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Further evidences concerning the systemic spreading of Agrobacterium tumefaciens in the vascular system of grapevines

by

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Earlier researches have shown the presence of pathogen, *Agrobacterium tumefaciens* (Smith et Townsend) Conn, in spring in the liquid of bleeding of vines which were infected naturally and which stood on self-roots (cuttings). It was moreover possible to provoke tumor formation on the above ground parts of the grapevines, wounded with an aseptic scalpel (Lehoczky 1968 a, Malenin 1970). An obvious conclusion could be drawn from the results that the pathogen can be transmitted by various methods of vegetative propagation and this can be one of the most important ways of spreading the disease (Lehoczky 1968 b).

The investigations and observations described below give further evidence about the systematic spreading of pathogens in the vascular system of grapevines and at the same time about their transmissibility by vegetative propagation methods.

Materials and Methods

Isolation of the pathogen was carried out using a slightly modified technique of an earlier described method (Lehoczky 1968 a). To prevent the canes and root pieces from moulding during their overwintering and long incubations in a thermostat, we soaked them for 4 hours in 0.5 percent solution of Chinosol W. (active ingredient: 8-hydroxyquinoline sulphate) and dried their surface in free air.

The surface of the tissue pieces, which were excised in about 3—4 mm diameter from different places of the callus, the buds and the fresh root tips, were sterilized quickly with ethyl alcohol. After washing they were macerated in 0.5 ml sterile water in a watch glass and stored for 20—24 hours at 8—10° C in a refrigerator. The tissue pieces were separated from the liquid which was then 10- and 100-fold diluted with sterile water and from this 0.15 ml was smeared with a glass-rod on to the surface of broth-agar, in 10 cm diameter Petri-dishes. The cultures were incubated for 5 days at 28° C in a thermostat, then the colonies characteristic to *A. tumefaciens* were isolated.

Identification and investigation of the pathogenicity of the isolates were carried out and proved on *Helianthus annuus*. The plants were inoculated 6 days later, when their cotyledons appeared. The hypocotyls were sterilized with ethyl alcohol and after washing with sterile water, the surface was smeared with a bacterial suspension made of 48 hour-old cultures, with the aid of a brush. Afterwards they were wounded with fine needles, many, but not deep, wounds were made, and the injured surface was repeatedly smeared with the bacterial-suspension. In order to prevent the wounded parts from drying, they were covered with cotton, moistened with sterile water. Four plants were inoculated by each isolate. The results were read after 20 days.

Table 1

Reactions of strains isolated from swelling bud tissue

Isolate number	Tumor formation on *Helianthus annuus*
Ka. 30. 1 — 3 — 1	—
Ka. 30. 1 — 3 — 2	+
Ka. 30. 2 — 1 — 1	—

Signs used: + Tumor formation mild, ++ tumor formation medium, +++ tumor formation strong, — without tumor formation.

Origin of the isolates and results of identification

a) Swelling buds of canes free of symptoms

Canes of self-rooted, 3-year-old cv. Rekord vines, naturally infected, were collected in autumn of 1968. After overwintering, cuttings were made and forced for 3 weeks in sterile river sand. Strains from swelling, but not yet bursting buds were isolated. Results of isolations are shown in Table 1.

b) Callus tissue of canes free of symptoms

One-year-old symptomless canes were collected from 4-year-old cuttings of naturally infected cv. Olimpia vines, in the course of the autumn of 1967. After overwintering, short cuttings were made and allowed to be rooted in sterile river-sand at 25° C. After four weeks, strains were isolated from the callus tissue. Results of isolation can be found in Table 2.

c) Fresh root-tips of symptomless canes

The canes originated from 2-year-old naturally infected cuttings cv. Rekord vines. They were collected in the autumn of 1969. After overwintering, we cut them

Table 2

Reactions of strains isolated from the callus tissue of cuttings

Isolate number	Tumor formation on *Helianthus annuus*
Ka. 20. 1 — 1 — 1	—
Ka. 20. 2 — 1 — 1	—
Ka. 20. 3 — 1 — 1	—
Ka. 20. 4 — 1 — 1	++

Table 3

Reactions of strains isolated from fresh root-tips of cuttings

Isolate number	Tumor formation on *Helianthus annuus*
Ka. 50. 1 — 1 — 0	++
Ka. 50. 1 — 1 — 2	—
Ka. 50. 1 — 2 — 1	—
Ka. 50. 1 — 3 — 1	—
Ka. 50. 1 — 3 — 2	—

Explanation see Fig. 1.

