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On the factors that may have influenced the esca epidemic in Tuscany in the eighties

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Summary. Tuscany is one of the viticultural regions in Italy most severely affected by esca. The epidemic started in 1986, and at the time it was explained as being caused by the great frost that hit Tuscany and many other parts of Italy the year before. Now, several years later, with a clearer understanding of the disease in the light of more recent research, we re-examined the cause of the epidemic and ask which factors could have been more likely explanations of that epidemic – factors such as the chemicals then used to control other vine diseases in Italy (active ingredients, dosages, times of application), or, contemporary, methods to produce propagation material, changes in cultural practices, or the rootstock that were then used in new vineyards and so on. We found little evidence that there was a direct correlation between the cold damage suffered in 1985 and the increase in esca disease later. We suggest that contributing factors causing the epidemic included the selective activity of fungicides used in the vineyard, which may have led to a build-up of inoculum of the fungi causing esca, and poor quality planting material arising from large scale propagation, resulting in vines more susceptible to weak pathogens such as *Phaeomoniella chlamydospora*.

Key words: grapevine, brown wood streaking, fungicides.

Until the mid-1980s, esca of grapevine in Tuscany as in the rest of Italy was a disease with which viticulturists had been familiar for centuries: it affected vines as they aged, and was in older vines more or less inevitable. After that date, however, esca gradually began to assume epidemic proportions, and became less a disease of older vines only but also increasingly affected younger vines (Larignon and Dubos, 1997; Mugnai *et al*., 1999).

The increase in esca incidence seems to have started from l986, and followed the very severe frost that occurred in Tuscany and other parts of Italy in the winter of 1984–1985 (with temperatures reaching -23°C in January 1985). One and a half years later, in the summer of 1986, esca reached an incidence of 15–20% in 15–20-year-old vineyards and also affected relatively young vines (7–10 years). The phenomenon was reported with due prominence in the Florentine newspaper *La Nazione* (15 August 1986) by U. Bruni, who also mentioned a surge of apoplexy that occurred in June 1986, after the second half of May of that year had been characterised by hot and dry weather. At the time it seemed obvious to attribute both this esca epidemic and the apoplexy upsurge to the damage

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the vines had suffered during the exceptionally cold winter of 1984–85: frost injury, it was thought, had weakened the vines and favoured invasion by *Phellinus igniarius,* the fungus which at the time was considered, together with *Stereum hirsutum*, to be the main cause of esca. Today, however, in the light of a better understanding of esca acquired over the last decade, it is perhaps possible to suggest some different causes.

In 1986 and following years, there was a dramatic increase in esca in almost all parts of Italy, including those that had not been affected by the great frost of 1985. Moreover, the incubation time of esca, from initial infection to symptom expression, although not yet precisely ascertained, certainly cannot be telescoped into the year and a half or so that intervened between the great frost of 1985 and the epidemic of 1986. Most importantly, it is a peculiar characteristic of esca that in some vines the external, visible symptoms of the disease may disappear completely during one or more successive growing seasons; those vines are during that time still diseased but are quite undistinguishable from healthy vines (Surico *et al*., 2000). This means that the vines found infected in 1986 could well have been diseased already in the year or indeed years preceding.

Lastly, high incidences of esca have also been found in vineyards established after 1985 and that did not experience the 1985 frost. It therefore seems evident that the high esca incidence of 1986 and later years was not caused, or not only caused, by the frost of 1985, but was the consequence of an increase in infection that had already started in the years preceding 1985. The question that then remains is: what caused those earlier infections that did not manifest themselves until 1986? To furnish an answer to this question the changes that Tuscan vineyards underwent from the 1960s to the 1980s were examined, with special emphasis on those changes that influenced, or may have influenced, esca development (Table 1). The following report considers some of the more important of these changes.

