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Istituto di Botanica dell'Università di Milano
Istituto Sperimentale di Viticoltura di Conegliano
Centro di Microscopia Elettronica del Politecnico di Milano, Italia

Ultrastructure of pollen of Vitis vinifera L. cv. "Picolit giallo" and its behaviour in experiments of self- and cross-pollination

by

Giuliana Lombardo, Luisa Carraro, G. Cargnello and Maria Bassi

Die Feinstruktur der Pollenkörner von Vitis vinifera L. cv. Picolit giallo und ihr Verhalten bei Versuchen zur Selbst- und Fremdbestäubung

Zusammenfassung. — Die *Vitis-vinifera*-Sorte Picolit giallo liefert nur geringe Erträge. Um hierfür verantwortliche Abnormitäten aufzufinden, wurde die Feinstruktur von Pollenkörnern und Narbe mit Hilfe der Raster- und Transmissionselektronenmikroskopie untersucht. Der Protoplast der Pollenkörner wirkt normal, aber die Pollenwand ist von kontinuierlicher Beschaffenheit und zeigt keine Furchen oder Keimporen wie bei typischen Pollenkörnern. Die Narbe macht einen normalen Eindruck. Sehr wahrscheinlich verhindert die nicht unterbrochene Pollenwand die Pollenkeimung und ist somit eine mögliche Ursache der geringen Fertilität von Picolit giallo. Diese Vermutung wird durch Versuche zur Selbst- und Fremdbestäubung gestützt, wobei Picolit-Pollen nie keimte, auch dann nicht, wenn er auf Narben anderer Rebensorten gebracht wurde; umgekehrt keimte der Pollen solcher Sorten regelmäßig, wenn er auf Picolit-Narben übertragen wurde.

Introduction

The productivity of the different varieties of grape vines is highly variable, and among the varieties with low productivity "Picolit giallo" is one with the lowest. In fact, generally only a few berries develop out of an inflorescence. This extremely low yield is due to the fact that fertilization occurs in a very few number of flowers (Cosmo and Sardi 1962, Candussio 1966—69). The low productivity of "Picolit giallo" was experimentally increased by pollinating its female flowers with pollen from other cultivars, such as "Pinot grigio" or "Verduzzo friulano", but experiments of self-pollination always gave negative results (Cargnello 1976). This suggested that the low yield of Picolit might be due to some impediment in pollen germination.

Since some of the mechanisms which plants possess to control pollination (Bateman 1952, Lewis 1954, Brewbaker 1957, Valdeyron 1972) are based on pure morphological grounds, we wanted to see if at the basis of the low incidence of fertilization of "Picolit giallo" there were alterations of either pollen or stigma structures. We confronted these structures with those of the pollen and stigma of other varieties with normal incidence of fertilization ("Pinot grigio" and "Verduzzo friulano"). Besides, we studied pollen behaviour in experiments of self-pollination and cross-pollination.

