563

JOURNAL
OF FRUIT
AND
ORNAMENTAL
PLANT
RESEARCH

VOL. IV
No. 4
1996

SKIERNIEWICE, POLAND

Piotr Sobiczewski
Research Institute of Pomology and Floriculture, Skierniewice, POLAND

ETIOLOGY OF CROWN GALL ON FRUIT TREES IN POLAND

ABSTRACT. All *Agrobacterium tumefaciens* isolated from tumors occurring on roots of various species of fruit trees originating from 6 nurseries located in various regions of Poland belonged to biovar 2. Of 41 isolates investigated, 29 were sensitive to agrocin 84, the bacteriotoxin produced by strain K84 of *A. radiobacter*. In spite of the various origins of tumorigenic bacteria - different host plants and localities - they showed no pathogenic specialization and caused crown gall both on sunflower and tomato seedlings and on sour cherry shoots cultured *in vitro*.

Key words: crown gall, *Agrobacterium tumefaciens*, fruit trees, biovars

INTRODUCTION. First reports of crown gall on roots as plant disease are dated from the second half of the 19th century. At the beginning of the 20th century Smith and Townsend had proved that the disease was caused by *Bacterium tumefaciens*, later on named *Agrobacterium tumefaciens* by Conn (Lippincott et al., 1983).

According to "Bergey's Manual of Systematic Bacteriology" within the genus *Agrobacterium*, belonging to *Rhizobiaceae* family, four species are distinguished (Kersters and De Ley, 1984): nonpathogenic (saprophytic) *A. radiobacter* (Beijerinck and van Delden) Conn.; *A. tumefaciens* (Smith and Townsend) Conn., causing galls on roots, *A. rubus* (Hildebrand) Starr and Weiss, causing galls on *Rubus* plants and *A. rhizogenes* (Riker, Banfield, Wright, Keitt and Sagen),

Determination of bacteria pathogenicity

a/On sunflower seedlings

Root of seven-day-old seedlings were rinsed in tap water and then the main root was wounded and inoculated with a needle dipped in a 24 hr old culture of bacteria grown on nutrient agar medium (2.3 % Difco Nutrient Agar, 0.5 % glucose). Then plants were planted into pots and placed in a greenhouse.

b. On tomato seedlings

Three-week-old Venture tomato seedlings grown in pots in a greenhouse were inoculated into a wound at the base of the stem with 24 hr old bacteria culture grown on nutrient agar.

c. On sour cherry shoot cultures

Five-week-old plantlets of sour cherry cv. English Morello propagated *in vitro* were removed from flasks and placed in sterile Petri dishes. The plantlets were punctured at the base, middle and top parts of the shoots and inoculated with 24 hr old bacteria culture grown on nutrient agar. Inoculated plantlets were transferred to new flasks with MS (Murashige Skoog) medium stimulating rooting.

In all the above tests, the control combination consisted of plants scarified in the same way as for inoculation but instead of bacteria they were treated with sterile water. Each inoculation was performed on three plants. The appearance of galls on sunflower and tomato seedlings was checked after 14 and 21 days but on sour cherry shoot cultures after 17 days. Additionally, on sour cherry shoot cultures the size of galls was measured and used as an index of bacteria virulence.

Assessment of physiological and biochemical characters of bacteria isolates

Isolates of bacteria pathogenic to sunflower and tomato seedlings and to sour cherry shoot culture were subjected at least twice to the following biochemical and physiological tests:

a. Gram reaction. Staining of bacteria with a water solution of crystalline violet, then with a water solution of safranine was performed according to method described by Bradbury (1970). Additionaly a Gram reaction was determined by mixing bacteria with 3 % KOH (Suslow et al. 1982).

b. Oxidation of lactose to 3-ketolactose (Bernaerts and De Lay, 1963). A medium containing 1 % lactose and solidified with agar was inoculated with bacteria and then incubated at 27°C. Two days later the surface was flooded with Benedict's reagent. The appearance of a characteristic yellow ring around bacteria growth was an indication of 3-ketolactose production.

Supplied by the British Library - "The world's knowledge" www.bl.uk

c. Ability to utilize citrate as a carbon source (Moore et a l., 1988, after Simons, 1926). The dark blue pigmentation of mineral substrate containing sodium citrate and bromothymol blue after 48 hr of bacteria incubation at room temperature indicates a positive result.

d. Ability to utilize L-tyrosine as a nitrogen source (Kersters et al., 1973). Growth of bacteria in liquid substrate containing L-tyrosine (demonstrated as a turbidity) was evaluated on the fourth day of incubation at room temperature.

e. Growth and pigmentation of liquid substrate containing ferric ammonium citrate (Moore et al., 1988, after Hendrickson et al. 1934). The appearance of reddish brown pellicle at the surface of the medium is typical of bacteria classified as biovar 1.