Production of healthy plant material

Until the 1950s or somewhat later, viticulture in Tuscany was with few exceptions a local undertaking. New vineyards were generally established with plant material derived from nearby fields in the same vineyard, collected in August when esca leaf symptoms were most evident, and avoiding any plants with such symptoms. The worker who selected the plant material was usually the same who later grafted the scion on the rootstock cuttings, rooted in the field the year before. This procedure of field grafting may not have been ideal from a plant health point of view, but the care with which it was done, the modest size of the vineyards and the skill and care of the grafters gave a reasonable certainty that the operations involved in establishing a new vineyard would be carried out well. But starting at the end of the 1960s, vineyard operations began to develop, becoming more modern, but also perhaps more chaotic. Two successive campaigns promoting vineyard renewal and the establishment of new fields, supported by contributions from the European Community, led to a significant increase in the area under vine cultivation, which in Tuscany passed from 11,743 ha in 1960 to 70,508 ha in 1990. As a result there was a substantial increase in the demand for propagating material, and the production of grafted rooted cuttings also expanded enormously, at the expense of field grafting, which was in any case more expensive. Such a vast increase in the demand for rooted cuttings, which had never been seen before, probably caught

Table 1. A list of factors that may be significant in esca development. Factors accompanied by a question mark (?) have not yet been shown to be relevant.

Quality of propagation material
Latent infections in propagation material
Rootstock characteristics (?)
Cultivar
Training system
Pruning
Wound protection
Discarding pruning residues (?)
Chemical treatments (?)
Climate
Vine age
Physiological state of the vine
Soil
Overall vigour (?)
Irrigation
Topography (?)
Spacing (?)
Exposure (?)

nursery operators by surprise, and they were forced to produce as much plant material they could, of whatever type, and including almost certainly also shoots that came from esca-infected vines. In justification of nursery operators it should be said that in those years a body of plant-health rules did not exist, and that they could not have been expected to realise that vine shoots that looked healthy could come from esca-diseased plants and contain fungal propagules that would spread the disease, which at that time was thought to be a type of rot affecting old vines only.

New vineyards continued to be planted throughout the 1970s. In the Chianti area, 72.4% of all vineyards existing in 1985 had been planted in only 9 years, from 1968 to 1976. Table 2 shows the area under grapevine cultivation in Tuscany in 1990 with some of the cultivars most common at that time. As a result, in 1985, the year of the great frost, about 70% of all Tuscan vineyards were between 10 and 20 years old (and more than 80% were 10 years or older), the optimal vineyard age for esca symptoms to appear. And this is exactly what happened. In the 1970s esca was still only an occasional phenomenon in Tuscany and elsewhere in Italy (most vineyards being fairly young), but in the early 1980s, reports of esca infections were already becoming more numerous (here it should be mentioned that in 1979 there had been another frost in Tuscany, though less severe than that in 1985), probably in vineyards that had been established in the 1960s, and then, as has been said, esca became particularly common after 1985, affecting the many vineyards that had been planted in the boom years of vineyard establishment.

In the early 1990s there was another campaign in Tuscany, designed to promote the replacement of old vineyards that had been partly destroyed by esca. This time it was in some cases possible to examine the health of batches of rooted cuttings before outplanting, focusing particularly on the occurrence of dark streaks colonised by *Pa. chlamydospora,* a condition called dark wood streaking of rooted cuttings (Mugnai *et al*., 1999). Although the production of propagating material in nurseries is much better now than it was, the percentage of rooted cuttings infected with this fungus was still found to be rather high, frequently around 20–30% but going up to 80% in some batches (Bertelli *et al*., 1997; Surico *et al*., 1997; Sidoti, 2001; Zanzotto *et al*., 2001). These examinations were carried out in 1997–2000; in years to come the health of the vineyards planted with these cuttings will be studied.

At this point it should be said that so far the only concern has rightly been to produce nursery material that is guaranteed free from viruses, and thanks to a notable effort of clonal selection, viral diseases now no longer represent a serious problem in vineyards. Nevertheless, the wood vessels of vines harbour many micro-organisms (Tables 3), most of which are saprophytes, but some also pathogens (Bell, 1985; Minervini and Bisiach, 1988; Contesini, 1996; Mugnai *et al*., 1996; Esseln and Weltzien, 1997; Larignon and Dubos, 1997). Among

Table 2. Distrubution of Tuscan vineyards by vineyard age group (No. of ha) for some of the main cultivars grown in the Chianti Classico area (Tuscany, Italy) in 1990. As a result of the extensive plantation in the 1960s, 84.4% of the vineyards of the cultivars listed were planted in the twenty years from 1960 to 1980 (IV Censimento Generale dell'Agricoltura, 1990, In: Periccioli, 1997).

