GRAPE DIEBACK IN ROMANIA INDUCED BY PATHOGENIC LIGNICOULUS FUNGI

Liliana Tomoiaga[1], Maria Oprea[2], Aurelia Podosu[3], I. Voiculescu[4], Carmen Stoica[5], V. Florian[6]*

[1]Research Station for Enology and Viticulture, Blaj, Alba
[2]Research-Development Institute for Plant Protection, Bucharest
[3]Research Station for Grapevine Odobeşti, Vrancea
[4]Research Institute for Grapevine Valea Călugărească, Prahova
[5]Research Station for Grapevine, Iaşi
[6]University of Agricultural Science and Veterinary Medicine, Cluj

[*] correspondence address:
[1]Research Station for Enology and Viticulture, Blaj, Alba
515400, Blaj, Str Gh.Baritiu, No 2, Alba County
el: 0258-711623, Fax: 0258-710620
E-mail: tomoiagaliliana@yahoo.com

Abstract. Biological decline of grapevine is determined under the pedoclimate conditions prevailing in Romania by the lignicoulus fungi parasiting the trunk and branches: *Eutypa lata, Phomopsis viticola, Stereum hirsutum, Cytospora vitis, Verticillium dahliae, Phoma uvicola, Diplodia viticola, Pestalozzia vitis, Sphaeropsis malorum* and telluric fungus which found on drying roots *Roesleria hypogea, Roesellinia necatrix.* Micromyceta involved in grapevine decline can attack during the autumn-spring period, when grapevine are in dormancy and temperatures are low for a more prolonged period. Evolution of the progress of early drying of grapevine, as results of the pathogenic action of lignicoulus fungi is influenced by the age of plants, pruning system and cultivar behavior.

KEYWORDS: *grapevine, biological decline, lignicoulus fungi*

INTRODUCTION

The cryptogamic diseases are almost entirely of multi-factorial etiology, the etiological agents are grouped in two large categories that are connected with the genetical or rised at the host plant level particularities and are influenced by external factors, among which are the ecological elements, the applied agrophytotechnical measures, so this means man's intervention over the plant in the created eco-system.

Early drying is a disease usually affecting grapevine, with the most obvious damaging. There is an wide information in the scientific literature of this field: in Australia – Adam (1938, 1952), Carter (1952, 1957, 1960, 1991), Uyemeto et Goheen (1985), Boubala (1982), Pearson (1983), Ramos (1980), Moor et Anderson (1988), Tarboh (1986), SUA - English (1976, 1985), France - Castelain (1964), Bonifaces (1980), Bonea (1980), Dubos et al. (1983), Switzerland – Boley (1977, 1985, 1986), Rumbos (1981), Italy - Cristianizo (1978), Hungary – Lehoszky (1981), Roznyai (1981), Germany – Gartel (1980), Greece - Chritzanidis (1976), Portugalia - Da Costa (1984), refered by Pearson and Goheen (1990).

In Romania, grapevine early drying has been described by Crişan (1963), Mărmurean et al. (1990), Rafailă and Oprea, Podosu et al. (1990, 1995), Tică et al. (1994), Ulea (1997). During the decade 1983 - 2006 the disease turned in calamity, leading to drying of more than 50% grapevine occurring in this country. This phenomenon subsequently diminished, due to resistant cultivars and to superior culture technologies, the extent of early of grapevine being 5-35 %.

MATERIAL AND METHOD

The researches regarding the apricot biological dieback in the Romanian ecological conditions were extended over a period of 10 years, including orchards from all crop zones. Biological samples were taken from the grapevine about to dry, out of which the lignicoulus fungi that play a role in the dieback process were isolated in lab conditions.

The biological parameters (temperatures, U%) were observed at the identified species by the current lab techniques (Tuit, 1968), regarding the fungi: *Eutypa lata, Phomopsis viticola, Cytospora vitis, Stereaum hirsutum, Roesleria hypogea.*

RESULTS

From investigations performed in vine plot of the country during 1996 – 2006, it was recorded that trees sensitive to early drying, 1-2 years before drying exhibited slight vegetation, sometimes leaves appeared before flowering or simultaneously, an abnormal fact in this species; leaves remained small, getting brown and drying, without falling. Fruits remained small, with sponging pulp. Sometimes wilt occurred in full season or a huge defoliation took place. Cross section in branches affected revealed browning of ligneous and cambium tissues.