f. Production of acid from erythritol (Hayward 1964). The yellow colouring of a medium containing erythritol and bromothymol blue after 4 days incubation with bacteria at room temperature indicates a positive result.

g. Tolerance to salt (Moore et al., 1988). Growth of bacteria was evaluated in nutrient broth containing 1, 2, 3, 4 and 5 % NaCl. Substrate inoculated with bacteria was incubated at 25°C for 14 days. Turbidity of substrate indicates bacteria growth.

h. Presence of oxidase c (Kovacs, 1956). Bacteria were incubated for 48 hr on King B medium and then a sample was transferred with a loop to a filter paper soaked with 1 % solution of tetramethylparafenylodiamin chloride. The appearance of purple-violet pigmentation around the bacterial streak after 10 sec indicates an oxidase-positive isolate.

Sensitivity of bacteria to agrocin

Sensitivity of tumorigenic bacteria to agrocin 84 produced by K84 strain of *Agrobacterium radiobacter* was tested by the method of Stonier modified by Cooksey and Moore (1982). Strain K84 was kindly supplied by Dr. A. Kerr of the University in Adelaide (Australia). It was inoculated onto medium in the middle of Petri dishes of 10 cm in diameter containing yeast extract and mannitol. After 3 day incubation at 24°C bacteria were inactivated with chloroform vapour, then layered with 4 ml 0.7% agar inoculated with bacteria under test. The presence of a growth inhibition zone was evaluated after 2 day of incubation at 22°C. Sensitivity of isolates to agrocin was assessed on the basis of the diameter and character of the inhibition zone around K84 growth. Strains with a distinct growth inhibition zone larger than 46 mm were recognized as very sensitive. Medium sensitive were these forming an inhibition zone narrower than 46 mm, and these

Supplied by the British Library - "The world's knowledge" www.bl.uk

with a lightly misty zone. Lack of an inhibition zone meant total resistance of the isolate to agrocin (Tab. 2).

RESULTS. After 3 days of incubation on Patel medium, colonies of both reference and selected isolates were regularly round, slightly convex, glistening, lightly mucous with light violet pigmentation and whitish in the middle, surrounded by a narrow (0.5 mm) transparent border. The colony diameter of the reference strain K27 and of 36 isolates (31, 32, 39, 40, 46, 60, 67, 69, 71, 74, 77, 81, 83, 84, 88, 89, 101, 103, 104, 104a, 107, 126, 129, 133, 137, 182, 192, 221, 262, 301, 307, 365, 387, 389, 400, 580) ranged from 2 to 3 mm. Reference strains B6, ACH5, C58 and 5 isolates (122, 125, 131, 392, 568) formed colonies which were more mucous with a tendency to merge into larger spots - their diameter was about 4 mm.

All isolates were pathogenic to sunflower and tomato seedlings and to sour cherry shoot cultures (Photo 1 a, b, c). Measurements of gall diameters on sour cherry shoot cultures allowed division into three groups of virulence (Tab. 1).

