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Further observations on the factors related to the low productivity of Picolit giallo[1])

by

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Weitere Untersuchungen über die Ursachen der geringen Ertragsleistung von Picolit giallo

Zusammenfassung. — Bei zwei Klonen der Rebsorte Picolit giallo mit unterschiedlicher Ertragsleistung (Picolit 31 A.A.U. mit hohen und Picolit F. mit schwachen Erträgen) wurden mittels Licht- und Elektronenmikroskop die Mikrosporogenese sowie die Entwicklung des Tapetums untersucht. Ferner wurde bei der Pfropfkombination Picolit F. auf Picolit 31 A.A.U. als Unterlage untersucht, wie sich die Pfropfung auf die Pollenentwicklung des Edelreises auswirkte. Die Befunde bei Picolit wurden mit Beobachtungen bei der Sorte Verduzzo friulano verglichen.

Die Mikrosporogenese läuft in allen untersuchten Fällen bis zum Stadium der reifen, Pollenkörner enthaltenden Anthere normal ab. Die Antheren der schwachtragenden Sorte Picolit F. können sich jedoch bezüglich ihres Inhaltes unterscheiden: Sie können mit zahlreichen kugeligen acolporaten Pollenkörnern gefüllt oder fast leer sein oder auch zahlreiche acolporate, aber kollabierte Pollenkörner enthalten.

In der Entwicklung des Tapetums wurde bei der schwachtragenden Sorte Picolit F. eine frühzeitige Degeneration der Zellen festgestellt; diese waren leer oder fehlten im Tetradenstadium sogar schon völlig. Dies hat zur Folge, daß die jungen Pollenkörner dieses Klons teilweise ohne die Wandenzyme sporophytischer Herkunft sind.

Da alle untersuchten Klone von Picolit giallo selbststeril sind und Trauben nur bei Fremdbestäubung entstehen, wurde versucht, die Anzahl keimfähiger tricolporater Pollenkörner in der Luft zweier Picolit-Weingärten mit unterschiedlicher Ertragsleistung zu bestimmen. Hierbei stellte sich heraus, daß die Anzahl keimfähiger Pollenkörner in dem reichtragenden Weingarten viel höher war als in der schwachtragenden Rebanlage.

Introduction

In previous works (Lombardo *et al.* 1976, 1978, Cargnello *et al.* 1980), we observed that, while pollen grains of *Vitis vinifera* cvs. normally appear tricolporated, pollen grains of the cv. Picolit giallo are viable, but without furrows and germinative pores. Such pollen grains seem therefore to be unable to germinate. This condition recurs in all the examined clones of Picolit (both in main branches and feathers), whatever their provenance and rootstock may be (Cargnello *et al.* 1980). Therefore, the absence of pores seems to be a genetic character of the cv. Picolit, that appears consequently self-sterile. While this character keeps always unchanged, the productivity of the main branches in all the examined clones of Picolit is highly variable (Cargnello *et al.* 1980); the feathers, however, always show normal productivity. Thus, it must be assumed that other factors influence the productivity rate of the various clones, as for instance the gametophyte development or the stigmatic receptivity (Carraro *et al.* 1979).