From branches of trees starting decline, pathogenic fungi with lignicoulus behavior (fig. 1) have been isolated, such as: *Eutypa lata* (42,7%), *Phomopsis viticol* (24,8%), *Cyrospora vitis* (8,4%), *Phoma uvicole* (4,8%), *Diplodia vitis* (4,8%), *Stereum hirsutum* (3,8%), *Verticillium dahliae* (3,6%%), *Sphaeropsis malorum* (4,7%), *Roesleria hypogea* (1,2%), *Pestalozzia vitis* (1,2%).

The vine infested by fungi, beside a diminished crop, up to the vine perish, are not recommended to be used as graft because of the high risks of spreading the disease and shortening the young grape vine life.

In the area where have been signaled the grape dieback, the inauspicious pedo-climatic conditions for the plant development showed to be, a clay soil (Blaj, Jidvei, Sard vineyards in Alba, Minis in Arad, Valea Calugareasca, Cotnari) a weak fertilization soil with strong erosions (Diosig and Siria in Arad), chlorites phenomena showed in almost all observation stations, the replace of the classical vine pruning with raised stump one, sandy soil structure favoring an insufficient watering condition, industrial pollution, (Valea Calugareasca), prolonged drought (Murfatlar and Cotnari vineyards), vicinity of the forest (Sard in Alba, Gaiceanca in Bacau).

The inadequate phytosanitary measures also favored the vine weakening process. By the classical vine pruning named “ardelean circle” replaced with the “raised stump”, have produced large arm wounds (perfect enter gates for lignocolous fungi), and on the other hand eliminating the autumn buried labors, the grape vine remained exposed to the cold temperatures during the winter. The repeated overproductions and the heavy loaded fruits on vine arms, as well as the wrong application of phytosanitary treatments against leaves diseases (blight, mildew, rot) have also weakened the vine.

On these ecologic backgrounds the vine becomes vulnerable to the lignicoulus fungi contamination, especially to *Eutypa lata.* Once entered into the vine wood, the fungus has a bad influence on the plant metabolism, determining a premature vine arms drying.

The disease is greatly disseminated in almost all vineyards from the country, and associated with ecological weakening factors finished up the drying process. As a result of the observations carried on between 1983-1993 in the main vineyards, the disease was found present in the following regions: Transilvania plateau, Chrish hills, Dobrogea hillock, Danube terraces, Muntenia, Oltenia and Moldova hills.

The percentage of infested vine arms was between 3-96%; the disease was set up on the vine older than 6 years of age, especially on the vine in 10-15 years of age. The most damaged vine varieties (sorts) were the beverage ones, especially Italian Riesling (Diosig – Bihor and Gaiceanca - Bacau vineyards) where on the certain lots, all vine arms showed specific symptoms of eutipoze. Other varieties very much damaged of the disease include: in proportion of 60% Cabernet Sauvignon, over 30% White Feteasca, between 10 - 89% Black Babeasca (much severe in Nicoresti-Galati vineyard), about 63% Royal Feteasca, 30% Muscat Ottonel (especially in Minis vineyard) and between 3 – 33% the native varieties: Grasa de Cotnari, Galbena de Odobesti and Sarba (fig.2).

The grape vine desert fruit varieties most damaged were Chasslas doree (68% in Valea Calugareasca vineyard) and Afuz Ali (in south country vineyards).

The fungus *Phomosis viticola* have been found present on all observed dieback vines. The most frequent presence of this fungus was found on the varieties like: Feteasca alba (20%), Chasslas doree (38%), Cabernet Sauvignon (26), Italian Riesling (15,6%), Cardinal (50%), Clairete (21,6%), Merlot (7,8%), Pinot Noir (11,2%), in the following vineyards: Odobesti, Coretesti – Vrancea, Valea Calugareasca, Pietroasele Dragasani. The grape vine affected by excoriose was growing on the lots presenting a clay compact acid soil, watered in excess and industrially polluted.

The fungus *Cytospora vitis* was found in proportion of 8,4% especially in Alba country vineyards (Sard and Bucerdea).

