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Xanthomonas campestris pv. populi, the causal agent of bark necrosis in poplar

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Abstract

A bacterium was isolated from superficial bark necroses on young poplars and its pathogenicity demonstrated by inoculation experiments. The organism was identified as *Xanthomonas campestris*. Cross-inoculations showed that a previously undescribed pathovar was involved. It is suggested to designate this organism *X. campestris* pv. *populi*.

Additional keywords: Xanthomonas populi subsp. *populi, Xanthomonas campestris* pv. *campestris, Xanthomonas pelargonii.*

Introduction

Bacteria of the genus *Xanthomonas* Dowson cause many plant diseases and although these diseases vary considerably in pathogenesis and symptom expression, many of them cannot be distinguished from each other by biochemical or serological methods (Dye, 1958). Thus, most of them are assigned to one single species: *Xanthomonas campestris* (Pammel) Dowson.

In 1980 a preliminary report was published on a new member of the *Campestris* group, that was suspected to cause a superficial bark necrosis on *Populus* (De Kam, 1980). The first symptoms of this disease appear in the course of the growing season on the 1-year-old shoots, especially in nursery stock. The attacked bark tissue becomes dark and subsequently necrotic in long streaks. Initially the damage is superficial and does not reach the cambinal zone, but when progressing, necrosis reaches the cambium and the bark may be killed. Cracks appear in the bark and the wood becomes exposed. Sap containing numerous bacteria exudes through the cracks and lenticels and dries to a white powder (Fig. 1).

Bacteria can readily be isolated from the invaded tissues and the exudate by plating on nutrient agar.

The aim of the present study is to verify the pathogenic nature of the isolated organism and provides information on the taxonomical position of the organism.

Material and methods

Isolates examined:
119, *Xanthomonas* sp., isolated April 1979 from *Populus* sp. with bark necrosis, Didam.

Fig. 1. Natural attack of 1-year-old *Populus* 'Robusta' caused by Xanthomonas campestris pv. *populi;* left a healthy shoot.

120, *Xanthomonas* sp., isol. May 1979 from *Populus* sp. with bark necrosis, Wageningen.
121, *Xanthomonas* sp., isol. May 1979 from *Populus* sp. with bark necrosis, Didam.
122, *Xanthomonas* sp., isol. May 1979 from *P. trichocarpa* with bark necrosis, Didam.
128, *Xanthomonas* sp., isol. June 1979 from *Populus* sp. with bark necrosis, Wageningen.
129, *Xanthomonas* sp., isol. August 1979 from *P.* 'Robusta' with bark necrosis, Roggebotsluis. Type strain.
88, *X. populi* subsp. *populi,* May 1976 from *Populus* sp. Lienden.
115, *X. populi* subsp. *populi,* April 1978 from *P.* 'Serotina', Wageningen.
89, *X. populi* subsp. *salicis* (= NCPPB 3038), June 1976 from *Salix dasyclada,* Biesbos.
102, *X. populi* subsp. *salicis* (= NCPPB 3038), June 1976 from *Salix dasyclada,* Biesbos.

102, *X. populi* subsp. *salicis* (= NCPPB 3039), June 1977 from *S. dasyclada,* Biesbos.
91, *X. campestris* pv. *campestris* (reveived from H.P. Maas Geesteranus, his no 102), Sept. 1972 from *Brassica oleracea,* the Netherlands.
124, *X. campestris* pv.*campestris* (NCPPB 1943), from *B. oleracea.*
125, *X. campestris* pv. *campestris* (NCPPB 1645), from *B. oleracea.*
126, *X. campestris* pv. *pelargonii* (received from H.P. Maas Geesteranus, no 273) from *Pelargonium* sp.

Inoculation experiments. In September 1980 seven poplar clones were inoculated with a pure culture of isolate 129. Of each clone four 1-year old plants growing in the nursery were inoculated, whereas two plants served as controls. Bacteria grown for three days on nutrient agar at 27 °C were suspended in demineralized water at a concentration of 10^9 cells/ml and introduced into the bark at several places using a syringe. Control plants were treated the same way with water.

In order to study the host specificity of the *Xanthomonas* isolates from poplar, nine *Xanthomonas* isolates among them four isolates from poplar were each inoculated into three current year shoots of *Populus candicans* and *Salix dasyclada,* and into three young *Brassica oleracea* and *Pelargonium zonale* plants. In addition three plants of each host were used as controls. These cross-inoculations were carried out in a greenhouse in April 1980. Assessments were made four weeks after inoculation. Re-isolations were carried out from a number of inoculated plants, hosts and non-hosts, 1, 2 and 8 months after inoculation.

