1. Average growth curve from annual rings of declining and healthy *Quercus robur* trees near Fagarè, Padova, Italy.

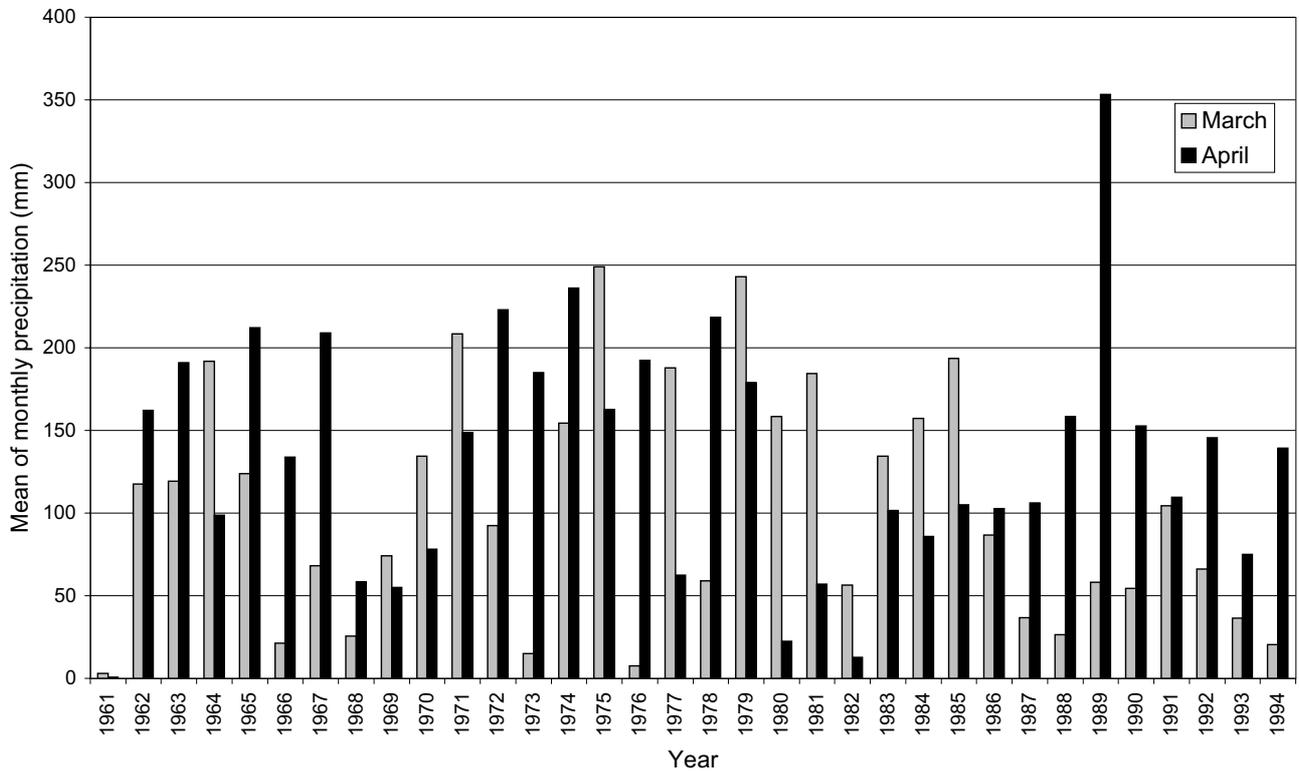


Fig. 2. Mean monthly precipitation during March and April in the Fagarè, Padova, Italy, area from 1961 to 1994.

these two months, the most important for vegetative resumption, was 100 mm or less in 1961 (1.8); 1966 (77.4); 1968 (41.9); 1969 (64.5); 1973 (99.8); 1976 (99.9); 1980 (90.2); 1982 (35.4); 1986 (94.6); 1987 (71.3); 1988 (92.2); 1993 (55.6); and 1994 (79.7).

Figure 3 shows variations in the mean temperature for March and April of each year in relation to the mean temperature for these months over the entire 1961–94 period: March 8.4°C, April 12.4°C.

Very warm springs occurred in 1989, 1990, 1991 and 1994.

The temperatures of the warmest months each year fluctuated between 20 and 23°C, which is a normal range for the Po-Veneto basin where Fagarè is located.