The fungus *Cytospora vitis* was found present on the lots in the vicinity of the forest, in a soil fall short of humus, and very humid conditions during the whole vegetation period. The damaged varieties were: Royal Feteasca (Transilvania plateau), Italian Riesling (Arad), Merlot (Minis),Cabernet Sauvignon (Dragasani), Pinot noir (Iasi).

The fungus *Stereum hirsutum* was present in two vineyards: Blaj, Prahova Iasi and Ostrov – Constanta in proportion of 8%, in a soil showing a high concentration of calcium carbonic, ferric chloroses and strong erosions. The disease of the vine was identified by carpophores apparition, or the specific leaves colour.

Symptoms

Due to the ecologic weakening and biological drying of grape vine, the aspect of the infested vine show specific aspects for the pathogenic action of each lignicoulus fungi.

" Dead arm" induced by *Eutypa lata* fungus are developed on the vine infested in the previous spring, at the beginning of the vegetation period, which was 10-14 days delayed compared with the healthy vines period.

Vine arms in the draying process showed longitudinal splits, ulcers surrounding the wounds produced by cuttings. Into a longitudinal section were observed xilem necroses, at the primary infection places. The infested areas edges turn to brown-red up to purple. The damaged wood showed well defined limits. In an advanced developed stage, the secondary libero-ligneous vessels walls were destroyed, the tissue becoming very frail. This aspect can be proved, by a very easy broking the tendril of vine, without remaining fibers around the broken section.

Excoriose produced by the *Phomopsis viticola* was present at the begining of the vegetation period in the observed vineyards. In Valea Calugareasca vineyard, before the budding period, the tendrils of vine showed dark coloured spots, isolated or associated, about 0,5-2 cm x 0,3-1,0 cm, usualy plased at the base of tendril. The budding period was 12-14 days delayed, and the buds placed at the tendril of vine base were dyed. A thorough observation on the damaged tendrils of vine revealed that the first 3-4 basal buds were withered, and only the buds placed on the top of the tendril of vine being viable.

Roesleria hypogea infested Merlot grape vine variety, in Oltenia sandy soil vineyard, lead to the plants weakening until dying. The next spring the vegetation period for the infested vine were weak, the development of tendrils of vine was limited, the diameter of the limb leaf was lesser than 5 cm; at the flowering period the sterile flowers percentage was greater than usual and the clusters (bunches) aspect were denudate and the yield diminished. The dieback aspect of vine progressed year by year. At a thorough observation on a transversal section in the stock-vine it was revealed brown necrotic areas in the libero-ligneous tissue. In an advanced disease stage the roots dried, and in the excessive humid conditions the stock-vine developed apothecia.

Biological parameters

Temperature

The development of the *Eutypa lata* colonies on CGA medium, were strongly influenced by the level of temperature. The fungi colonies start to grow at +8^{o}C, showing a white, lax mycelium, yellow on reverse. After 15 days the colonies reach 20 mm diameter. In these conditions were not formed fructifications. By temperature rising, the colonies diameter increase, the mycelium become dense and takes a felt like aspect. The optimal temperature for fungi development was between 18-26^{o}C. At the mycelium surface appeared black crusts, where due to the abundant hyphen appeared the gray pycnidia. The pycnidium maturation takes 12-14 days long, and was included in a gelid mass that is eliminated via ostiols. Some of the cultures kept at 18^{o}C formed perithecium.

The maximum temperature for the development of *Eutypa lata* showed to be 34^{o}C. For this fungus 36^{o}C may be considered the lethal level (fig.3). The Petri plates colonies moving from 36^{o}C to the 22^{o}C, seized to develop the mycelium. The temperature level is determining the *Eutypa lata* germination of spores. In the amorphous stage pycnospora do not germinated on the medium culture (water, agar or malthoza 5%), unless were not exposed to the UV radiation for 15 minutes. Germination started two hours after irradiation, in a low percentage (2%), the length of the

germination tube was 1,5-2 µ. The germination percentage increased with the length of the irradiation exposure time, and as the result, after 24 hours the spores germination was 46% and germination tube length reached 52-65 µ (fig.4).

Ascospora present in the teleomorph stage germinated in the usual conditions of water-agar medium. The germination starts at 10°C and kept going till 30°C, the optimum temperature was between 18-28°C. The maximum germination temperature may be considered 32°C, and lethal temperature 34°C.