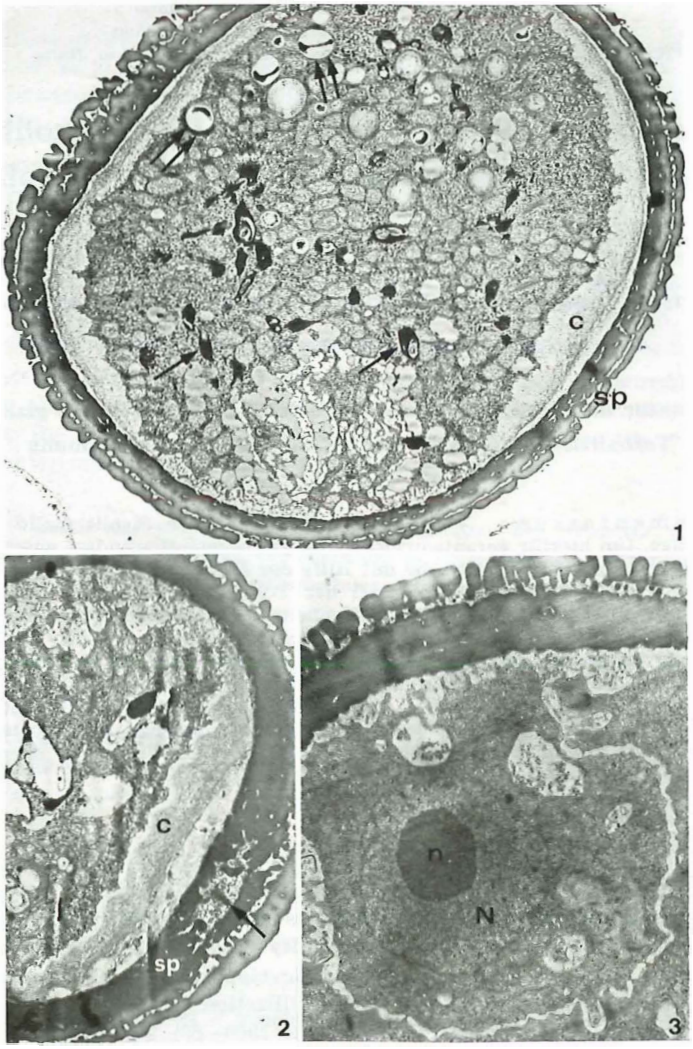

Fig. 1: TEM micrograph of a Picolit pollen grain. The sporopollenin wall (sp) is continuous and compact; the cytoplasm is dense and full of organelles. The proplastids have a dark matrix (arrows) and often contain starch granules (double-arrows). c = callose layer. ×5,250.

Fig. 2: TEM micrograph of a Picolit pollen grain. The sporopollenin wall (sp) shows a rarefied area (arrow); the underlying callose layer (c) forms a lenticular thickening. ×5,625.

Fig. 3: TEM micrograph of a Picolit pollen grain, showing the generative cell delimited by a layer made presumably of callose. The generative nucleus (N) is clearly visible. n = nucleolus. ×7,500.

Abb. 1: TEM-Aufnahme eines Pollenkornes von Picolit. Die Sporopolleninwand (sp) ist kompakt und ohne Unterbrechungen; das Cytoplasma ist dicht und von Organellen erfüllt. Die Proplastiden besitzen eine elektronendichte Matrix (Pfeile) und enthalten oft Stärkekörner (Doppelpfeile). c = Kalloseschicht. 5.250×.

Materials and Methods

Transmission electron microscopy (TEM). — Fully developed pollen grains of "Picolit giallo", "Pinot grigio" and "Verduzzo friulano", taken from the inflorescences of the main branches, were fixed in cacodilate-buffered 3% glutaraldehyde, pH 6.9, at 4 °C for 24 h. After washing in 0.1 M cacodilate buffer, the specimens were postfixed in cacodilate-buffered 1% osmium tetroxide for 2 h, at 4 °C, dehydrated in ethanol, and embedded in araldite. When in 75% ethanol, the samples were impregnated with uranyl acetate in semisaturated solution.

Fully developed stigmas of the three varieties after experimental self- and cross-pollination were treated in the same way.

Ultrathin sections were cut with an LKB Ultrotome III, stained with lead citrate and examined in a Hitachi H11B electron microscope.

Light microscopy. Semi-thin (1 μm) sections of the araldite-embedded stigmas were cut with a glass knife, stained with toluidine blue and viewed with a light microscope.

Scanning electron microscopy (SEM). — Both the mature anthers and the pollinated stigmas were fixed immediately after sampling in phosphate-buffered 3% glutaraldehyde, pH 6.9, for 24 h. They were then rinsed and exposed to vapours of 1% osmium tetroxide for further 24 h, dehydrated in ethanol and critical point dried with CO_2. The samples were coated with carbon and gold in a Balzers BAE 121 coating unit provided with a tilting-rotating stage, and examined with a Jeol JSM U3.

Results

Morphology of Picolit pollen and stigma. — When observed by SEM, pollen grains appeared subspherical but more or less collapsed, and did not show the three furrows typical of *Vitis* pollen grains (Fig. 4). In ultrathin sections, they appeared spherical. Their sporopollenin wall was continuous, compact, with a limited number of sculptures (Fig. 1). It showed no thinnings, such as are generally seen at the level of the furrows. In one case only, did it present a rarefied area (Fig. 2). The callose layer was generally of uniform thickness, with the exception of rare cases in which it formed lenticular thickenings (Fig. 2).

The vegetative cell showed a very dense cytoplasm containing an extraordinary great number of mitochondria and proplastids (Fig. 1). The latter had a dark stroma and often contained starch granules. The generative cell was always visible, delimited by a callose wall (Fig. 3).

The stigma had a normal aspect, with well developed papillae (Fig. 6).