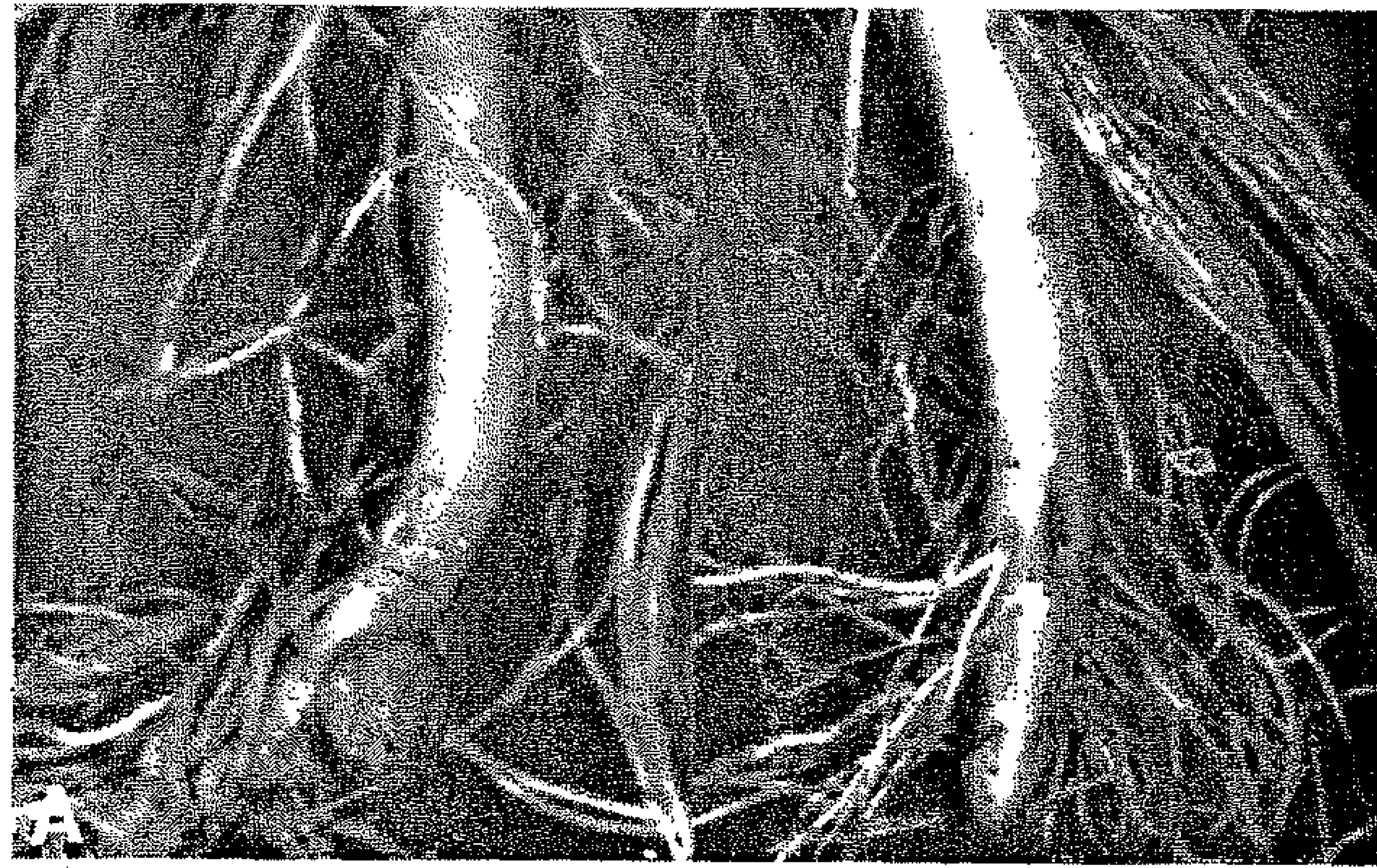
Table 1. Virulence of *Agrobacterium tumefaciens* strains to sour cherry cv. English Morello shoots cultivated *in vitro*

Type of virulence	Strain	No. of strains
High	32, 71, 74, 77, 81, 83, 84, 104a, 133, 192, 22, 301, 307, 387, 400	15
Medium	31, 39, 60, 69, 88, 89, 103, 104, 126, 129, 131, 182, 262, 389, 392, 568, 580	17
Low	40, 46, 67, 101, 107, 122, 125, 137, 365	9

Explanation: Low, medium and high virulence were assessed to galls in diameters: 1, 2 and 4 mm respectively as measured 17 days after inoculation

Fifteen isolates inducing galls about 4 mm in diameter were clasified as highly virulent, 17 inducing galls about 2 mm as having medium virulence and these inducing galls about 1 mm in diameter as having low virulence. Two reference strains, C58 and K27 were highly virulent. Virulence did not correspond with sensitivity to agrocin 84. Within each group of isolates characterised by their virulence, isolates with different sensitivity to agrocin were represented. As shown in Table 2, 16 of isolates were very sensitive and 12 resistant to agrocin. All the reference strains were very sensitive to agrocin (Photo 2). Sensitivity to agrocin did not correlate with the origin of isolates (Tab. 2).