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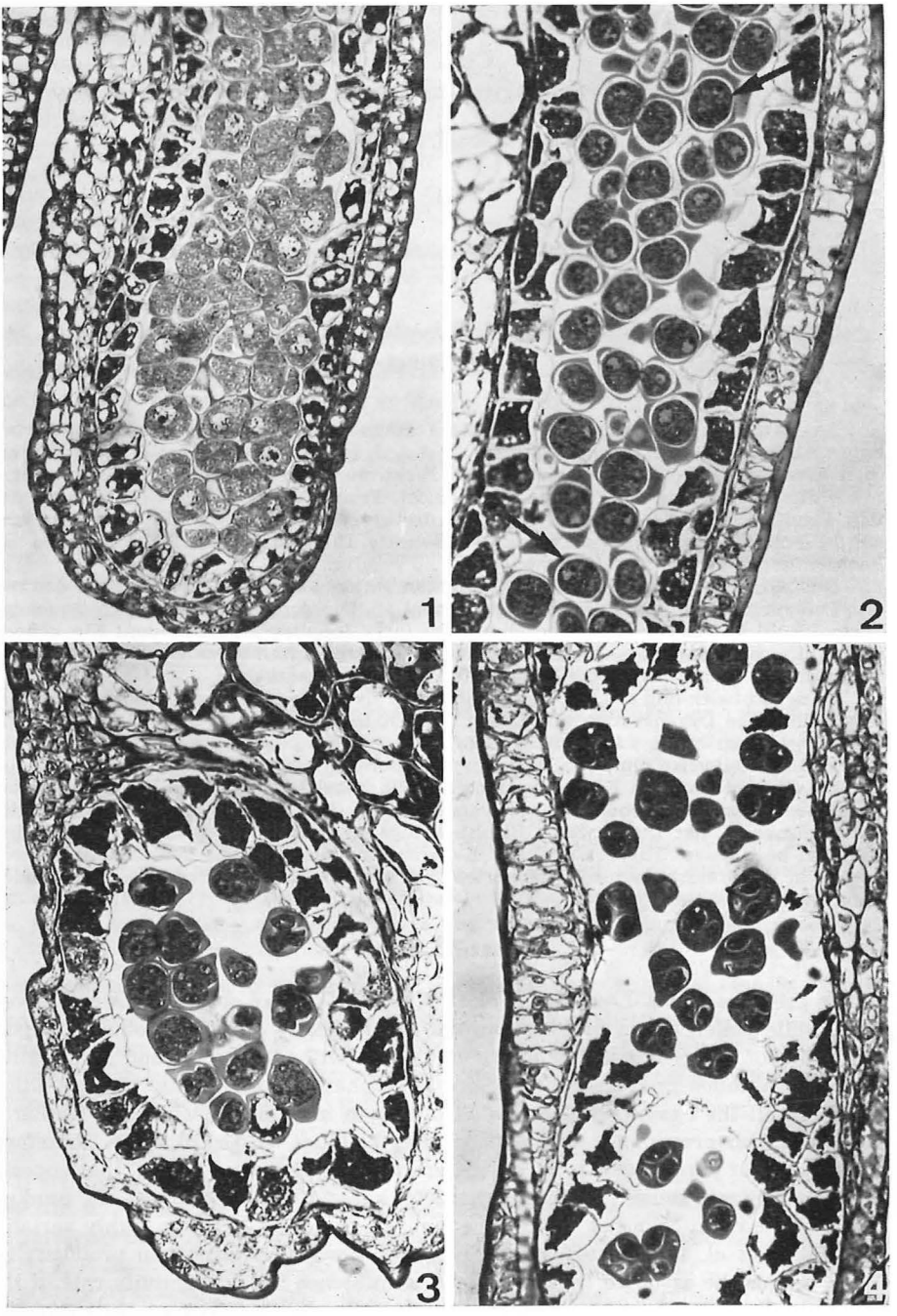

Fig. 1: Longitudinal section of a young anther of Picolit 31 A.A.U. The anther cavity is filled with pollen mother cells showing dense cytoplasm and large nuclei, surrounded by normally developed tapetal cells. × 350.

The studies reported in this work concern the evaluation of the whole amount of pollen grains that can presumably reach the stigmatic surface of the flowers of Picolit. More precisely, we tried to determine the amount of germinable pollen grains present in the air of vineyards with different productivity and the amount of pollen grains produced in the anthers of Picolit that will reach the stigmatic surface at the fall of the calyptra. In fact, it is known that well developed pollen grains, viable and germinable, do not germinate neither on the stigmatic surface nor *in vitro* if they are not present in sufficient amount (Howlett *et al.* 1975).

Further investigations were carried out on the microsporogenesis in order to detect the pollen morphology in subsequent developmental stages and the relationship between pollen grains and tapetal cells. As already known, the tapetum nourishes the young microspores during their development and, also after the tetrads release, the tapetal cells supply the microspores with sporopollenin and wall enzymes (Knox and Heslop-Harrison 1969, Heslop-Harrison *et al.* 1973, 1975, Heslop-Harrison and Heslop-Harrison 1973, Knox and Howlett 1973, Knox *et al.* 1975). Moreover, the proteins released by the pollen wall on the stigmatic surface are essential to pollen tube emission.