Morphology of pollen and stigma of Pinot and Verduzzo. — When observed by SEM, the pollen grains appeared subspherical but tri-

Abb. 2: TEM-Aufnahme eines Pollenkornes von Picolit. Die Sporopolleninwand (sp) besitzt eine verdünnte Zone (Pfeil); darunter bildet die Kalloseschicht (c) eine linsenförmige Verdickung. 5.625×.

Abb. 3: TEM-Aufnahme eines Pollenkornes von Picolit. Die generative Zelle wird von einer Schicht umhüllt, die vermutlich aus Kallose besteht. Der generative Kern (N) ist deutlich sichtbar. n = Nucleolus. 7.500×.

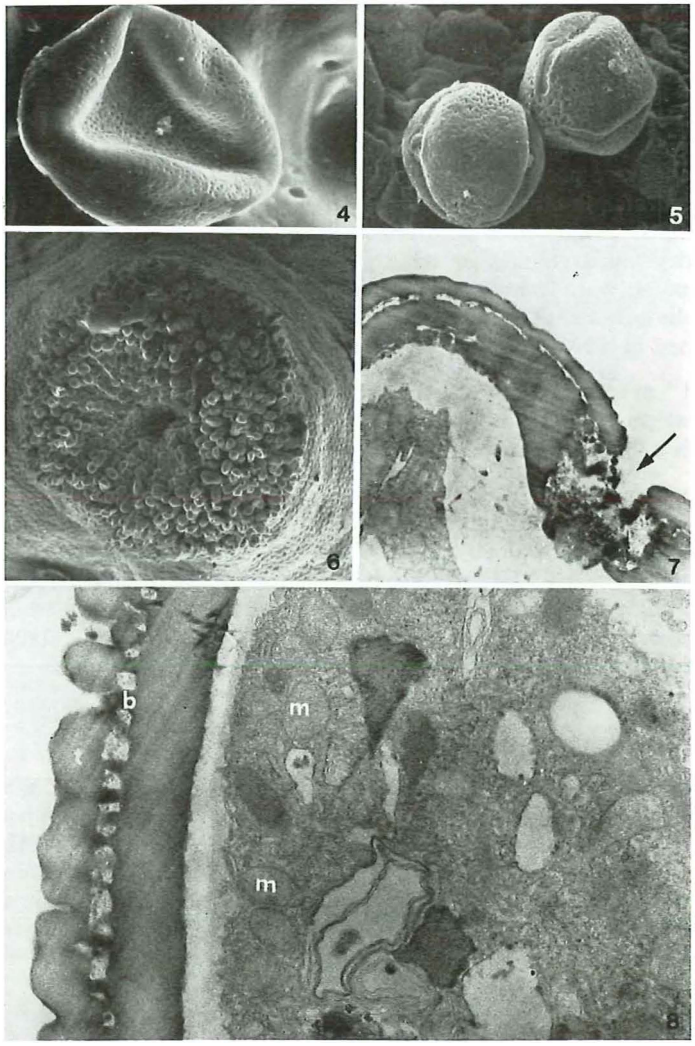

Fig. 4: SEM micrograph of a Picolit pollen grain. It appears subspherical but collapsed. ×3,750.

Fig. 5: SEM micrograph of Verduzzo pollen grains. They appear subspherical and trilobated owing to the presence of three furrows in their wall. ×1,875.

Fig. 6: SEM micrograph of Picolit stigma. Well developed papillae surround the opening of the stylar channel. ×2,250.

Fig. 7: TEM micrograph of a portion of the pollen wall of Pinot. The sporopollenin wall is interrupted at the level of a germinative pore (arrow), while the underlying callose appears thickened. ×9,000.

Fig. 8: TEM micrograph of a Pinot pollen grain. The sporopollenin wall shows evident bacula (b) and tegmina (t). The cytoplasm is dense and contains many organelles. m = mitochondria. ×15,000.

Abb. 4: SEM-Aufnahme eines Pollenkornes von Picolit. Seine Form ist sphäroidisch, aber eingedrückt. 3.750×.

lobated due to the presence of three furrows on their surface (Fig. 5). In ultrathin sections, the wall appeared thick, with bacula and tegmina well evident (Fig. 8), but often interrupted at the level of the furrows (Fig. 7). The callose layer was continuous and particularly thick at the level of the wall interruptions. The cytoplasm of the vegetative cell appeared less dense and contained fewer organelles than that of the vegetative cell of Picolit pollen. The generative cell was always visible, delimited by a callose wall.