Supplied by the British Library - "The world's knowledge" www.bl.uk



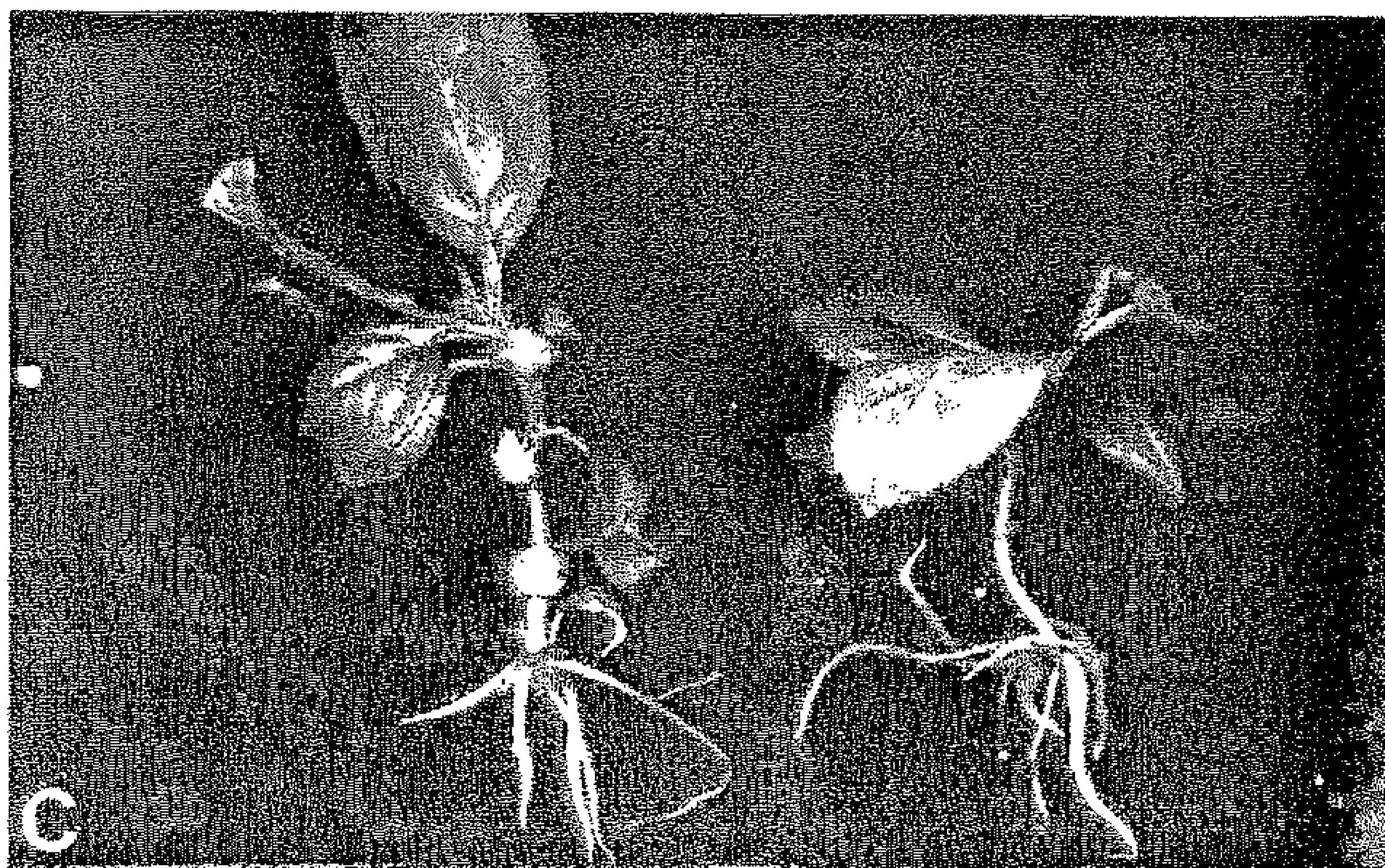


Photo 1a, b, c. Pathogenicity test on: a - sunflower, b - tomato, c - sour cherry shoot cultures; to the left - infected plant (with gall tissue); to the right - control plant