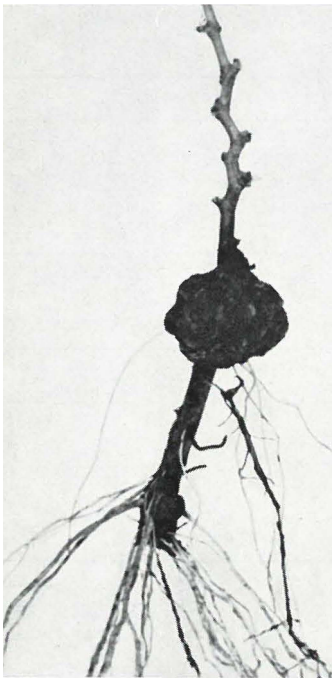

Fig. 1

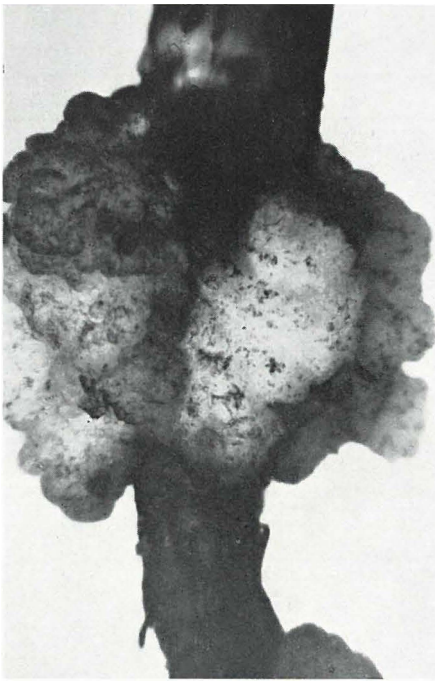

Fig. 2

Fig. 1: Origin of root pieces: root-system of 2-year-old, infected cutting.
Fig. 2: Site of isolation: callus tissue developed on 2-year-old root pieces, induced by wounding.

in March 1970 to short cuttings, which were then placed in Petri-dishes of 20 cm diameter as in a hygrostat, covered inside with moist filter paper and forced at 25° C for 3 weeks. Strains were isolated from 4 mm of the tips of the fresh roots, developed in the course of forcing time. Results are given in Table 3.

d) Callus tissue of symptomless root pieces

One and two year-old roots were collected from two year-old, naturally infected cuttings of cv. Rekord vines, in winter of 1968 (Fig. 1). Roots were cut into 10—15 cm pieces and their surface was sterilized quickly with ethyl alcohol. After washing, 3—4 mm diameter tissue pieces were excised from surface forming concave wounds with a sterile scalpel, in order to get plenty of callus tissue. The wounded roots were then placed in Petri-dishes and kept at 28° C in a thermostat for 3 weeks. Strains were isolated from callus tissue appearing on wounds (Fig. 2). Results can be found in Table 4.

e) Fresh root-tips developed on symptom-free root pieces

The one year-old roots were collected in the spring of 1969, from one year-old naturally infected cuttings of cv. Olimpia vines. The roots, after sterilization with ethyl alcohol, were cut into pieces of 10—15 cm, placed in 20 cm diameter Petri-dishes and covered inside with wet filter paper. They were stored at 25° C in a thermostat for 3 weeks. Strains were isolated from the tips of fresh roots, developed on the root pieces. Results of isolation are shown in Table 5.

