Vitis **20**, 341—346 1981)

Istituto di Patologia Vegetale, Università degli Studi di Catania, Italia

Decline and death of young grapevines by infection of Phoma glomerata on the rootstock

by

G. Granata and E. Refatti

Deperimenti di giovani piante di vite indotti da infezioni di Phoma glomerata sul portinnesto

Riassunto. — Su giovani piante di vite (*Vitis vinifera* L.) cv. Italia, innestate in campo sui portinnesti *V. berlandieri* Planchon × *V. rupestris* Scheele 140 Ru., sono stati notati — in un'area viticola della Sicilia — gravi fenomeni di deperimento. Le viti presentavano aree necrotiche sul portinnesto, con conseguente scarsa vegetazione e clorosi progressive delle foglie, a cui seguiva spesso la morte dell'intera pianta.

Dalle piante ammalate è stato costantemente isolato il fungo *Phoma glomerata* (Corda) Wollenw. et Hochapf. (= *Peyronellaea glomerata* (Corda) Goidanich). L'isolato ha riprodotto la sindrome in seguito ad inoculazione artificiale, su barbatelle dei portinnesti *V. berlandieri* × *V. rupestris* 140 Ru. e 1103 P.; *V. rupestris* du Lot e *V. riparia* Michaux × *V. berlandieri* Kober 5BB. A distanza di tempo è stato possibile reisolare dalle piante con infezione artificiale lo stesso agente patogeno; *P. glomerata* è pertanto da considerare l'agente del deperimento delle giovani piante di vite. Si è potuto appurrare che l'infezione avviene attraverso la ferita praticata sul tronco per asportare lo sperone del portinnesto.

In prove *in vitro*, *P. glomerata* è risultato molto sensibile ai fungicidi Captafol, Ziram e Carbendazim.

Introduction

In June 1978, we detected in a grape area of the province of Agrigento (Sicily) decline and death of young grapevines (*Vitis vinifera* L. cv. Italia). The plants, grafted on the rootstock hybrid *V. berlandieri* Planchon × *V. rupestris* Scheele 140 Ru. and grown in the "tendone" system, were showing heavy necrotic areas on the rootstock. Researches tending to define the aetiology of the disease have been carried out since 1978. The related results are the object of the present paper.

Materials and methods

1. Diagnostic trials and observations

In 1978 and 1979, a series of surveys have been carried out in the grape area in order to follow the evolution of the disease. Meanwhile, information concerning the history of the disease and the local techniques of tillage and planting has been acquired. From tissues close to the necrotic areas attempts to isolate the causal agent of the disease onto an artificial medium have been executed.

2. Pathogenicity tests

Trials have been made on 2-year-old grapes, grown in steam-sterilized soil. The test plants included five rooted cuttings each of two cultivars of *V. vinifera* (Nerello mascalese and Bombino nero) and five of the most common rootstocks used in Sicily (*V. berlandieri* × *V. rupestris* 140 Ru. and 1103 P., *V. rupestris* DU LOT, *V. riparia* MICHAUX × *V. berlandieri* KOBER 5BB). As inoculum suspensions in sterile water of conidia of an isolate constantly obtained in the trials mentioned in (1) have been employed. Concentrations of conidia, obtained from colonies grown in Petri dishes, in carrot-agar, in a thermostat graduated at 21 °C, were adjusted approximately to 5×10^6/ml.

For the artificial inoculations transversal cuts were made on the stems of the young potted plants with a knife contaminated with a few drops of the conidial suspension. The control plants were treated in the same manner with only sterile water. The cuts were then covered with a piece of cotton dampened by sterile water. After inoculation, the plants were transferred to a controlled climate chamber (temperature 20 ± 2 °C, relative humidity 80 % and light intensity at the plant level 3.000 lx for a period of 12 h/d.

3. *In vitro* assays with various fungicides

The sensitivity of the isolate to various fungicides has been tested with two different techniques: germination inhibition of conidia and growth inhibition of the fungus by paper discs.