The weather data revealed that there was a relationship between spring precipitation and radial growth in both healthy and declining trees. In declining trees, annual growth was less than 1 mm in most years in which average March–April rainfall was 100 mm or less. Mean precipitation for March–April was correlated with the growth indexes: analysis of variance of the linear regression of March–April precipitation on the growth increments of declining trees gave an F-Ratio of 16.87 ( $P \leq 0.01$ ); since the resulting F value was higher than the P value for 5% and 1% probability, it can be stated that regression was highly significant (Table 1).

Years when healthy trees had below-average growth rates (i.e. below the overall 1961–1994 average of 3.57 mm) were: 1961 (3.11), 1966 (2.81), 1968 (2.11), 1969 (2.43), 1973 (2.31), 1976 (2.41), 1982 (2.37), 1987 (2.11), 1988 (1.96), 1993 (1.64), and 1994 (1.91).

In each of these years, however, healthy trees had higher growth rates than declining trees, whose growth rates in these same years were: 1961 (0.9), 1966 (0.4), 1968 (0.1), 1969 (0.2), 1973 (1.7), 1976 (0.8), 1982 (0.1), 1987 (0.5), 1988 (0.1), 1993 (0.1) and 1994 (1.2).

#### Occurrence of *Fusarium eumartii*

The growth rings of declining trees exhibited gum deposits in 1962, 1963, 1967, 1969, 1970, 1972, 1973, 1977, 1978, 1983, 1985, 1988, 1989, 1990, 1994, and *F. eumartii* was always isolated from these deposits. In other years gum deposits were not observed, not even in rings with a very low annual growth increment (Fig. 4). The most numerous deposits were in years that immediately followed a year in which the trees had shown strongly reduced growth: 1962, 1967, 1969, 1970, 1977, 1983, 1988, 1989 and 1994 (Fig. 1). In these years, *F. eumartii* colonies occurred in numbers ranging from 2 to 40. *F. eumartii* was never found in healthy trees.

Table 1. Simple linear regression of growth increments on precipitation in March–April (1961–1994).

Parameters	Regression <sup>a</sup>				
	Coefficient	Standard error	T-value	P	
Intercept	-12.67348	6.64321	-1.90773	0.00295	
Angular coefficient	0.68443	0.11824	5.78848	0.00018	
ANOVA					
Variation	Df	Deviance	Variance	F	P
Model	1	29004.321	29004.321	16.87	3.77
Error	13	22341.660	1718.5892		
Total	14	51345.981			
Correlation coefficient = 0.51					

<sup>a</sup> Dependent variable: growth increment; independent variable: precipitation in March–April period.