Relative atmospheric humidity

The observations carried on "in vitro" experiments, showed that the development of *Eutypa lata* fungi colonies are influenced by the levels of the relative atmospheric humidity. The values under 30,5 RH seized the mycelium development. The increased RH values between 39% RH to 74% RH, without fructification showed that mycelium become abundant. Morphological changes occur at humidity level greater than 80%. The fungus fructified, and after 4 weeks showed pycknidia, maturing after 7-11 days (fig, 5).

pH values

The observed lignicoulus fungi (*Eutypa lata, Phomosis viticola, Cytospora vitis, Roesleria hypogea*) developed in acid (4) to basic (11) pH growing medium. At the acid level (4) the fungi showed limited growth colonies with a low vegetative mass. From the low acide up to strong basic pH (5,5-11) the fungi developed colonies with an abundant vegetation mass, specific for each species. Some of them fructified forming pycknidia (*Eutypa lata, Phomosis viticola, Cytospora vitis*) (fig.5).

The energetic resources

Carbon source influence

The results obtained „in vitro" experiments, established that the carbon was the most important element for the lignicoulus fungi colonies development. Component of the molecule carbohydrates, this element can be asimilated in diferent ways, depending on the source, and the carbon chemical link (connection) in that molecule. The carbon is slighty hydrolised from the monosaharides like: glucosis, fructosis, trehalosis, ribosis, arabinosis, levulosis and rhamnosis. Among disaharides are melobiosis and zaharosis and from polysaharides are celulosis and lignine. The colonies growth medium including these carbon resources developed at greatest dimensions and fructified well. *Eutypa lata, Phomosis viticola, Cytospora vitis* showed a weaker development on sorbosis growth medium, also *Eutypa lata* and *Roesleria hypogea* on galactosis medium, all fungi on maltosis, *Cytospora vitis* on celulosis, and *Eutypa lata* and *Cytospora vitis* on inulinae. *Eutypa lata* grows well on manitosis, galactosis, levulosis, melobiosis, and starch culture medium, but do not fructify. *Phomosis viticola* fructified on all culture medium were fungus mycelium developed.

In the absence of carbon, the development of *Eutypa lata, Phomosis viticola* fungi colonies were inhibited, *Roesleria hypogea* and *Cytospora vitis* showed a lax mycelium, in 13 and respectively 30 mm diameter, without fructifying.

Light influence

The influence of light on the lignicoulus fungi development was tested „in vitro" experiments. The CGA growth medium on Perty plates with fungi colonies were exposed to a permanent source of light, to light/dark alternate (8 hs with 16 hs or 12 to 12 hs) and to continous dark. The fungi colonies in permanent dark condition developed the best vegetation mass; the colonies showed a dense, felt like aspect. But were developed weak fructifications. *Eutypa lata* and *Phomosis viticola* fructified very well in light dark alternate of 8 to 16 hs.

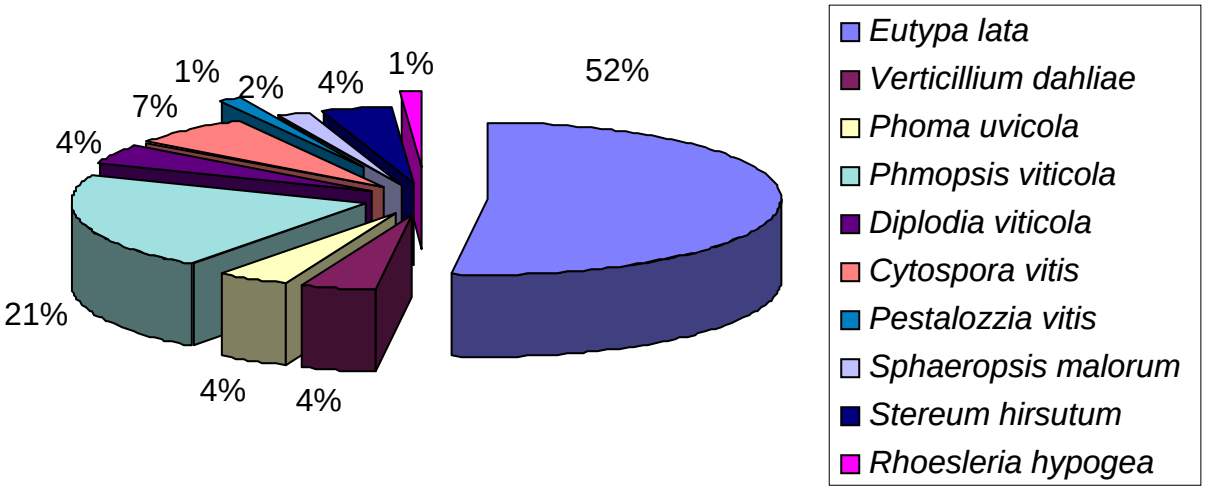

Fig. 1 The frequency of lignicoulus fungi associated to vine dieback among in Romanian vineyards