Germination inhibition of conidia. — Suspensions of conidia from pycnidia differentiated in carrot-agar cultures were put on the hole of hanging drop slides and water suspensions of the fungicides (1:1) at various concentrations (see Table 1) were added. The final concentration of conidia in the drops was about 5×10^5/ml. The checking of the germinated conidia has been made after 48 h of incubation at 21 °C, examining 500 conidia per drop. The data acquired were corrected in respect to the lack of germination in the water check and to the germination in the standard

Table 1

Average data attained in the hanging drop slide assays for testing germination inhibitions of conidia of the *P. glomerata* isolate with six concentrations (1:2) of various fungicides

Dati medi ottenuti nei saggi di inibizione della germinazione dei conidi di *P. glomerata* su vetrini a goccia pendente con sei concentrazioni (1:2) di diversi fungicidi

Fungicide	Germination (%) of conidia at various dilutions (ppm a. i.)					
	5	10	20	40	80	160
Benomyl	10	8	3	0	0	0
Captafol	0	0	0	0	0	0
Carbendazim	10	4	2	0	0	0
Copper oxychloride	76	65	58	48	3	0
Zineb	68	65	61	58	55	24
Ziram	0	0	0	0	0	0
Check (water)	92					

Table 2

Inhibition halos of growth of *P. glomerata* obtained in the paper disc test with four concentrations of various fungicides

Aloni di inibizione dello sviluppo di *P. glomerata* ottenuti nei saggi su dischetti di carta bibula con quattro diverse concentrazioni (ppm/s. a.) di sei fungicidi

Fungicide	Inhibition halos (mm) at various concentration (ppm a. i.)			
	150	300	600	1200
Benomyl	0	0.2	0.4	0.4
Captafol	2.0	2.2	2.6	3.0
Carbendazim	4.5	6.0	6.5	7.2
Copper oxychloride	0	0.1	0.1	0.1
Zineb	0	0	0	0
Ziram	0.5	1.0	2.0	4.0

fungicide (Ziram) at the inhibitive concentration, according to the Abbott formula, adopting the Healy tables (Ciferri 1961).

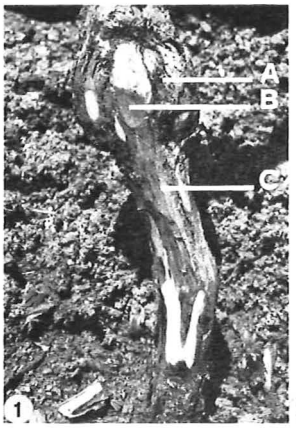
Growth inhibition of the fungus by paper discs. — 0.03 ml of the fungicide suspensions were put on discs of paper "Selecta" (Schleicher and Schüll No. 589[3]), diameter 1.1 cm, set on glass surfaces. After drying, the discs were laid on the surface of a layer of carrot-agar in Petri dishes, previously seeded with a conidial suspension of the isolate. For each fungicide four dilutions (1:2), as reported in Table 2, have been tested. The fungicidal activity was evaluated on the basis of the inhibition halos of the fungus around each disc, after a 64-h-period of incubation in a thermostat regulated at 21 °C.

The fungicides tested were:
Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate);
Captafol N-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide);
Carbendazim (methyl-2-benzimidazolecarbamate);
Copper oxychloride;
Zineb (zinc ethylenebisdithiocarbamate);
Ziram (zinc dimethyldithiocarbamate).

Results

1. Symptomatology

Since June, plants showing scarce growth and chlorotic leaves have been noticed in some vineyards, in the 1st or 2nd year after grafting. The affected grapevines were alone or grouped and scattered here and there in the vineyard. The colour alteration generally started with a noticeable yellowing of main leaf veins, that tended to spread gradually all over the leaf blade. Progressive wilting and necrosis phenomena till the death of the entire plant followed the initial symptoms. At the graft union heavy necroses were present on the affected vines. Examining plants in different stages of decline, it was possible to clarify that infection takes place in correspondence to the wound practised on the trunk by removing the rootstock



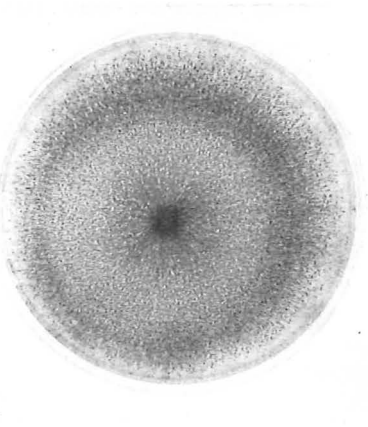


Fig. 1: Necrosis of the rootstock tissues caused by *Phoma glomerata*. A = graft union; B = wound made for removing the rootstock spur; C = necrotic area on the rootstock.