Grape cultivar	Period of vineyard establishement				
	Before 1960	1961–1980	1981–1987	1988–1990	Total
Brunello (Sangiovese Grosso)	43.32	379.59	134.75	114.08	671.74
Vernaccia di S. Gimignano	6.14	446.12	246.53	44.16	742.95
Malvasia bianca lunga	162.73	1,516.31	115.98	18.46	1,813.48
Canaiolo nero	148.26	1,971.06	125.95	23.02	2,268.29
Trebbiano toscano	292.62	3,163.60	242.79	39.63	3,738.64
Sangiovese	924.10	15,802.21	1,266.76	339.09	18,332.16
Total	1,577.17	23,278.89	2,132.76	578.44	27,567.26

Table 3. Endophytic fungi and bacteria isolated from diseased and healthy grapevines by at least one of the following authors: Bell, 1985; Minervini and Bisiach, 1988; Contesini, 1996; Mugnai *et al*., 1996; Esseln and Weltzien, 1997; Larignon and Dubos, 1997.

Acremonium alternatum
Acremonium berkeleyanum
Acremonium kiliense
Acremonium murorum
Acremonium sp.
Alternaria spp.
Aphanocladium spp.
Aschochyta sp.
Aspergillus flavus
Aspergillus niger
Aspergillus sp.
Aureobasidium sp.
Beauveria bassiana
Bispora sp.
Botryosphaeria obtusa
Botrytis cinerea sp.
Camarosporium flaccidum
Cephalosporium sp.
Ceratocystis stenocreas
Chaetomella
Chetomium globosum
Cladosporium cladosporioides
Cladosporium elatum
Cladosporium sp.
Coniothecium sp.
Coniothyrium sp.
Cylindrocarpon destructans
Cylindrocarpon obtusisporum
Dendrophoma pleurospora f. *vitigena*
Discosia sp.
Epicoccum purpurascens
Epicoccum sp.
Eutypa lata
Fomitiporia sp.
Fusarium oxysporum
Fusarium spp.
Gliocladium roseum
Gliocladium sp.
Graphium sp.
Hainesia sp.
Hendersonula sp.
Humicola brunnea
Humicola fuscoatra var. *longispora*
Humicola grisea var. *thermoidea*
Lecythophora sp.
Mucor sp.
Mycelia sterilia
Paecilomyces farinosus
Papulospora sp.
Penicillium sp.
Pestalotia coccoli
Pestalotia sp.
Pestalotia truncata
Phaeoacremonium aleophilum
Phaeoacremonium chlamydosporum
Phellinus igniarius
Phellinus punctatus
Phialophora malorum
Phialophora melinii
Phialophora parasitica
Phialospora sp.
Phoma eupyrena
Phoma spp.
Phomopsis sp.
Phomopsis viticola
Pyrenochaeta sp.
Rhizopus sp.
Scytalidium sp.
Sesquicillium candelabrum
Sphaeropsis malorum
Sphaeropsis sp.
Spicaria sp.
Sporotrix schenkii
Stachybotrys sp.
Stemphylium sp.
Stereum hirsutum
Tolypocladium cylindrosporum
Tolypocladium geodes
Torula sp.
Trichoderma hamatum
Trichoderma harzianum
Trichoderma koningii
Trichoderma longibrachiatum
Trichoderma piluliferum
Trichoderma pseudokoningii
Trichoderma sp.
Trichoderma viride
Valsa sp.
Verticillium psalliotae
Verticillium sp.

Gram negative bacteria
Comamonas terrigena
Enterobacter agglomerans
Enterobacter cloacae
Klebsiella ozaenae
Klebsiella pneumoniae
Moraxella bovis
Pantoea agglomerans
Pseudomonas cichorii
Pseudmonas corrugata
Pseudomonas marginalis
Pseudomonas putida
Pseudomonas spp.
Pseudmonas syringae pv. *lacrymans*
Pseudomonas syringae pv. *tabaci*
Rahnella aquatilis
Xanthomonas campestris pv. *dieffenbachiae*
Xanthomonas campestris pv. *flaccumfaciens*

Gram positive bacteria
Bacillus fastidiosus
Bacillus insolitus
CDC group 2
Clavibacter michiganensis
Curtobacterium flaccumfaciens pv. *flaccumfaciens*
Curtobacterium pusillum
Rhodococcus luteus
Staphylococcus spp.