Identification. Biochemical and physiological characteristics were examined, mainly using the methods described by Pelczar et al. (1957) and Dye (1962). As *X. populi* has a low tolerance of NaCl and does not grow in media without a suitable carbon source, NaCl was omitted in the media and 10 $g.l^{-1}$ dextrose added. Incubation was carried out at 24 °C unless otherwise indicated. The determination of the maximum temperature for growth was carried out on nutrient dextrose agar (NDA) slants in a water bath. NaCl tolerance was determined in nutrient dextrose broth, shaker incubated at room temperature. For the formation of acid from carbohydrates, Dye's medium C was used without NaCl (Dye, 1962). Flagella were stained by Gray's method (Gray, 1926). Hypersensitive reaction was studied using suspensions of 10^9 cells per ml which were injected into leaves of *Nicotiana tabacum* 'White Burley'. As a positive control a *Pseudomonas syringae* isolate from poplar was added. The serological relations of the isolates were studied using the double diffusion technique in agar gel (antiserum dilution 1 : 4) and the micro-agglutination reaction (antiserum dilutions 1 : 32 to 1 : 1024). The antisera used in this study were prepared against isolates 88, 102, 91, 124, 125 and 129 and obtained after whole-cell injections in rabbits. Antisera were absorbed by adding an excess of cross-reacting antigens to the antiserum and incubating for 2h at 28 °C after which the absorbed antiserum was centrifuged for 10 minutes at 13 000*g*, the pellets being discarded.

Results

Inoculation experiment. Of the 28 inoculated plants indicated in Table 1, 11 developed disease symptoms in the course of 1981. The symptoms were identical with those in

nature from which the bacterium originally had been isolated. No symptoms occurred in the control plants.

Plants in the cross-inoculation experiment reacted as indicated in Table 2. Characteristic bacterial cankers developed exclusively on *P. candicans* upon inoculation with isolate 115, whereas isolate 102 caused disease symptoms only on *S. dasyclada*. The *B. oleracea* plants were severely attacted by isolates 91 and 124, whereas *P. zonale* showed characteristic symptoms only after inoculation with isolate 126. So far all isolates consistently reacted host-specifically. The *Xanthomonas* isolates from poplar caused superficial bark necroses not only on poplar, but also on *S. dasyclada*. The bacteria were re-isolated from the bark necroses which had developed upon in-

Table 1. Results of inoculations of seven poplar clones with *Xanthomonas* sp. isolate 129 1 year after inoculation.

Clone	Number of plants			
	inoculated		control	
	total	diseased	total	diseased
Populus candicans	4	3	2	0
Populus 'Brabantica'	4	3	2	0
Populus 'Grandis'	4	3	2	0
Populus 'Robusta'	4	0	2	0
Populus 'Heidemij'	4	1	2	0
Populus 'Geneva'	4	0	2	0
Populus nigra 'Italica'	4	1	2	0
	28	11	14	0

Table 2. Results of cross-inoculations of four different hosts, with 9 *Xanthomonas* isolates 4 weeks after inoculation.

Isolate no.	Host			
	Populus candicans	*Salix dasyclada*	*Brassica oleracea*	*Pelargonium zonale*
115	+[1]	−	−	−
102	−	+	−	−
91	−	−	+	−
124	−	−	+	−
126	−	−	−	+
119	+	+	−	−
120	+	+	−	−
121	+	+	−	−
128	+	+	−	−

[1] + = typical disease symptoms after inoculation, − = no disease symptoms.

Table 3. Re-isolation of Xanthomonads from hosts and non-hosts at various times after inoculation in the greenhouse.

Isolate no.	Months after inoculation											
	Populus Candicans			*Salix dasyclada*			*Brassica oleracea*			*Pelargonium zonale*		
	1	2	8	1	2	8	1	2	8	1	2	8
115	+[1]	+	+	+	−	○	−	−	−	+	−	−
102	−	−	○	+	+	○	−	−	○	+	+	−
91	+	−	○	−	+	○	+	+	+	+	+	−
126	+	−	○	+	−	○	+	+	○	+	○	○
119	+	+	○	+	+	○	+	+	○	+	+	−
120	○	○	○	○	○	○	○	○	+	○	○	+
121	○	○	○	○	○	○	○	○	+	○	○	+

[1] + = re-isolation successful; − = re-isolation failed; ○ = not tested.

oculation, but sometimes also from the bark near the inocultion place of inoculated non-hosts (Table 3). They were identified as the pathogen that had been inoculated.

Taxonomy. All isolates examined were Gram-negative, strictly aerobic rods, oxidase-negative, catalase-positive. They caused an alkaline reaction in litmus milk and showed mucoid growth on nutrient agar containing 10 $g.l^{-1}$ dextrose. With the exception of the *X. populi* isolates, they formed yellow colonies on nutrient agar and NDA. Motility was observed in aqueous suspensions in all but the two *X. populi* subsp. *populi* strains and their movement was shown to be accomplished by one polar flagellum (monotrichic). In isolate 126, however, no flagella could be found in spite of the fact that movement was observed. A hypersensitive reaction in tobacco was caused by *P. syringae* within 17 h. All other isolates did not cause a reaction within 24 h, after which chlorotic spots slowly developed. After 14 days these spots became necrotic. Other metabolic characteristics have been compiled in Table 4.