The stigma aspect was identical to that of Picolit.

Self-pollination. — In the case of Picolit, no germination of pollen grains was observed. In the other two cases (Pinot and Verduzzo), the emission of pollen tubes was frequently seen both in semi-thin sections and in SEM or TEM preparations (Figs. 9, 10, 11 and 12). In ultrathin sections of pollinated stigmas, pollen tubes were seen among the stigma cells (Fig. 12). The cytoplasm of the pollen tubes was dense, with many organelles (Figs. 11 and 12).

Cross-pollination. — When Pinot pollen grains were deposited on Picolit stigmas, they germinated regularly, by emission of a pollen tube clearly visible both in semi-thin sections (Fig. 14) and in TEM preparations (Fig. 15).

When Picolit pollen was deposited on Pinot stigmas, in one case a small protuberance was detected in one grain (Fig. 13), but true pollen tubes were never seen. In ultrathin sections the aspect of the pollen grains was similar to that described for the pollen grains in the anthers.

Discussion

The content of Picolit pollen grains is apparently normal, and, in any case, if a slight difference is found between the ultrastructure of their vegetative cell and that of the vegetative cell of Pinot and Verduzzo, it consists in a higher content of cytoplasmic organelles in the former. The presence of a generative cell is always recognizable, so that it seems that we are in the presence of a normal male gametophyte, at least as regards its protoplast. Actually, when Picolit pollen grains are stained with Alexander method to check their vitality, they stain normally (Cargnello and Candussi 1976). On the contrary, the wall does not seem perfectly normal, because it is of uniform thickness and aspect along the whole surface of the pollen grains, without those areas of rarefaction that are normally seen in pollen grains and that generally correspond to the sites of emission of pollen tubes (germinative pores). The only visible structure that may suggest the formation of germinative pores is a thickening of the callose layer in restricted areas, which, how-

Abb. 5: SEM-Aufnahme von Pollenkörnern der Sorte Verduzzo. Sie sind sphäroidisch und besitzen in ihrer Wand drei Furchen. 1.875×.

Abb. 6: SEM-Aufnahme einer Narbe von Picolit. Die Öffnung des Griffelkanals ist von gut entwickelten Papillae umgeben. 2.250×.

Abb. 7: TEM-Aufnahme eines Ausschnittes aus der Pollenwand von Pinot. Die sporopolleninwand ist in Höhe einer Keimpore unterbrochen (Pfeil), während die darunterliegende Kalloseschicht verdickt ist. 9.000×.

Abb. 8: TEM-Aufnahme eines Pollenkorns von Pinot. Die Sporopolleninwand zeigt deutliche Bacula (b) und Tegmina (t). Das Cytoplasma ist dicht und enthält zahlreiche Organellen. m = Mitochondrien. 15.000×.