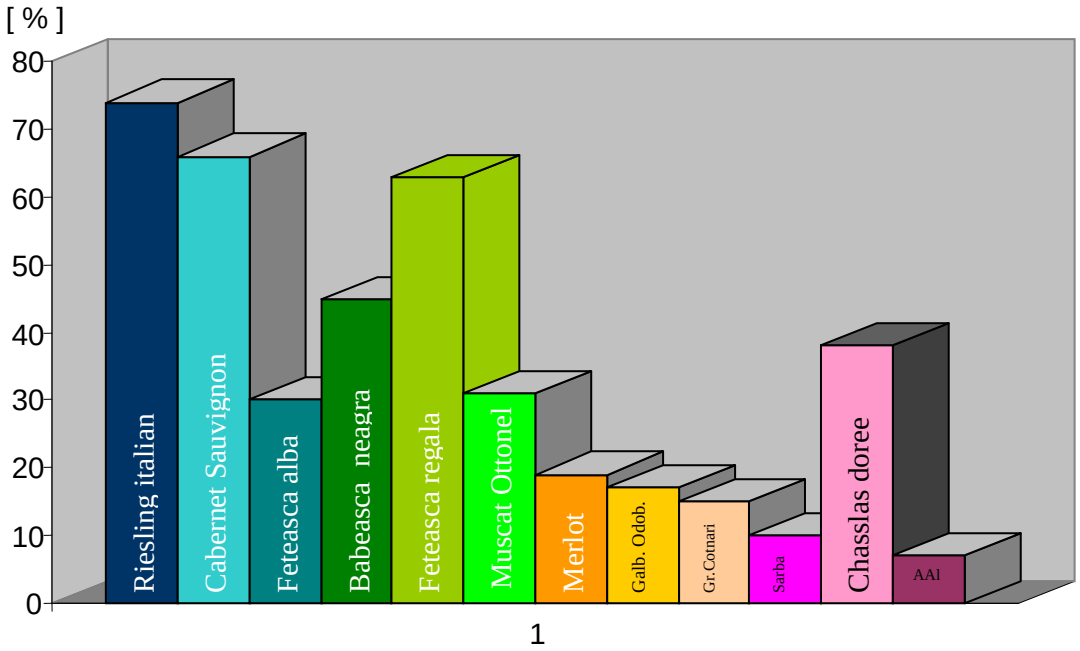

Fig. 2 The behaviour answer of varieties in Romanian vinegards under the influence of pathogenic fungus *Eutypa lata*