Some of the fungal species have been recently reclassified to new genera and species.

these last are *Agrobacterium tumefaciens* (which, on grapevine, is known to be mainly represented by strains of *A. vitis*), the causal agent of crown gall of grapevine, and those fungi, most notably *P. chlamydospora*, that are associated with various types of deterioration in vines: brown wood streaking, Petri disease, and esca.

In this situation it may be a good idea to start thinking also about protecting rooted cuttings against infection with *A. vitis* and *P. chlamydospora*.

Esca and young vines

Today esca is much more frequent in young vines (less than 10 years old) than it used to be. Though esca of young vines was not unknown to growers in the past, it was rare. The French researchers who first studied esca properly stated that when a young vine became infected it was always a vine that had been outplanted to a site from which adult vines that had died of apoplexy had been uprooted (Ravaz, 1922). Quite apart from the accuracy of these statements, which dates back some 80 years ago, the question remains why so many vines now show symptoms already at 5–6 years, or somewhat later, or, indeed, even earlier. One obvious reason why vines show symptoms of esca earlier is that they become infected earlier. This earlier infection could be due to an abnormal increase of fungal inoculum in the vineyard environment, or to vineyards being planted with nursery material that is already infected. In the nursery the mass of inoculum could have increased in response to the various control measures adopted from time to time against various other diseases (see next paragraph), this may have made esca more frequent, first in the nursery and then in the vineyards. The possibility that infected propagating material was marketed by the nursery could be linked to the great demand for such material in the mid-1980s and later, just when esca was becoming much more common. This last possibility gains added weight from the fact that, as has now been shown, the conidia of some important esca agents, *P. chlamydospora* and *Phaeoacremonium aleophilum,* are able to survive undetected in the xylem vessels of vines, and hence in the propagating material collected from the canes of those vines (Bertelli *et al*., 1997; Edwards *et al*., 2004; Whiteman *et al*., 2004).

Treatment with fungicides

The chemical control of grapevine diseases is directed against three main ones: downy mildew (*Plasmopara viticola)*, powdery mildew (*Uncinula necator)* and grey mold (*Botrytis cinerea*). To these can be added, at least in Italy, esca and, more rarely, escoriosis.

Downy mildew was until shortly after 1945, controlled basically by Bordeaux mixture or other copper-based compounds applied to the entire plant, and providing continuous protection or as required by the downy mildew life cycle. These agents were followed by the dithiocarbamates: zineb, ziram and maneb and, towards the late 1960s and early 1970s, by captan and folpet, and then the other dithiocarbamates: mancozeb, propineb, metiram, etc.

Control of powdery mildew in the 1960s and 70s relied on 8–9 sulphur treatments per growing season in areas where disease pressure was high: a first application at the end of April or in the first week of May, a second about the phase of fruit set, a third in mid-June, and 5 or 6 more during the summer depending on the severity of the infection.

Against grey mold the recommended control compounds were TMTD and captan, then folpet or dichlofluanide (a first application shortly after colour-change, a second in late June and a third between the tenth and the twentieth of August; after this date growers relied on the secondary effect of copper compounds.

Escoriosis was dealt with by winter treatments with dinitroorthocresol (DNOC) or sodium arsenite; and, lastly, esca was combated by sodium arsenite: 1250 g hl^{-1}, 150–170 l ha^{-1} in winter within 15 days of pruning and at least 21 days before shoot development.

In summary, then, the three main diseases of grapevine were all countered with broad-spectrum contact sprays, while escoriosis and esca were countered with winter applications of DNOC or sodium arsenite. This last compound was banned in 1977. With the disuse of sodium arsenite and later also DNOC, prohibited in European countries from 1999 on (Council Directive 91/414/EEC of 15 July 1991), winter sprayings of grapevine were discontinued.

From the first half of the 1980s the cytotropic and systemic fungicides began to be successful on the market, and the control effort began to switch from broad-spectrum contact sprays to pathogen-

specific and site-specific products (Tables 4). Dinocap joined sulphur in the control of powdery mildew and was followed by triarimol, the first endotherapic fungicide, and in 1980 by triadimefon. After that other compounds inhibiting sterol biosynthesis were approved for use: diclobutrazole, fenarimol, penconazole, propiconazole, triadimenol and others, and more recently compounds containing other chemical groups (strobilurin) and quinoxyfen (phenoxyquinoline).