Supplied by the British Library - "The world's knowledge" www.bl.uk

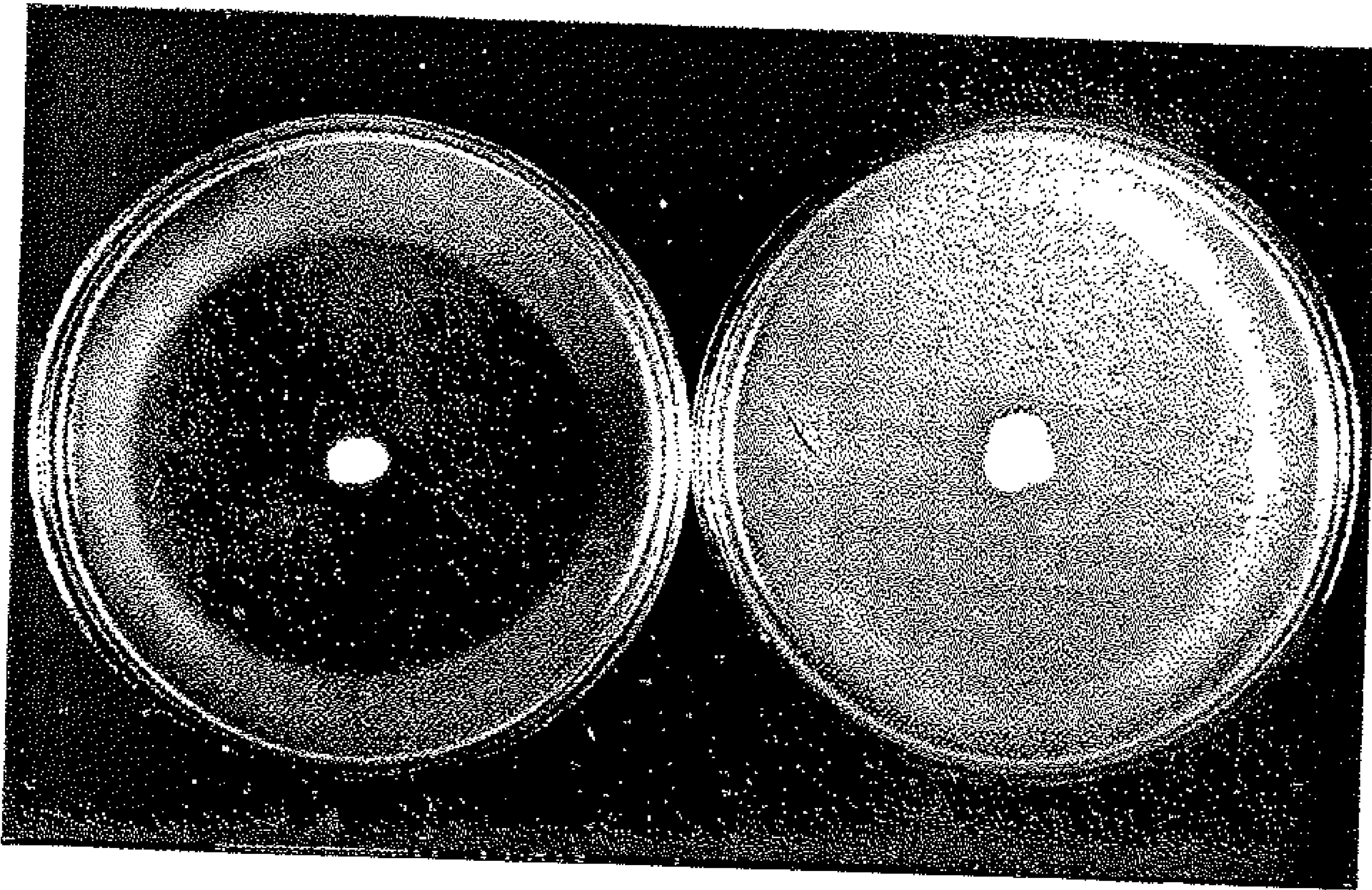


Photo 2. Growth inhibition zone of *A. tumefaciens* isolate sensitive to agrocin 84 (left); unlimited growth of strain resistant to agrocin (right)

Table 2. Sensitivity of *Agrobacterium tumefaciens* strains to agrocin 84

Type of sensitivity	Strain	No. of strains
High	67, 77, 83, 84, 88, 89, 122, 125, 126, 131, 133, 137, 182, 301, 387, 400	16
Medium	39, 40, 60, 69, 74, 81, 107, 129, 221, 262, 307, 365, 392	13
Nonsensitive	31, 32, 46, 71, 101, 103, 104, 104a, 192, 389, 568, 580	12

Explanation: See Material and Methods

Supplied by the British Library - "The world's knowledge" www.bl.uk

Table 3. Phenotypic characters of *Agrobacterium tumefaciens* isolated from fruit trees in Poland

Origin and symbol of isolate	No. of isolates	Gram reaction	3-keto-lactose	Utilization of sodium citrate	Utilization of L-tyrosine	Ferric ammonium citrate	Acid from erythritol	Tolerance to NaCl(%)	Oxidase c
Sour cherry *Prunus avium* 31D*, 32D, 39A, 40A,88A,101C, 107C, 125D, 126D, 129C, 133C, 137C, 301C, 387D, 389D, 392D, 400D	17	-	-	+	+	-	+	1	+
Cherry F12/1 60C, 67C, 69C, 71C, 74,C, 77C, 81C, 83C, 84C, 89C,221B, 365C	12	-	-	+	+	-	+	1	+
Pear *Pyrus communis* 46B, 182C, 192C, 262A	4	-	-	+	+	-	+	1	+
Apple *Malus* 131C, 307E, 580F	3	-	-	+	+	-	+	1	+
Peach *Prunus cerasifera* 103D, 104D, 104aD	3	-	-	+	+	-	+	1	+
Plum *Prunus cerasifera* 122D, 568F	2	-	-	+	+	-	+	1	+

Explanation: Letters refer to the origin of isolates: A - Prusy, B - Dąbrowice, C - Wróblowice, D - private nursery Julków, E - Gołębiew, F - Kamień;
+/– reaction positive/negative

Supplied by the British Library - "The world's knowledge" www.bl.uk

Table 4. Phenotypic characters of *Agrobacterium tumefaciens* reference strains

Origin code	Gram reaction	3-ketolactose	Utilization of sodium citrate	Utilization of L-tyrosine	Ferric ammonium citrate	Acid from erythritol	Tolerance to NaCl(%)	Oxidase c
B6 (biovar 1) tomato (P. Manigault, France)	-	+	-	-	+	-	4	+
ACH5 (biovar 1) unknown (M. van Montagu, Belgium)	-	+	-	-	+	-	4	+
C58 (biovar 1) sweet cherry (R. Dickey, USA)	-	+	-	-	+	-	4	+
K27 (biovar 2) poplar (A. Kerr, Australia)	-	-	+	+	-	+	1	+