Fig. 2: Colony of the isolate of *P. glomerata* on artificial medium.

Fig. 3: Necrotic areas in correspondence of the buds of a cane of V. rupestris DU LOT artificially inoculated with the *P. glomerata* isolate.

Fig. 1: Necrosi dei tessuti del portinnesto causati da *Phoma glomerata*. A = punto d'innesto; B = ferita di potatura; C = necrosi del portinnesto.

Fig. 2: Colonia del fungo in coltura artificiale.

Fig. 3: Necrosi, in corrispondenza delle gemme, su un tralcio del portinnesto *V. rupestris* DU LOT inoculato artificialmente.

spur[1]) (Fig. 1). In some cases necroses were limited to small portions of the rootstock, close to the spur wound, but frequently they extended all around the trunk with the consequent decline of the entire plant.

The phenomenon has been noted in the grape area in the last 3 or 4 years and only on young plants.

2. Isolation of the specific pathogen

From the trials carried out by recovering the causal agent of the disease, a fungus species has been consistently isolated. Its colonies produced on carrot-agar abundant pycnidial fructifications (Fig. 2), containing hyaline, unicellular, ovoid conidia, together with clamidospores of the *Alternaria* type. On the basis of its morphological characteristics, the fungus was identified as *Phoma glomerata* (CORDA) WOLLENW. et HOCHAPF. (MORGAN-JONES 1967, BOEREMA *et al.* 1977)[1]).

3. Pathogenicity test

Inoculations made on plants of the five rootstocks were positive. After 90 d the young rooted cuttings were showing a heavy necrotic area (20—30 cm long) in correspondence to the inoculation points. In some cases necroses were evident also near

[1]) It is a custom in the grape area to plant the rooted cuttings of the rootstock in the spring, to budgraft such plants in the summer, to cut the rootstock at 5—10 cm from the grafted bud the next spring, and to eliminate the residual spur the following winter.

the buds of young canes (Fig. 3). Reisolation attempts from inoculated plants always yielded a fungus identical to the one isolated from naturally infected plants. In the *V. vinifera* rooted cuttings, artificial inoculations have induced only mild necrotic areas around the knife cuts and just in one case out of ten it was possible to reisolate the fungus.

4. Activity assays of some fungicides on the isolate

The data obtained with the various fungicides in the germination trials of conidia of the isolate of *P. glomerata* are reported in Table 1. The average data concerning the inhibition halos around the paper discs induced by four concentrations of the fungicides are summarized in Table 2.

Discussion

The results obtained in the attempts to isolate on an artificial medium the causal agent of the disease, the reproduction of the syndrome in the artificial inoculations of the isolate on healthy rooted cuttings and the reisolation of the same agent from the artificially inoculated plants have satisfied the Koch postulates, basal condition for considering a microorganism responsible for an infective disease. As a consequence, *Phoma glomerata* (Corda) Wollenw. et Hochapf. (= *Peyronellaea glomerata* (Corda) Goidanich) is to be considered the specific agent of the decline and death of the young grapevines noticed in the Agrigento grape area. It has also been possible to clarify that penetration of the pathogen takes place through the wound practised on the trunk by removing the rootstock spur.

The rootstock hybrids 140 Ru., 1103 P., Kober 5BB and *V. rupestris* du Lot appeared to be more sensitive to the infection than the *V. vinifera* cultivars tested. This confirms the fact that the heavy necroses of the rootstock are at the origin of the decline and death phenomena.