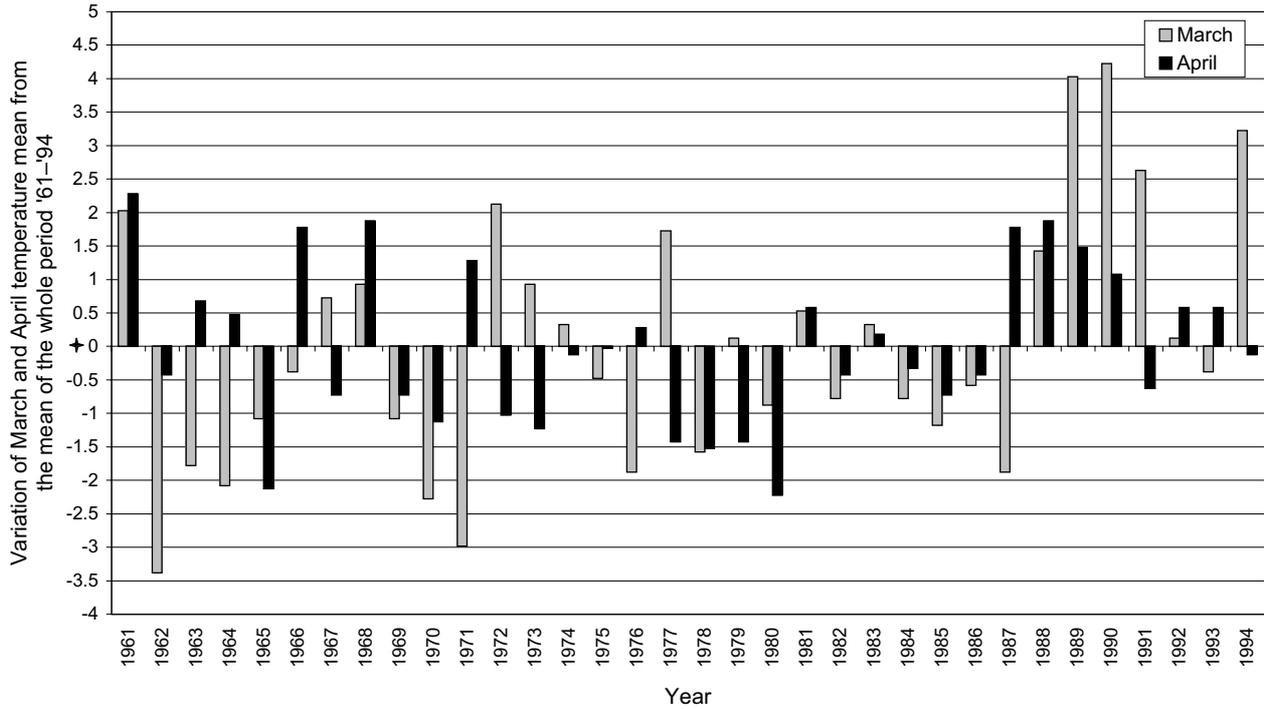


Fig. 3. Variation of the mean temperature in March and April each year from 1961 to 1994 in relation to the temperature mean over the whole period 1961-1994.

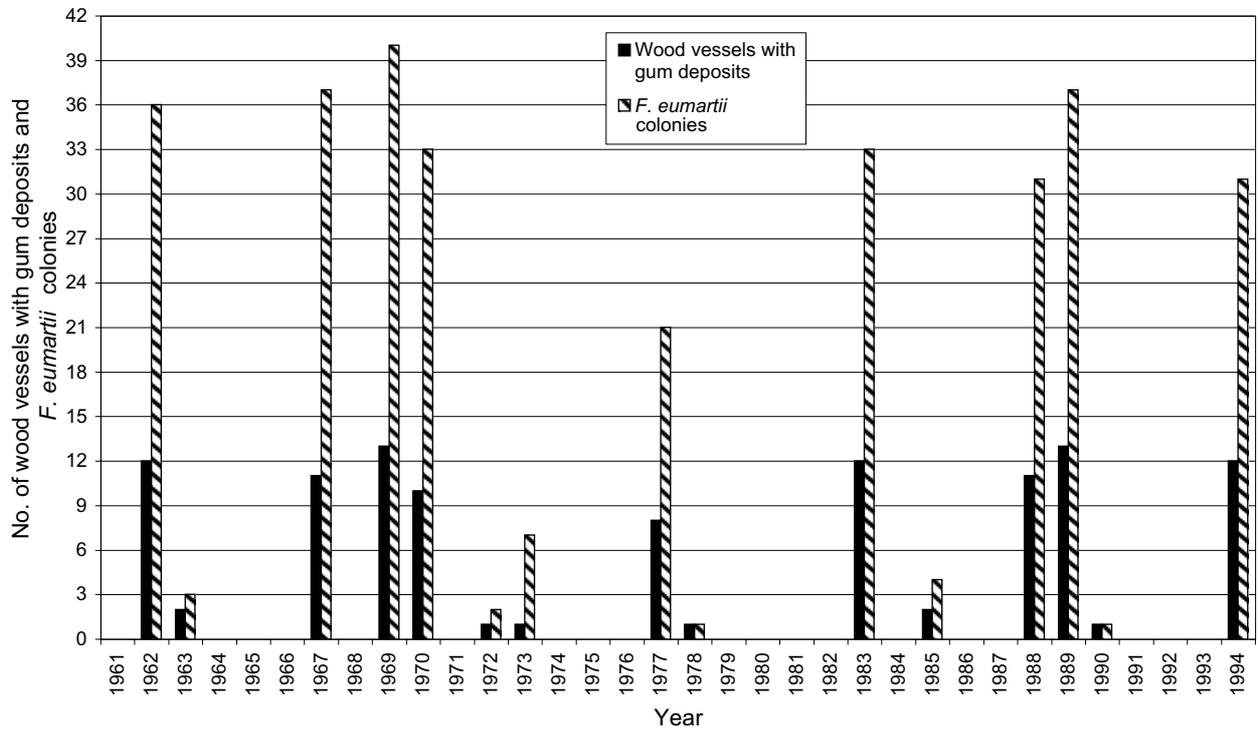


Fig. 4. Number of wood vessels filled with gum deposits from which *Fusarium eumartii* was isolated, and number of *F. eumartii* colonies obtained from gum deposits.