Materials and methods

Microsporogenesis

Anthers have been examined in various developmental stages, taken from flowers of the main branches of: a) the high producing Picolit 31 A.A.U.[1]), b) the low producing Picolit F.[1]), c) the normal producing cv. Verduzzo, d) Picolit F. grafted on Picolit 31 A.A.U.

The samples have been fixed *(in loco)* and embedded, following procedures of electron microscopy earlier described (Lombardo *et al.* 1976, 1978). Ultrathin sections

[1]) Picolit 31 A.A.U. derives from the clone 31 A.A. and is cultivated in the wine farm G. B. Cragnolini near Cividale del Friuli (Udine), Picolit F. grows in the wine farm L. Felluga near Oleis (Udine) (see Lombardo *et al.* 1978).

Fig. 2: Beginning of meiosis in an anther of Verduzzo. The pollen mother cells are embedded in a thin callose sheet and always surrounded by a well developed tapetum. It is already possible to observe some meiocytes with two nuclei (arrows). × 400.

Fig. 3: Tetrad stage in an anther of Picolit 31 A.A.U. The microspores are embedded in a thick callose matrix and surrounded by a continous layer of large tapetal cells with dense cytoplasm. × 400.

Fig. 4: Tetrad stage in an anther of Picolit F. The tapetal layer appears discontinuous, in some portions absent and with highly collapsed cytoplasm. × 350.

Abb. 1: Längsschnitt durch eine junge Anthere von Picolit 31 A.A.U. Sie enthält Pollenmutterzellen mit dichtem Cytoplasma und großen Kernen, die von normal entwikkelten Tapetumzellen umgeben sind. 350 ×.

Abb. 2: Beginnende Meiose in einer Anthere von Verduzzo. Die Pollenmutterzellen sind in eine dünne Kallosehülle eingebettet und stets von einem wohlentwickelten Tapetum umgeben. Einige Meiocyten mit zwei Kernen sind bereits sichtbar (Pfeile). 400 ×.

Abb. 3: Tetraden in einer Anthere von Picolit 31 A.A.U. Die Mikrosporen sind von einer dicken Kalloseschicht umhüllt und von einem ununterbrochenen Tapetum umgeben, dessen Zellen ein dichtes Cytoplasma zeigen. 400 ×.

Abb. 4: Tetraden in einer Anthere von Picolit F. Die Tapetumschicht ist unterbrochen, und ihre Zellen zeigen ein stark kollabiertes Cytoplasma. 350 ×.