According to the synthesis made by Morgan-Jones (1967) and the studies carried out by Boerema *et al.* (1977), *P. glomerata* has been isolated from various species of plants (woody and herbaceous), as well as from animals, man, and inorganic materials. The fungus is present everywhere, mainly in the temperate regions. The various strains included in the population of the species show remarkable variation in the shape and dimension of the spores.

Infection of *P. glomerata* on grapevines have been reported by Šarić-Sabadoš *et al.* (1960) for Yugoslavia and by Picco (1962) for North Italy. In both cases the fungus induced necroses of young organs (bunches, leaves and sprouts), and their partial or total dryness. Such a syndrome differs from the one we have noticed in Sicily. It is to presume that in our case infections on the rootstock are promoted by the mentioned scissor's intervention, in favorable environment conditions for the pathogen.

The *in vitro* assays carried out with six different fungicides have proved that the *P. glomerata* isolate is quite sensitive to Captafol, Ziram and Carbendazim. Benomyl, having an activity of the same order in the conidia germination test, has

[1]) The identity of the species has been confirmed by Dr. E. Punithalingam, of the Commonwealth Mycological Institute, Kew (England), to whom we express our gratitude.

shown a very low activity in the agar assays. Modest action has been noted for copper oxychloride. Zineb proved to be practically inactive.

In order to prevent infections of P. *glomerata* at the union, we are suggesting to disinfect the wounds deriving from removing the rootstock spurs, with one of the three more active fungicides and to practise such cuts in the late winter, when plants are close to bud-breaking.

Summary

Decline and death of young grapevines *(Vitis vinifera L.)* cultivar Italia, grafted on the rootstock *V. berlandieri* PLANCHON × *V. rupestris* SCHEELE 140 Ru., have been detected in a grape area of Sicily. The phenomena were initially characterized by necrotic areas on the rootstock and consequent scarce growth and progressive chlorosis of the leaves.

From the affected plants a fungus, identified as *Phoma glomerata* (CORDA) WOLLENW. et HOCHAPF. (= *Peyronellaea glomerata* (CORDA) GOIDANICH), has been consistently isolated. Such an isolate has reproduced the syndrome when inoculated on rooted cuttings of the rootstocks *V. berlandieri* × *V. rupestris* 140 Ru. and 1103 P., *V. riparia* MICHAUX × *V. berlandieri* KOBER 5BB and *V. rupestris* DU LOT. From the plants with artificial inoculations it was possible to reisolate the same fungus. On such a basis, *P. glomerata* has to be considered the causal agent of the disease. Infections take place through the wounds made on the young trunks by removing the rootstock spurs from the field-grafted plants.

In vitro assays have proved that the isolate of *P. glomerata* is quite sensitive to the fungicides Captafol, Ziram and Carbendazim.

References

BOEREMA, G. H., DORENBOSCH, M. M. J., and VAN KESTEREN, H. A., 1977: Remarks on species of *Phoma* referred to *Peyronellaea*. Kew Bulletin 31, 533—544.

CIFERRI, R., 1961: Tavola di Healy per la correzione della mortalità naturale secondo Abbott. In: Note di biometria applicata alla fitoiatria, 96—98. Società Italiana di Fitoiatria, Pavia.

MORGAN-JONES, G., 1967: *Phoma glomerata*. C. M. I. Descriptions of Pathogenic Fungi and Bacteria No. 134, 2 pp. Commonwealth Mycological Institute, Kew (Surrey), England.

PICCO, D., 1962: La *Peyronellaea glomerata* su piante erbacee di vigneti con viti infette. Riv. Patol. Veg. 2, 311—333.

ŠARIĆ-SABADOŠ, A., MILATOVIĆ, I. und MASTEN, V., 1960: *Peyronellaea glomerata* als Erreger der Vertrocknung der Blüten und jungen Trauben der Weinrebe in Jugoslawien (ital.). Atti Ist. Bot. Lab. Critt. Univ. Pavia 18, 101—107.

Eingegangen am 2. 6. 1981

Prof. Dr. E. REFATTI
Università degli Studi di Udine
Facoltà di Agraria
Istituto di Difesa delle Piante
Via Chiusaforte, 54
33100 Udine
Italia