## Discussion and conclusions

In recent years many studies in Europe and North America have adopted a dendroclimatic approach in order to explore the relation between environmental factors and annual tree growth. Such an approach seeks to understand the start and time-course of decline phenomena (Tessier, 1986; Eckstein and Sass, 1988; Vannini, 1990; Becker *et al.*, 1990; Amorini *et al.*, 1996).

As is known (Zahner, 1968), the greatest annual growth increments in *Quercus* spp. occur during the early spring months (March and April); consequently, any climatic factors whose action is exerted during this brief period become crucial for the annual growth of the tree. For *Q. robur*, the climatic parameters that significantly correlate with annual growth increments are those expected for other oak species in the Mediterranean area, such as *Q. cerris*. In particular, precipitation may play a major role, since the months with low precipitation, March and April, are also the months of vegetative resumption, during which *Q. robur* requires a mean monthly precipitation of more than 100 mm (Borghetti, 1992). *Q. robur* has large vessels (200–300  $\mu\text{m}$ ), especially in the spring wood; it is also the oak species with one of the highest water-flow speeds in the vessels, 43.6  $\text{m h}^{-1}$  (Zimmermann and Brown, 1971). Moreover, in these months the leaves are not yet protected by a tomentose covering, so that water loss may also occur through the cuticle.

In the Fagarè stand, the low precipitation in March–April of 1961, 1966, 1968, 1969, 1973, 1976, 1982, 1987, 1988, 1993 and 1994, precisely at a time when water requirements were high at the start of vegetative growth, may explain the low growth increments of declining trees in those years. The modest snow cover in the winters of those years did not trigger tree activity to protect the rhizosphere, but it also did not enable trees to accumulate a water reserve on which to draw when the temperature rose above freezing and vegetative growth began. Together with the low precipitation, three subsequent years with very warm springs (1989, 1990, 1991) must also be taken into account, as well as the higher temperatures in the second half of the 80s as compared with the first half.

However the decline recorded by dendroclimatic analysis may not be due solely to low precipita-

tion during the period of resumption of growth. A more complex pattern of drought and precipitation may be involved in *Quercus* spp. For example, Tainter *et al.* (1984) found that the decline of *Quercus borealis* Michx observed in North Carolina in 1979 followed a number of drought-stricken summers; in addition, precipitation in July was correlated with decline and depressed the annual growth increments. Vannini (1990) stated that drought in August was a contributing factor to decline of *Q. cerris* in central Italy. Other studies have shown that periods of low precipitation result in a slowing of the tree's water potential, leading to lower growth and, in particular, scant production of summer wood (Kramer and Kozłowski, 1979).

Amorini *et al.* (1996) observed in a 30-year-old oak stand near Tolfa (Rome, Italy), that healthy trees had greater annual increments than declining trees; that study reported a correlation between annual growth and several climatic parameters, above all water stress in particular months, in this case May and June.

Dendroclimatic analysis thus suggests that low precipitation in March–April (mean less than 100 mm), just when the water requirement of *Q. robur* is very high, causes a water imbalance that results in lower growth. It is likely that this phenomenon predisposes the tree in the following year (1962, 1967, 1969, 1970, 1977, 1983, 1988, 1989 and 1994) to attack by *F. eumartii*, which is always present in the soil and survives even at low temperatures (Ragazzi *et al.*, 1993). This hypothesis is in agreement with numerous reports that water stress leads to a reduction in host vigour and vitality, predisposing the tree to attack by aggressive or nonaggressive microorganisms (Gäumann, 1950; Yarwood, 1959; Schoeneweiss, 1978). The vessel occlusions by gum deposits further limit the already impaired water flow, and the activity of *F. eumartii* and its metabolites then aggravates any existing decline.

In support of this explanation it should be noted that *Q. robur* suffers severely from dry years in the alluvial plains. The larger adult trees (>25 m high) are particularly affected, as they have a wide crown exposed to light and are therefore more subject to transpiration (Becker and Levy, 1983; Durand *et al.*, 1983). Large-size *Q. robur* trees also experience more difficulty with water uptake because of the greater distance from the roots to the

leaves (Walter, 1968). Finally, *Q. robur* roots are shallow and are therefore more immediately affected by the soil as it dries out.

In this interpretation, a succession of inciting factors, here represented by drought, followed by contributing factors, here the activity of *F. eumartii*, would explain the decline now occurring in the *Q. robur* population near Fagarè. Such a succession of factors is consistent with the model of oak decline posited by Manion (1991).

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