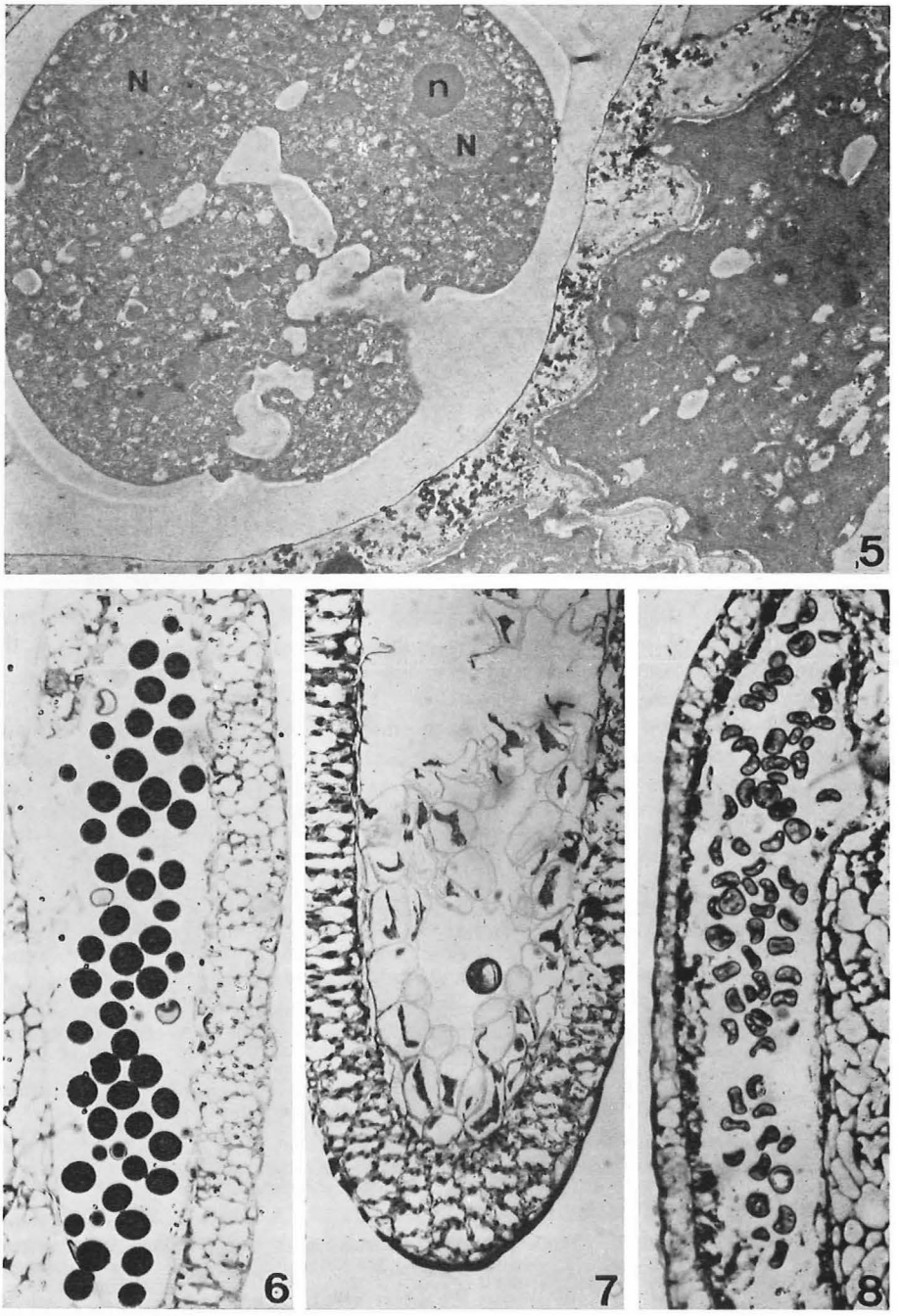

Fig. 5: Electron micrograph of a young tetrad and a tapetal portion of Picolit F. grafted on Picolit 31 A.A.U. The microspores of the tetrad appear plenty of organelles with evident nuclei (N) and nucleoli (n) and show a limiting wall not yet completed. The tape-

were cut with an LKB Ultrotome III, stained with lead citrate and examined in a Hitachi H 11 B electron microscope. Semi-thin (1 μm) sections were cut with a glass knife, stained with toluidine blue and observed with a light microscope.

Evaluation of the pollen amount

Glass slides coated with silicon have been exposed in two different vineyards with high and low productivity, respectively: the wine farm G. B. Cragnolini and the wine farm L. Felluga (see LOMBARDO *et al.* 1978). Several slides have been distributed in the vineyards randomly, to evaluate the whole pollen amount present in the air; others have been situated at increasing distances from vines with colporated pollen grains (the cv. Picolit giallo and the cvs. Verduzzo friulano or Tocai friulano are planted together), to estimate the amount of available pollen grains in relation to the distance from the pollination source. After 2 d the slides were collected and numbered with a light microscope, the tricolporated pollen grains adhering on a surface of 400 mm^2.

Results

The subsequent microsporogenesis steps of anthers have been examined in different developmental stages beginning with the pollen mother cells.

The pollen mother cells in all the examined Picolit clones, as well as in Verduzzo, show normal aspect, dense cytoplasm, and are so numerous as to fill all the anther cavity (Figs. 1 and 2). These cells are always surrounded by their nourishing tissue, the tapetal cells, which have also a dense cytoplasm and are normally structured (Figs. 1 and 2).

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tal cells also contain numerous organelles; roundish electron-dense bodies are to be seen along the tapetal portion facing the anther cavity. × 3,800.