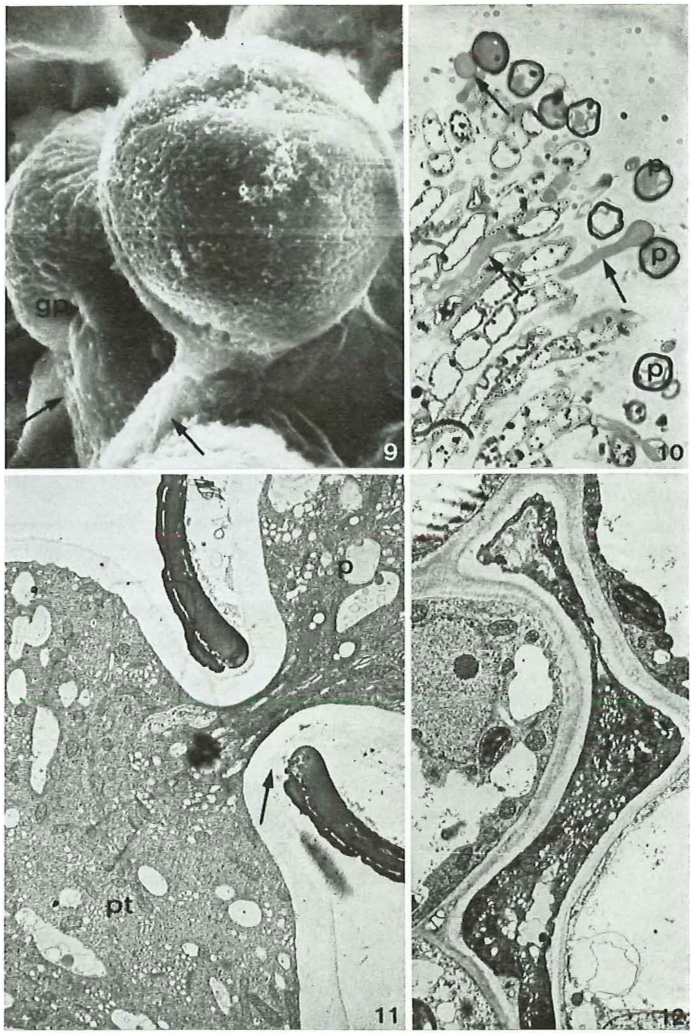

Fig. 9: SEM micrograph of germinating pollen grains of Pinot after self-pollination. Pollen tubes (arrows) are clearly visible. gp = germinative pore. ×3,750.

Fig. 10: Light micrograph of a 1 μm section of Pinot stigma after self-pollination. Many pollen grains (p) have germinated and the pollen tubes (arrows) are visible among the stigma papillae. ×240.

Fig. 11: TEM micrograph of a pollen grain of Pinot after self-pollination. The emission of a pollen tube is clearly visible at the level of a germinative pore (arrow). p = pollen grain; pt = pollen tube. ×5,250.

Fig. 12: TEM micrograph of a pollen tube insinuating among the stigma cells. The tube cytoplasm is very dense. ×5,250.

Abb. 9: SEM-Aufnahme keimender Pollenkörner von Pinot nach Selbstbestäubung. Die Pollenschläuche sind deutlich sichtbar (Pfeile). gp = Keimpore. 3.750×.

Abb. 10: Lichtmikroskopische Aufnahme eines 1 μm dicken Schnittes durch eine Narbe von Pinot nach Selbstbestäubung. Zahlreiche Pollenkörner (p) haben gekeimt, und die Pollenschläuche (Pfeile) sind zwischen den Narbenpapillen sichtbar. 240×.

Abb. 11: TEM-Aufnahme eines Pollenkornes von Pinot nach Selbstbestäubung. Der Austritt eines Pollenschlauches durch eine Keimpore ist deutlich sichtbar (Pfeil). p = Pollenkorn; pt = Pollenschlauch. 5.250×.

Abb. 12: TEM-Aufnahme eines Pollenschlauches, der zwischen die Zellen der Narbe eindringt. Das Schlauchcytoplasma ist sehr dicht. 5.250×.