Against grey mold the dicarboximides (iprodione, vinclozolin, procymidone) began to be applied in 1979–1980, followed at a later date by anilinopyrimidines (pyrimethanil, mepanypirim, cyprodinil mixed with fludioxonil) and fenhexamid.

Downy mildew always received the greatest quantities of fungicide. This disease began to be treated with Curzate (cymoxanil) alone or mixed with traditional fungicides (copper-based, mancozeb, folpet) in 1980; this was the first curative fungicide. After 1980 various products were introduced: metalaxyl, benalaxyl, oxadixyl and fosetyl-Al (mixed with mancozeb, folpet, or copper), while strobilurin and similar products were used from the second half of the 1990s onwards.

By 1982 31 active ingredients were approved for use on grapevine, including folpet, alone or in combination with other fungicides: captafol, sulphur, mancozeb, benomyl, copper oxychloride, thiophanate methyl, cymoxanil, metalaxil, zineb, maneb, thiram. From this it will be seen that the combination of folpet and copper (which has recently been declared by Boubals in France [2002] to be highly effective against esca and Eutypa dieback) was in common use for a considerable time, and also that, from the late 1970s/early 1980s onwards, the use of organic contact fungicide sprays gradually began to decline, being replaced by curative and selective fungicides, generally with a narrower spectrum of action even though they were often used in mixtures with copper or with organic contact fungicide sprays. Is it a legitimate assumption that the novel fungicides now being used in viticulture, applied as they are in different ways and sometimes at different times during the growing season, are causing the increases in the inoculum of the fungi implicated in esca? This assumption could be correct, but it is at present very difficult to prove.

Grapevine growing practices

Cultural practices have also changed over time: vines are now planted closer together (facilitating multiplication of the pathogen or pathogens and disease spread), there is an increased use of machines for harvesting, pruning and removal of side-shoots (with increased risk of injury to the vines) and pruning methods are also different: in Tuscany cordon training and pruning has given way to Guyot (simple or double). As regards the mode of pruning, a French research report from 1921 found that a vineyard trained to free-standing espalier and pruned with double Guyot had an incidence of esca of 15–20%; in a vineyard trained and pruned with simple Guyot the incidence was 10–25%, whereas in vineyards pruned with Gobelet or Robat it was 0–5%, and in vineyards with vertical cordon pruning only 0–1%. It seems logical to deduce from this that prun-

Table 4. List of the fungicides admitted in Italy for the control of grapevine diseases in 1983. In bold, systemic fungicides, in italics, localized systemic fungicides, in romans contact fungicides.

Barium polysulphide	Dinocap	**Fosethyl-Al**
Benomyl	Dinitroortocresole (DNOC)	Procymidone
Brandol	*Dodina*	Propineb
Captafol	Etem	Sulphur
Captan	**Fenarimol**	**Thiophanate methyl**
Carbendazim	Folpet	Thiram
Chinosol	Iprodione	**Triadimefon**
Chlorothalonil	Mancozeb	**Triforine**
Copper	Maneb	Vinclozolin
Cymoxanil	**Metalaxyl**	Zinc sulphate (in combination)
Dazomet (soil application)	Metiram	Zineb
Dichlofluanide	Nabam	

ing systems that involve more extensive cutting are at a greater risk of esca. A more widespread use of cordon training with spur pruning at the expense of Guyot (the former involves larger pruning cuts, at least when the spurs or the cordon are being renewed) may therefore have favoured the spread of esca.