Fig. 6: Mature anther of Picolit F. containing numerous acolporated and roundish pollen grains. The tapetal layer has disappeared. × 200.

Fig. 7: Mature anther of Picolit F. containing only a single, acolporated, roundish pollen grain. × 200.

Fig. 8: Mature anther of Picolit F. containing numerous, collapsed, acolporated pollen grains. × 150.

Abb. 5: Elektronenmikroskopische Aufnahme einer jungen Tetrade und eines Teils vom Tapetum des Klons Picolit F. bei Pfropfung auf Picolit 31 A.A.U. Die Mikrosporen der Tetrade enthalten zahlreiche Organellen, sie zeigen große Kerne (N) sowie Nucleolen (n). Ihre Wand ist noch unvollständig. Auch die Tapetumzellen enthalten zahlreiche Organellen; gegen die Innenseite der Anthere hin sind rundliche elektronendichte Partikel sichtbar. 3.800 ×.

Abb. 6: Reife Anthere von Picolit F. mit zahlreichen acolporaten und runden Pollenkörnern. Die Tapetumschicht ist verschwunden. 200 ×.

Abb. 7: Reife Anthere von Picolit F. mit einem einzigen acolporaten, runden Pollenkorn im Inneren. 200 ×.

Abb. 8: Reife Anthere von Picolit F. mit zahlreichen kollabierten acolporaten Pollenkörnern. 150 ×.

At the tetrad stage, the normally developed microspores appear embedded in a thick callose matrix (Fig. 3). Their cytoplasm is dense, highly colourable with evident nucleus and nucleolus. The tetrads are always numerous and degenerated microspores are only seldom to be observed. This condition is a constant feature of all the examined Picolit clones as well as of Verduzzo. At this stage, however, the tapetum appears like a continuous layer of large cells filled with a dense cytoplasm, both in Picolit 31 A.A.U. and in Verduzzo (Fig. 3); in Picolit F., the tapetal layer is discontinuous, in some portions even absent, in others formed by cells with highly collapsed cytoplasm or completely empty (Fig. 4). In contrast to this, Picolit F. grafted on 31 A.A.U. in the vineyard G. B. Cragnolini at the tetrad stage shows a tapetum built by large cells with dense, highly colourable cytoplasm, like those of Picolit 31 A.A.U. These cells, observed in the electron microscope, appear plenty of organelles with large nuclei; numerous roundish electron-dense bodies are to be seen along the portion of their walls facing the anther cavity (Fig. 5).

In mature anthers still closed, the pollen grains show different aspects in all the examined samples. In Verduzzo, the pollen grains are tricolporated and in large quantity. In Picolit 31 A.A.U., the pollen grains are without furrows and germinative pores, showing a thick and continuous wall; they are present in considerable amount and fill a large portion of the anther cavity. In Picolit F., however, the mature anthers have different histological aspects. Some anthers are filled with numerous acolporated, round pollen grains (Fig. 6); others contain the same kind of roundish acolporated pollen grains in extremely reduced amounts up to one or two grains per anther (Fig. 7). It is also possible to find anthers filled with

Table 1

Total amount of germinable pollen grains in the examined vineyards
Gesamtmenge keimfähiger Pollenkörner in den untersuchten Rebanlagen

Vineyard	Number of tricolporated pollen grains
G. B. Cragnolini	54**
L. Felluga	4

** According to the statistical analysis of the variance the difference between the two averages is highly significant.

Table 2

Germinable pollen amount in relation to the distance from the pollinating source (vineyard G. B. Cragnolini)
Anzahl keimfähiger Pollenkörner in Beziehung zur Entfernung von der Pollenquelle (Rebanlage G. B. Cragnolini)

	Distance (m)				
	0.25	1.40	5	25	50
Number of tricolporated pollen grains	144	34	22	11	6

acolporated and highly collapsed pollen grains (Fig. 8). In Picolit F. grafted on Picolit 31 A.A.U. cultivated in the vineyard G. B. Cragnolini, the mature anthers are always filled with numerous roundish pollen grains, acolporated and with dense cytoplasm.