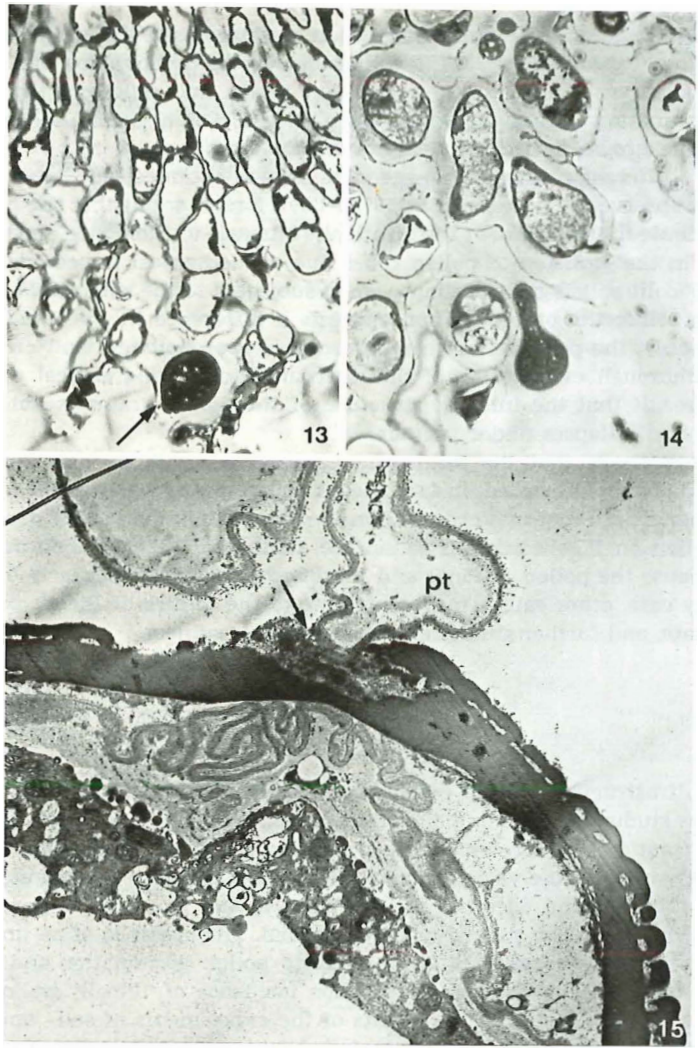

Fig. 13: Light micrograph of a 1 µm section of Picolit pollen grain after cross-pollination on Pinot stigma. This is the only case in which an initial budding could be seen in a pollen grain (arrow). ×375.

Fig. 14: Light micrograph of a 1 µm section of Pinot pollen after cross-pollination on Picolit stigma. The pollen grain has germinated and the pollen tube insinuates among the stigma cells. ×525.

Fig. 15: TEM micrograph of a pollen grain of Pinot after cross-pollination on Picolit stigma. A germinative pore (arrow) is visible, through which a pollen tube (pt) originates. ×7.500.

Abb. 13: Lichtmikroskopische Aufnahme eines 1 µm dicken Schnittes durch ein Pollenkorn von Picolit auf einer Narbe von Pinot. Nur in diesem einen Fall konnte die Anfangsphase eines Pollenschlauches bei einem Pollenkorn von Picolit beobachtet werden (Pfeil). 375×.

Abb. 14: Lichtmikroskopische Aufnahme eines 1 µm dicken Schnittes durch ein Pollenkorn von Pinot auf einer Narbe von Picolit. Das Pollenkorn hat gekeimt, und der Pollenschlauch ist zwischen die Zellen der Narbe eingedrungen. 525×.

Abb. 15: TEM-Aufnahme eines Pollenkornes von Pinot auf einer Narbe von Picolit. Durch eine Keimpore (Pfeil) tritt ein Pollenschlauch (pt) aus. 7.500×.

ever, is not accompanied by an interruption or thinning of the sporopollenin wall. Besides, when examined by SEM, Picolit pollen grains do not show the longitudinal furrows which are a normal feature of *Vitis* pollen and represent the sites along which germination occurs. Actually, when Picolit pollen grains, taken from different plants grown in different environmental conditions, were put to germinate *in vitro* on different substrates, no germination was obtained, but bursting of many grains was observed (CARGNELLO *et al.* 1976). Therefore, it seems that one of the causes that contribute to the failure of fertilization is intrinsic to the pollen and consists in a difficulty in the emission of pollen tubes due to mechanical impediment. Also the fact that Picolit pollen grains collapse when subjected to the usual SEM preparation procedure, while the others do not, suggests a difference in their wall structure. Most probably, the presence of an uninterrupted sporopollenin wall creates a barrier to a thorough evaporation of the internal fluids during critical point drying, with the result that the internal structure of the pollen grains is not completely stabilized and collapses under vacuum.

The hypothesis that the failure in fertilization might be due to autoincompatibility has to be discarded; in fact, Picolit pollen grains were unable to germinate also on stigmas of Pinot or Verduzzo. On the other hand, the lack of germination of Picolit pollen on Picolit stigmas cannot be attributed to some abnormality of the latter, because the pollen of Pinot and Verduzzo germinated regularly on them.

In any case, other causes may contribute to the failure of Picolit pollen grains to germinate, and further studies are needed on this subject.

Summary

The ultrastructure of the pollen and stigma of *Vitis vinifera* L. cv. "Picolit giallo" was studied by scanning and transmission electron microscopy, with the aim of finding out if they presented abnormalities which could account for the low productivity of this vine variety. The pollen protoplast has a normal aspect, but its wall is continuous and does not present furrows and germinative pores as pollen grains generally do. The stigma appeared normal. The presence of an uninterrupted wall most probably constitutes an obstacle to pollen germination and is likely to be one of the causes of the low fertilization incidence of "Picolit giallo". This hypothesis is supported also by the results of the experiments of self- and cross-pollination, because Picolit pollen always failed to germinate, even if put on stigmas of other vine varieties, while the pollen of these varieties germinated regularly when put on Picolit stigmas.

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Dr. Giuliana Lombardo
Istituto di Scienze Botaniche
Università degli Studi
Via G. Colombo 60
20133 Milano
Italia