Table 4

Reactions of strains isolated from callus tissue of root pieces

Isolate number	Tumor formation on *Helianthus annuus*
La. 16. 1 — 1 — 1	+++
La. 16. 1 — 2 — 1	+++
La. 16. 2 — 1 — 1	+++
La. 16. 2 — 2 — 1	+++

Table 5

Reactions of strains isolated from fresh root tips of one year-old root pieces

Isolate number	Tumor formation on *Helianthus annuus*
Ka. 27. 5 — 1 — 1	—
Ka. 27. 5 — 1 — 2	+++
Ka. 27. 5 — 2 — 1	—
Ka. 27. 5 — 2 — 2	+
Ka. 27. 5 — 3 — 1	+++

Explanation see Fig. 1.

Results and Discussion

The results indicate that the pathogen spreads systemically in the host vascular system. In addition, it is proved that a danger exists for the transmissibility of the pathogen and in particular for disease spreading by means of vegetative propagation, in a similar way to the pathogen of bacterial wilt (*Erwinia vitivora* [BACC.] DU PL.) which was established by DU PLESSIS (1940, cited in: ANDERSON 1956).

Evidences of the natural occurrence of *A. tumefaciens* in practice, sometimes abundant, which prove a spreading of pathogen with propagating material, are:

a) Abnormally big, induced callus on short cuttings forced in river-sand (Fig. 3).

b) Tumors on one year-old rooted short cuttings forced in sand, then reared in a glasshouse (compare Fig. 1).

c) Tumor tissue developed on bud unions of grafts instead of healthy callus tissue, and on account of structural difference often inhibited essentially the perfect union formation between the scion and rootstock (Fig. 4). It leads to a weaker conditional growth later.

d) Tumors on scions of such vines were made by green-wood grafting on Kober 5 BB rootstock.

In the last case the shoots for green-wood grafting were obtained from a vineyard which was in $> 25\%$ diseased by crown gall. The 3-year-old green-wood grafted vines were surveyed visually based on symptoms, and we have found more often smaller (Fig. 5 and 6) and rarely bigger tumors on scions. 120 vines were evaluated symptomically in the vineyard, and results are to be seen in Table 6.

There are publications on such an extensive occurrence of crown gall disease in certain vineyards that it caused early death of a major part of young grapevines (RÁGALA 1960, cited in: STAPP 1968, RÁGALA 1968, ZINCA 1968). These cases demonstrate