Explanation: +/– reaction positive/negative

Supplied by the British Library - "The world's knowledge" www.bl.uk

All isolates were Gram negative and had the same phenotypic character (Tab. 3). They did not oxidize lactose, did not form a dark-brown pellicle of ferric ammonium citrate and did not multiply in salinity above 1 %. However, they were able to utilize sodium citrate and L-tyrosine, produced acid from erythritol and contained cytochrome oxidase. The above characteristics allowed classification of all isolates, in the same was as reference strain K27, as belonging to biovar 2 *A. tumefaciens*. None of them was similar to reference strains: B6, ACH5 and C58 classified as biovar 1 (Tab. 4).

DISCUSSCION. The present investigations show that all the isolates of *Agrobacterium tumefaciens* originating from 6 fruit tree nurseries belonged to biovar 2. Similarily, in several other countries this biovar was the only one or dominating taxon among *A. tumefaciens* isolated from several fruit species (Alconero, 1980; Bouzar et al., 1983; Dhanvantari, 1978; Kerr, 1969; Lopez et al., 1983; Moore and Allen,1977; Panagropoulos and Psallidas, 1973; Süle, 1978). However, in Holland and New Zealand only biovar 1 of this bacterium was isolated (Moore et al., 1988; Spiers, 1979).

Tests used to determine the physiological and biochemical characters of bacteria and to recognize biovars were chosen on the basis of the Moore's et al. (1988) guide which in turn was founded on investigations of Keane et al. (1970). Despite its wide application and acceptance, there are some doubts about such classification. Du Plessis et al. (1984) think that testing a greater number of bacteria will lead to the formation of new phenotypic groups within proposed biovars, and traditional methods are not always adequate. So, the question remains, what are the practical and theoretical implications of the observed variability of bacteria and of the value of their individual characters in their taxonomy. Keane et al. (1970) are of the opinion that *A. tumefaciens* should not be recognized as a separate species because the phenotypic traits of these isolates classed to that species (coded on chromosome) do not differ from nonpathogenic bacteria. Thus only *A. radiobacter* should be recognized as a species to which a new one-word denomination can be added describing the pathogenicity of a certain isolate when needed. Holmes and Roberts (1981) suggest adopting the species names *A. tumefaciens* and *A. rhizogenes* for the two main groups of agrobacteria and to reject *A. radiobacter*. Du Plessis et al. (1984) are of the same opinion, accepting the employment of terms such as: tumorigenic, rhizogenic and saprophytic, to describe the actual state of isolates tested. Nevertheless, the suggestion of Keane et al. (1970) seems to be more proper.

Supplied by the British Library - "The world's knowledge" www.bl.uk

Despite the similarity of our own isolates of *A. tumefaciens* in respect to phenotypic features, determining their place in the biovar, they varied in sensitivity to agrocin 84, a bacteriotoxin produced by K84 strain of *A. radiobacter.* It was proved that 29 out of 41 isolates appeared to be sensitive to a varying extent to this toxin. The width and character of growth inhibition zone of bacteria tested were employed as criterion of sensitivity by other authors (Alconero, 1980; Du Plassis et al., 1984; Lopez et al., 1983; Mirow and Knösel, 1986; Moore, 1977). It is only the rating scale which is not precise and can not be employed to compare strain sensitivity in various countries. But the percentage share of sensitive isolates could serve as a comparison. Lopez et al. (1983) in Spain have proved that 70% of isolates belonging to biovar 2 were sensitive to agrocin and 84% of those belonging to biovar 1 were also. Similar sensitivity of bacteria belonging to biovar 2 is reported from Greece (Kerr and Panagopoulos, 1977). Among over 20% of resistant isolates most was derived from grapevine and belonged to biovar 3. Similar resistance to agrocin was shown by isolates from grapevine in Israel (Haas et al., 1991) and Germany (Bien et al., 1990) as well as becteria isolated in Germany from various plant species (Mirow and Knösel, 1986). According to Dhanvantari (1983) all Canadian isolates originating from grapevine were also resistant to agrocin. In Australia, Kerr and Htay (1974) classified all isolates belonging to biovar 2 and originating from stone fruit trees as sensitive, but 30 % of biovar 1 isolates were resistant. Among South African isolates more than half appeared to be sensitive (Du Plessis et al., 1985).

The present review shows that *A. tumefaciens* isolates were sensitive to agrocin to a varied degree and the proportion of sensitive isolates among Polish isolates is comparable. Generally, isolates classified as biovar 2 were more sensitive than these belonging to biovar 1, whereas these originating from grapevine and belonging to biovar 3 were resistant to agrocin.