When estimating the tricolporated germinable pollen grains present in the air of the two examined vineyards, the following observation was made: In the vineyard G. B. Cragnolini, whose high productivity is well known, their amount is nearly 13 times that in the low producing vineyard L. Felluga (Table 1). Moreover, always regarding the vineyard G. B. Cragnolini, the amount of tricolporated germinable pollen grains rapidly decreases as the distance from the pollination source enlarges from 0.25 to 1.4 m; at distances of 5, 25, 50 m, the decrease of germinable pollen grains is less remarkable (Table 2).

Discussion

The course of the microsporogenesis seems completely normal in the first steps in all the examined cultivars; pollen mother cells and tapetal cells show a dense cytoplasm and numerous organelles, as it is the rule in *Vitis vinifera* (Kozma 1974). These tissues of the anther appear uniform in all the examined samples until the tetrad stage; the tetrads are always numerous with a thick callose layer and only seldom some of them appear degenerated. In the subsequent developmental stages, the normal producing cultivars possess anthers filled with numerous pollen grains of normal appearance, while in Picolit F., with extremely reduced productivity, many anthers are nearly deprived of pollen grains or partially empty. This remarkable degeneration following the tetrad stage seems probably independent of genetic factors and somewhat related to nourishing or environmental conditions. In fact, graftings of Picolit F. on Picolit 31 A.A.U., cultivated in the vineyard G. B. Cragnolini, show normal development of the anthers, which are filled with numerous acolporated pollen grains. Picolit F. cultivated in the wine farm L. Felluga, besides this peculiar lack in pollen grains inside the mature anthers, also shows an early degeneration of more or less extended tapetal portions (in the graft, the tapetum behaves like in Verduzzo).

Now, it is known that the tapetal cells act as nourishing tissue towards the pollen grains and also provide for the formation of the exine (external layer of the microspore wall) and of peculiar proteins that will be distributed among the bacula of the exine. These proteins are of great importance to the interactions between pollen grains and stigmatic papillae and are concerned in the regulation of the pollen tube growth. Pollen grains that mature in the anthers of Picolit F. are extremely reduced in amount compared with the other cultivars and should also be lacking in those proteins generally present among the exine bacula. Therefore, the reduced number of pollen grains able to reach the stigmatic surface and the possible lack of these in recognition proteins could explain their incapability to act as "pollen mentor" towards the few colporated pollen grains coming from normal producing cultivars. This condition could represent a further reason of the very reduced productivity of the Picolit vineyards cultivated in this region.

This hypothesis is also supported by the results exposed in the tables. The amount of germinable pollen in the low producing vineyard L. Felluga is extremely reduced and, therefore, inadequate to overcome the self-sterility of the cultivar.

The problem becomes worse considering that the evaluation of the pollen amount has been carried out on a slide surface of 400 mm², the stigmatic surface, however, where these pollen grains will adhere measuring only about 2 mm². Moreover, pollen amount rapidly decreases with the increasing distance from the pollination source.

Summary

Using light and electron microscopy, we have studied the microsporogenesis and the tapetal development in two different clones of Picolit giallo, the high producing Picolit 31 A.A.U. and the low producing Picolit F. Further, the influence of grafting Picolit F. on Picolit 31 A.A.U. rootstock was examined. All the results have been compared with those observed in the cv. Verduzzo friulano.

The microsporogenesis proceeds normally in all the examined samples until the stage of mature anther filled with pollen grains. Only the low producing Picolit F. shows anthers with different contents; some are filled with numerous roundish acolporated pollen grains, others are nearly empty, others contain numerous acolporated pollen grains of collapsed aspect.

As regards the tapetal development, an early degeneration was observed in the low producing clone Picolit F., whose tapetal cells are empty or even absent already at the tetrad stage; thus, the microspores in this clone will be partially deprived of the wall enzymes of sporophytical origin.

Besides, since all the clones of Picolit giallo are self-sterile and the production of grapes is exclusively due to cross-pollination, we tried to evaluate the amount of germinable, tricolporated pollen grains present in the air of Picolit vineyards at different productivity. The observations have shown that the amount of germinable pollen grains was much higher in a high producing vineyard than in a low producing one.

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