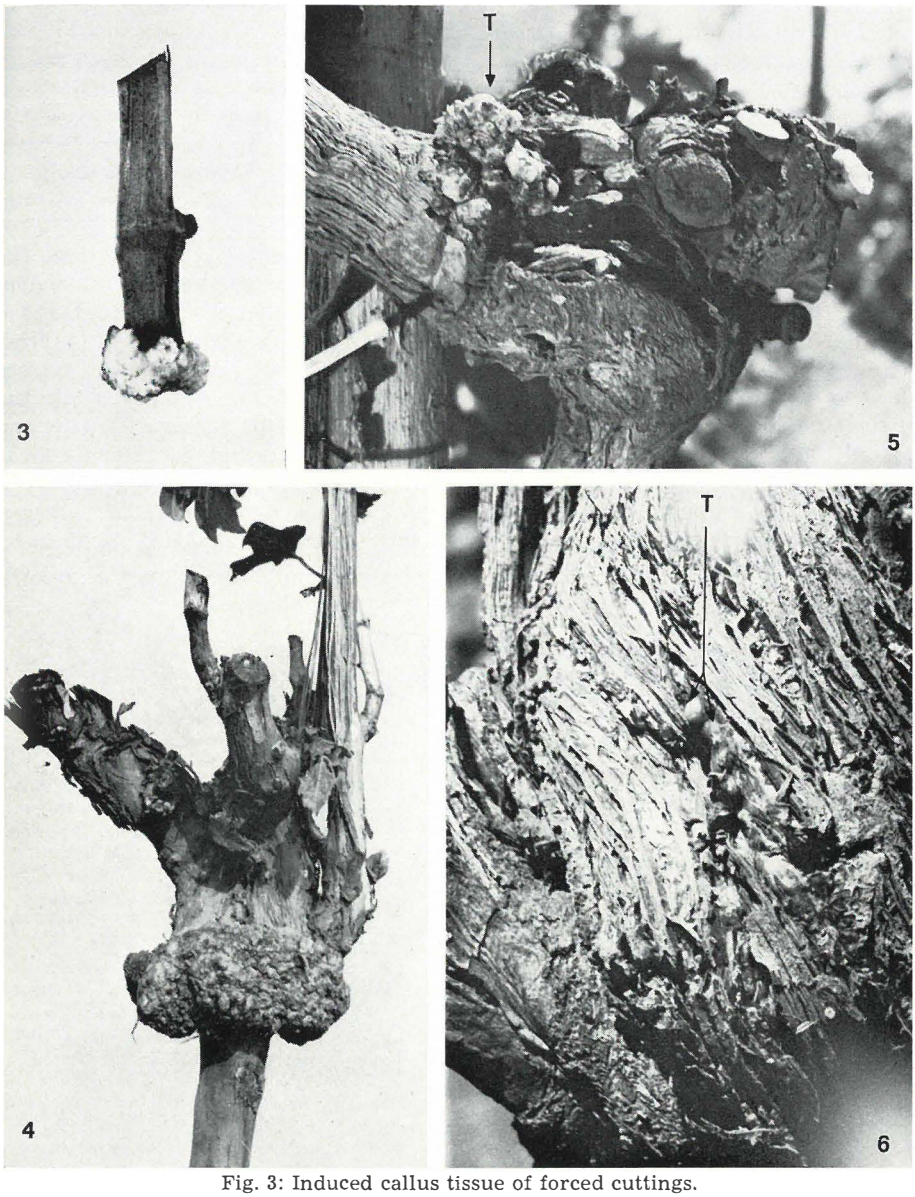

Fig. 3: Induced callus tissue of forced cuttings.
Fig. 4: Tumor tissue on site of bud union of a 5-year-old graft.
Figs. 5 and 6: Small tumors (T) on the scion of green-wood graft.

with great probability that their propagating material originated from an infected vineyard. The importance of the disease in the high-culture probably increases as the trunks and branches can be damaged seriously and can perish early (Figs. 7 and 8).

Histological investigations for keeping track of systemical spreading of pathogen were not carried out, but we consider it moves on the analogy of *Pseudomonas*

Table 6

Rate of diseased vines in a green-wood grafted vineyard

Cultivar and rootstock	Vines surveyed	Measure of tumor-formation			Percent of diseased vines
		mild	medium	strong	
Olimpia	120	5	2	1	6,7
Kober 5 BB		0	0	0	0,0

savastanoi (E. F. SM.) STEVENS in vessels of *Nerium oleander* L. In this case, the pathogen issues from the xylem vessels and passing through the cortical region, reaches the latex cells, fills them and later these cells become destroyed and a cavity is formed. A few cells adjacent to the cavity begin to divide and the process of the tumor-formation is started. It is characteristic that the secondary tumors develop on the stem along the same line where the pathogen penetrated into the tissue. They develop also on the midrib and the lateral veins of leaves (WILSON and MAGIE 1964). Other histological researches have shown that the *Pseudomonas caryophylli* (BURKH.) STARR et BURKH. spreads also systemically in the vessels of the carnation. Through the pit cavity membrane of xylem vessel, the pathogen exudes into adjoining xylem parenchyma cells, fills them and this is followed by the collapse of cortical and epidermal cells and cracks appear in the stem surface (NELSON and DICKEY 1966).

Fig. 7

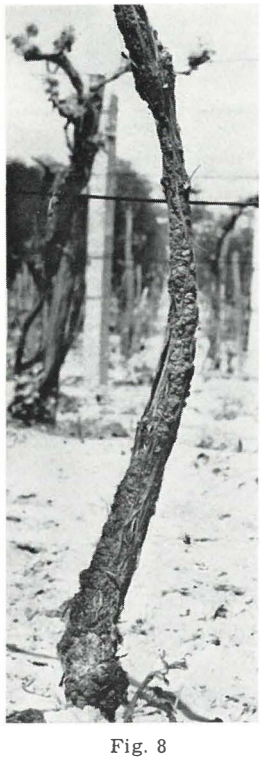

Fig. 8

Figs. 7 and 8: Tumorous deformation of trunk of high cultivated vines.