Das et al. (1978) relate toxic action of agrocin 84 to blocking synthesis of DNA in sensitive strains, but Moore and Warren (1979) relate it also to a change of cell membrane permeability. Bacteria resistance to agrocin may also be caused by the presence of degrading enzymes or inhibitors of agrocin synthesis. It was proved that agrocin is specifically transported to sensitive cells through the system agrocinopin-permease (Hayman and Farrand, 1988). Results obtained by Kerr and Roberts (1976) showed close relationship between bacteria pathogenicity, their ability to catabolize opines and their sensitivity to agrocin, but it is not a rule. Moore and Warren (1979) suggest that the above mechanism is coded on a chromosome of pathogenic bacteria.

Supplied by the British Library - "The world's knowledge" www.bl.uk

ACKNOWLEDGMENT. The author is grateful to Ing. St. Berczyński for his help during this research and to Prof. Dr B. Borkowska for kind supplying of cherry cultures.

REFERENCES

Alconero R. 1980. Crown gall of peaches from Maryland, South Carolina and Tennessee and problems with biological control. PLANT DIS. 64: 835-838.

Anderson A.R., Moore L.W. 1979. Host specificity in the genus *Agrobacterium*. PHYTOPATH. 69: 320-323.

Arsenijevic M., Stojic J., Panic M. 1974. Contribution to the study of bacterial canker of the grapevine *(Agrobacterium tumefaciens)*. ZASTITIA BILJA 25: 257-264.

Bernaerts, M.J., De Ley J. 1963. A biochemical tests for crown gall bacteria. NATURE 197: 406-407.

Bien E., Lorenz D., Eichhorn K., Plapp R. 1990. Isolation and characterization of *Agrobacterium tumefaciens* from the German vineregion Rheinpfalz. Z. FUR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ 97, 3: 313-322.

Bouzar H., Moore, L.W., Schaad N.W. 1983. Crown gall of pecan: A survey of *Agrobacterium* strains and potential for biological control in Georgia. PLANT DIS. 67: 310-312.

Bradbury J.F. 1970. Isolation and preliminary study of bacteria from plants. REV. PLANT PATHOL. 49: 213-218.

Cleveland G.L., Goodman R.N. 1987. A proposed basis for varietal differences in sensitivity of grapes to crown gall disease. PROC. 6 TH INT. CONF. PLANT PATH. BACTERIA, Beltsville, USA: 101-102.

Cooksey D.A., Moore L.W. 1982. High frequency spontaneous mutations to agrocin 84 resistance in *Agrobacterium tumefaciens* and *A. rhizogenes*. PHYSIOL. PLANT PATHOL. 20: 129-135.

Das P.K., Basu M., Chatterjee G.C. 1978. Studies on the mode of action of agrocin 84. J. ANTIBIOT. 31: 490-492.

Dhanvantari B.N. 1978. Characterization of *Agrobacterium* isolates from stone fruits in Ontario. CAN. J. BOT. 56: 2309-2311.

Dhanvantari B.N. 1983. Etiology of grape crown gall in Ontario. CAN.J. BOT. 61: 2641-2646.

Supplied by the British Library - "The world's knowledge" www.bl.uk

Du Plessis H.J., Hattingh M.J., van Vuuren H.J.J. 1985. Biological control of crown gall in South Africa by *Agrobacterium radiobacter* strain K84. PLANT DIS. 4: 302-305.

Du Plessis H.J., van Vuuren H.J.J., Hattingh M.J. 1984. Biotypes and phenotypic groups of strains of *Agrobacterium* in South Africa. PHYTOPATH. 74: 524-529.

Haas J., Zveibil A., Zutra D., Tanne E., Manulis S. 1991. The presence of crown gall of grape incited by *Agrobacterium tumefaciens* biovar 3 in Israel. PHYTOPARASITICA 4: 311-318.

Hayman G.T., Farrand S.K. 1988. Characterization and mapping of the agrocinopine-agrocin 84 locus on the nopaline Ti plasmid pTiC58. J. BACTERIOL. 170: 1759-1767.

Hayward A.C. 1964. Characteristics of *Pseudomonas solanacearum*. J. APPL. BACTERIOL. 27: 265-277.

Holmes B. 1988. Taxonomy of *Agrobacterium*. ACTA HORT. 225: 47-52.

Holmes B., Roberts P. 1981. The classification, identification and nomenclature of agrobacteria. J. APPL. BACTERIOL. 50: 443-467.

Kado C.I. 1991. Molecular mechanisms of crown gall tumorogenesis. CRITICAL REV. IN PLANT SCI. 1: 1-32.

Katz B.H., Yanofsky M., Burr T.J., Nester E.. 1987. Host range, virulence and genetic variability of *Agrobacterium tumefaciens* strains from New York vineyards. PROC. 6TH INT. CONF. PLANT PATH. BACTERIA, Beltsville, USA: 103-104.