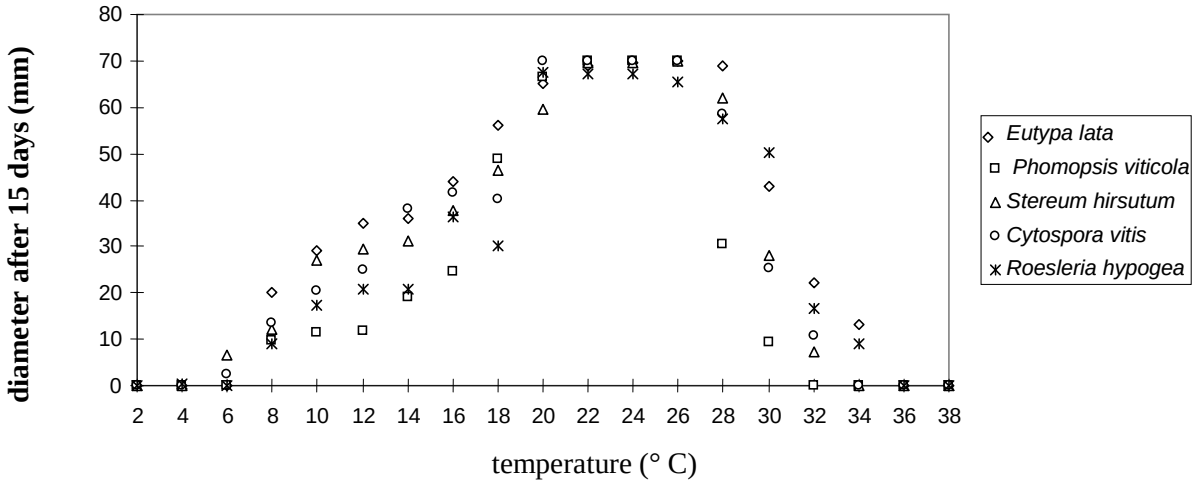

Fig. 3 The influence of temperature on the development of fungi responsible for vine dieback

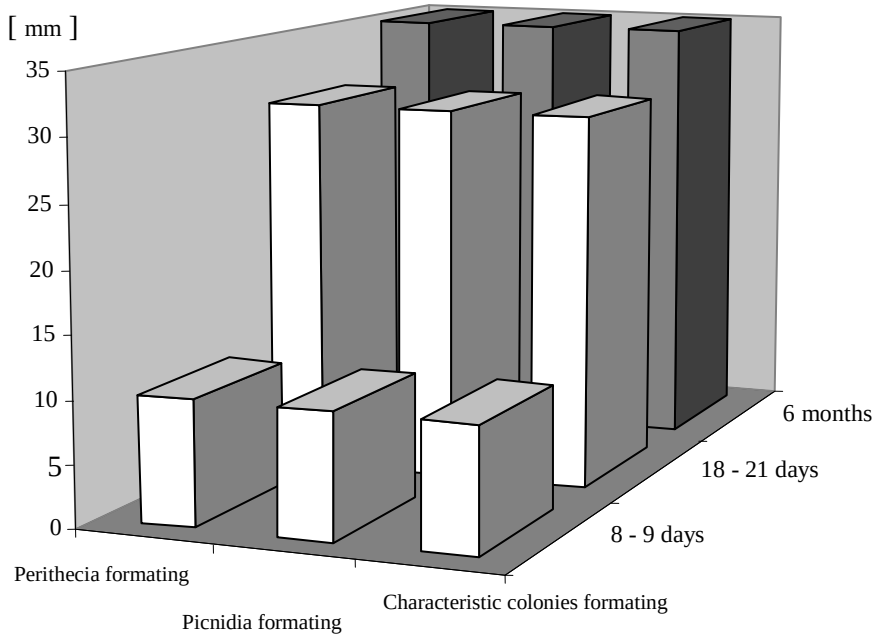

Fig. 4 Fructification formation on the culture medium

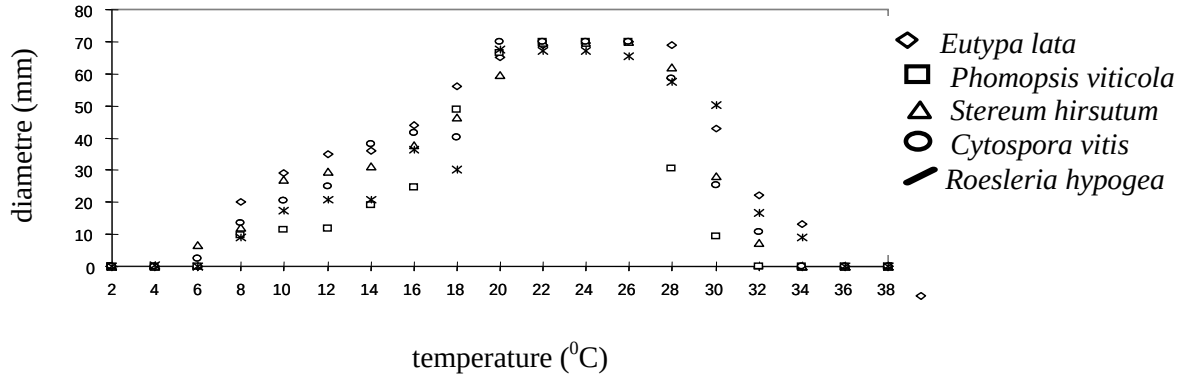

Fig. 5 The influence of pH range on the development of lignicoulus fungi

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