It can be supposed that, on the analogy of the above mentioned cases, the *A. tumefaciens* can also issue from the xylem vessels and penetrate into the adjoining xylem parenchyma and into the cambial and ray parenchyma, which are, according to BRAUN and STONIER (1958), all able to form tumors by induced cell division. The stem, after the pathogen has invaded the adjoining cells, remains free of symptoms unless the wounds of any injury induce the tumorigen process. This can occur rarely on green stems and more often on wooded canes, on which the wounds of frost injury or of mechanical twisting, or of pruning can induce the beginning of tumor-formation.

Summary

The isolation of pathogen, *A. tumefaciens*, was successful first from the buds, callus tissue and fresh root-tips of forced short cuttings made from symptom-free canes, and furthermore from fresh root-tips and callus tissue of forced, symptom-free root pieces.

Both the canes and roots were collected from natural infected, self-rooted vines (cuttings). The results give further evidence regarding systemic spreading of the pathogen in the vascular system of grapevine and to its transmissibility by vegetative propagating material. The tumor-formation on green-wood grafted vines, of which the shoots were collected in a vineyard which was more than 25 percent infected with crown gall, gives further convincing evidence.

Literature Cited

ANDERSON, H. W., 1956: Disease of Fruit Crops. 385—388 p. McGraw-Hill Book Company, Inc., New York.

BRAUN, A. C. and STONIER, T., 1958: Morphology and physiology of plant tumors. Protoplasmatologia. Handb. d. Protoplasmaforsch. Bd. 10: Pathologie des Protoplasmas, 5a. 93 pp. Springer Verl., Wien.

DU PLESSIS, S. J., 1940: Bacterial blight of vines (vlamsiekte) in South Africa caused by *Erwinia vitivora* (Bacc.) Du Pl. Dept. Agric. S. Afr. Sci. Bull. 214, 1—105.

LEHOCZKY, J., 1968 a: Spread of *Agrobacterium tumefaciens* in the vessels of the grapevine, after natural infection. Phytopath. Z. 63, 239—246.

— —, 1968 b: Bacterial tumor, a malignant disease of the grapevine (in Hung.). Orsz. Szőlész. Borász. Kutató Int. Évkönyve, 13, 115—126.

MALENIN, I., 1970: Etude de la propagation de *l'Agrobacterium tumefaciens* le long des vaisseaux de bois de la vigne et la formation de tumeurs secondaires. Gradinarska i Lozarska Nauka (Sofia) 7, 127—135.

NELSON, P. E. and DICKEY, R. S., 1966: Pseudomonas caryophylli in carnation. II. Histological studies of infected plants. Phytopathology 56, 154—163.

RÁGALA, M., 1968: Tumeurs bacteriennes de la vigne *(Bacterium tumefaciens):* rôle du gel: influence d'autres facteurs; méthodes du lutte contre la maladie. XIIe Congr. Internatl. de la Vigne et du Vin, Bucarest. Travaux Scientif., I. Viticult. 259—271.

STAPP, C., 1968: Fortschritte der bakteriellen Pflanzenkrebsforschung. Die neuere Literatur über das Gesamtgebiet. Zentralbl. Bakteriol., Parasitenk., Infektionskrankh., Hyg., 2. Naturwiss. Abt. Allgem. Landwirtsch. u. Tech. Mikrobiol. 122, 723—763.

WILSON, E. E. and MAGIE, A. R., 1964: Systemic invasion of the host plant by the tumor-inducing bacterium *Pseudomonas savastanoi*. Phytopathology 54, 576—579.

ZINCA, N., 1968: Tumeurs bacteriennes de la vigne *(Bacterium tumefaciens):* rôle du gel; influence d'autres facteurs; méthodes de lutte contre la maladie. XIIe Congr. Internatl. de la vigne et du Vin, Bucarest. Travaux Scientif., I. Viticult., 273—282.

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