Keane, P.J., Kerr A., New P.B. 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. AUST. J. BIOL. SCI. 23: 585-595.

Kerr A. 1969. Crown gall of stone fruit. I. Isolation of *Agrobacterium tumefaciens* and related species. AUST.J. BIOL. SCI. 22: 111-116.

Kerr A., Htay K. 1974. Biological control of crown gall through bacteriocin production. PHYSIOL. PLANT PATH. 4: 37-44.

Kerr A., Panagopoulos P.G. 1977. Biotypes of *Agrobacterium radiobacter var.tumefaciens* and their biological control. PHYTOPATH. 90: 172-179.

Kerr A., Roberts W.P. 1976. *Agrobacterium*: Correlations between and transfer of pathogencity, octopine and nopaline metabolism and bacteriocin 84 sensitivity. PHYSIOL. PLANT PATHOLOGY 9: 205-211.

Kersters K., De Ley J. 1984. *Agrobacterium* Conn 1942. In:N.R. Krieg, (ed.), Bergey's Manual of Systematic Bacteriology. vol.1. The Williams and Wilkins Co., Baltimore, pp. 244-254.

Supplied by the British Library - "The world's knowledge" www.bl.uk

Kersters K., De Ley J., Sneath P.H.A., Sackin M.1973 Numerical taxonomic analysis of *Agrobacterium*. J. GEN. MICROBIOL. 78: 227-239.

Kovacs N. 1956. Identification of *Pseudomonas pyocyana* by the oxidase reaction. NATURE 178: 703.

Lippincott, J.A., Lippincott B.B., Starr M.P. 1983. The genus *Agrobacterium*. In: Phytopathogenic bacteria. Springer Verlag, New York, pp. 842-855.

Loper J.E., Kado C.I. 1979. Host range conferred by the virulence specifying plasmid of *Agrobacterium tumefaciens*. J. BACTERIOL. 139: 591-596.

Lopez M.M., Miro M., Salcedo C.I., Orive R.J., Temprano F.J. 1983. Caracteristicas de aislados espanoles de *Agrobacterium radiobacter* pathovar *tumefaciens*. An. INIA/Ser. Agric./N. 24: 239-249.

Mirow H., Knösel D. 1986. Differential diagnostische Untersuchung von Erregerstammen der Wurzelkröpfkrankheit, *Agrobacterium tumefaciens*, in Zusammenhang mit Versuchen zur Biologischen Bekämpfung. Z. FUR PFLANZENKRANKHIETEN UND PFLANZENSCHUTZ 2: 153-162.

Moore L.W. 1977. Prevention of crown gall on *Prunus* roots by bacterial antagonsits. PHYTOPATH. 67: 139-144.

Moore L.W., Allen J. 1977. Comparison of selective and differential media for *Agrobacterium* species. PROC. AM. PHYTOPATH. SOC. 4: 209.

Moore L.W., Warren G. 1979. *Agrobacterium radiobacter* strain 84 and biological control of crown gall. ANN. REV. PHYTOPATHOL. 17: 163-179.

Moore L.W., Kado C.I., Bouzar H. 1988. *Agrobacterium*. In: Laboratory guide for identification of plant pathogenic bacteria, N.W. Schaad, 2nd edition, The American Phytopathological Society, St. Paul, Minnesota pp. 16-36.

Panagopoulos C.G., Psallidas P.G. 1973. Characteristcs of Greek isolates of *Agrobacterium tumefaciens* (E.F. Smith and Towsend) CONN. J. APPL. BACTERIOL. 36: 233-240.

Patel M.K. 1926. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. PHYTOPATH. 16: 577.

Sawada H., Leki H., Takikawa Y. 1990. Identification of grapevine crown gall bacteria. ANN. PHYTOPATH. SOC. JAPAN. 2: 199-206.

Sonoki S., Ireland C.R., Loper J.E.,. Baraka M., Kado C.I. 1978. New genetic determinants of the virulence plasmid of *Agrobacterium tumefaciens*. PROC. 4TH INT. CONF. PLANT PATH. BACTERIA. Angers: 133-142.

Spiers A.G. 1979. Isolation and characterization of *Agrobacterium* species. N.Z.J. AGRIC. RES. 22: 631-636.

Süle S. 1978. Biotypes of *Agrobacterium tumefaciens* in Hungary. J. APPL. BACTERIOL. 44: 207-213.

Suslow T.V., Schroth M.N., Isaka M. 1982. Application of a rapid method for Gram-differentiation of plant pathogenic and saprophytic bacteria without staining. PHYTOPATH. 72: 917-918.

Supplied by the British Library - "The world's knowledge" www.bl.uk