Other factors

As long as wine-growing in Tuscany was the activity, and the fortune, of relatively few growers, there was a kind of natural selection that led to only the best soils being chosen for this valuable plant, but when viticulture was thrown open to a much greater number of farmers, vineyards began to be established even in soils that were less than ideal for that purpose in terms of soil composition and texture, geographic location and field placement. All this was made possible by using types of rootstock, such as for example Kober 5BB, different from those in use in Tuscany in the 1950s and 1960s. When a scion of one variety is grafted onto the rootstock of another, a composite plant is created with physiological characteristics that may differ widely from either of the original constituent plants. Rootstocks also differ in the characteristics they confer on the entire plant (Marchi, 2001), such as plant vigour, growth rate, adaptability to different soils, capacity to absorb minerals and water, resistance to active calcium, sensitivity to soil exhaustion, resistance to viruses carried by nematodes and, of course, to *Phylloxera* and so on. It is therefore clear that a given rootstock gives a vine scion – though the vine still remains the same – particular physiological characteristics, and it is well-known that vine physiology, in ways not yet ascertained, affects esca symptom expression. Otherwise the fluctuations in the foliar symptoms of esca in individual vines could not be explained, nor the fact that in some vine-growing areas esca leaf symptoms do not appear even though the trunk is certainly colonised by esca fungi.

As regards the soils used for vineyards, studies exploring the link between soil type and esca have shown that esca symptom expression is facilitated in heavy moist soils (this may also be due to the peculiar root-microflora in these soils, which affects the absorption capacity of the vines) (Corti *et al*., 2004) while in less steep soils esca incidence tends to be lower (Surico *et al*., 2000).

In conclusion it seems a reasonable supposition that the use of some rootstocks, or planting vineyards in less suitable soils may favour esca development.

Conclusions

The ideas presented in this article are the outcome of a discussion held by the authors, which include some plant pathologists attached to the Tuscan Regional Government. The discussion arose from a report published in France by Denis Boubals on the unexpected effectiveness of copper fungicides against esca. In this article, published in *Progrès Agricole et Viticole* (2002), Boubals wrote that between 1978 and 1986, when he was at the Laboratoire de Recherches Viticoles de l'École Nationale Supérieure Agronomique de Montpellier, he had found that the fungicide Vifolcuivre, a compound containing copper (15%) and folpet (25%), was highly effective against both *Eutypa lata,* the causal agent of Eutypa dieback, and *Stereum hirsutum,* which at the time was thought to be the main cause of esca wood rot. On the basis of his findings Boubals advised some viticulturists who experienced problems with Eutypa dieback in their vineyards to uproot all Eutypa dieback-infected vines, and to treat pruning wounds of healthy vines with 5 kg ha^{-1} Vifolcuivre. Some of these growers were in any case already using this fungicide against downy mildew all the year round, and also after harvesting by machine. Some years have passed since Boubals's original findings and he has recently been back to have another look at the vineyards which had had problems with Eutypa dieback and esca before. This is what he found:

- in the south of France most vineyards that are at least 20 years old and that are planted with Cabernet-Sauvignon and Sauvignon have a high percentage of dead vines;
- vineyards with Cinsaut treated annually with Vifolcuivre on their crowns and pruning wounds showed no signs of either esca or Eutypa dieback;
- a 25-year-old vineyard of 5 ha planted with Cabernet-Sauvignon and treated with Vifolcuivre ever since it was established, likewise did not show any apparent signs of Eutypa dieback or esca, while a 27-year-old vineyard of the same cultivar located 2 km away and not so treated had lost several vines (although Boubals does

not say how these last vines had been protected from downy mildew if they were not treated with Vifolcuivre).

On the basis of these findings Boubals in his article renewed his suggestion that vines can be protected from Eutypa dieback, esca, or any other wood-rotting fungi, by treating them with Vifolcuivre immediately after pruning, during the growing season and after any grape harvesting machines have passed. This finding seemed little short of sensational considering that there is currently such an urgent need for a means to control esca. Boubals is a highly respected and able researcher of grapevine diseases; however, his study lacked some of the elements necessary for a proper assessment of the diseases he had examined, and folpet was used in Italy for quite a long period in the 1960s and 1970s against downy mildew, without ever being reported to be able to solve the esca problem.

This article has presented some suppositions regarding esca which it is hoped will be of use to researchers currently trying to understand that disease, and to technicians employed to make wine-growing a success in countries new to this crop. As regards the findings of Boubals in his study, we are convinced that, at least on the strength of our present knowledge, Vifolcuivre used alone cannot replace sodium arsenite as a means to control esca. Nevertheless, in the current emergency created by the need to find an agent to control esca, Boubals's study requires to be verified with care, if only for the strategic approach it may offer to the problem of esca.

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