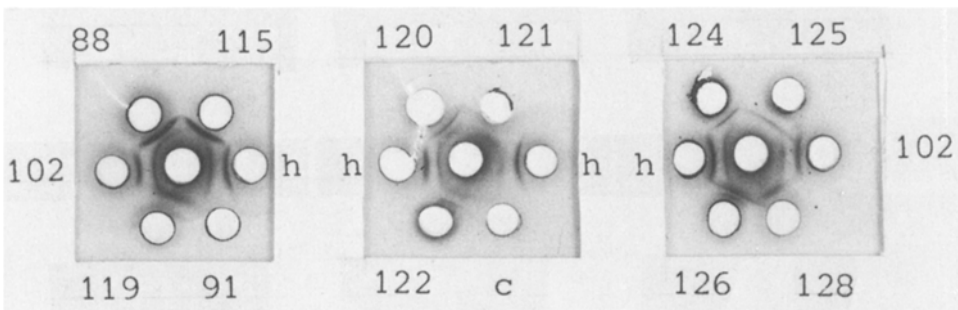

Fig. 2. Precipitation pattern of various Xanthomonads after 48h diffusion in agar gel with an antiserum prepared against *X. populi* subsp. *salicis* isolate 102 (staining with amidoblack). c = control well without antigen, h (homologue) = isolate 102.

Table 4. Physiological characteristics of six *Xanthomonas* isolates from poplar, four isolates of the *Campestris* group and three *X.populi isolates.*

Characteristics	Isolates												
	88	115	102	91	124	125	126	119	120	121	122	128	129
growth on NA	–	–	–	+	+	+	+	+	+	+	+	+	+
max. temp. (°C)	29	29	29	36	37	36	37	35	37	35	37	36	36
NaCl tolerance (%)	0.6	0.6	1.6	>2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
TTC tolerance (%)	0.01	0.02	0.02	0.02	0.1	0.1	0.02	0.05	0.05	0.05	0.05	0.05	0.05
starch hydrolysis	+	+	w[2]	+	+	+	+	+	+	+	+	+	+
gelatinase	–	–	–	+	+	+	+	+	+	+	+	+	+
urease	–	–	–	–	–	–	–	–	–	–	–	–	–
esculin hydrolysis	–	–	–	+	+	+	+	+	+	+	+	+	+
nitrate reduction	–	–	–	–	–	–	–	–	–	–	–	–	–
asparagin[1]	–	–	–	–	–	–	–	–	–	–	–	–	–
tributyrin	–	–	–	+	+	+	+	+	+	+	+	+	+
H_2S (cystein)	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S (peptone)	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S (Na-thiosulphate)	+	+	+	+	+	+	+	+	+	+	+	+	+
breakdown of:													
dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+
galactose	+	+	+	+	+	+	+	+	+	+	+	+	+
xylose	–	–	–	+	+	+	+	+	+	+	+	+	+
sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
sorbose	–	–	–	–	–	–	–	–	–	–	–	–	–
arabinose	+	+	–	+	+	+	+	+	+	+	+	+	+
cellobiose	–	–	+	+	+	+	+	+	+	+	+	+	+
trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+
melibiose	–	–	–	+	+	+	+	+	+	+	+	+	+
raffinose	–	–	–	+	–	–	+	+	+	+	+	+	+
rhamnose	–	–	–	–	–	–	–	–	–	–	–	–	–

lactose	–	–	–	–	–	–	+	+	+	+	+	+	+
ribose	–	–	–	w	w	w	–	+	+	+	+	+	+
mannose	+	+	+	+	+	+	+	+	+	+	+	+	+
melezitose	–	–	–	w	–	–	–	w	w	w	w	w	w
adonitol	–	–	–	–	–	–	–	–	–	–	–	–	–
mannitol	–	–	–	+	w	w	–	+	+	+	+	+	+
inositol	–	–	–	–	–	–	–	–	–	–	–	–	–
malate	+	+	+	+	+	+	+	+	+	+	+	+	+
malonate	–	–	–	+	+	+	+	+	+	+	+	+	+
pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+
gluconate	w	w	–	w	w	w	w	w	w	w	w	w	w
glutarate	+	+	–	+	+	+	+	+	+	+	+	+	+
glutamate	+	+	–	+	+	+	+	+	+	+	+	+	+
lactate	–	–	–	+	+	+	+	+	+	+	+	+	+
acetate	–	–	–	+	+	+	+	+	+	+	+	+	+
salicin	–	–	–	–	–	–	–	+	+	+	+	+	+

[1] As a sole source of carbon and nitrogen.
[2] w = weakly positive or delayed reaction.

Table 5. Results of agglutination (aggl.) and double diffusion (dd) reactions with unabsorbed antisera.

Isolate no.	Antisera against indicated isolates													
	88		102		91		124		125		126		129	
	aggl. titre	dd	aggl. titre	dd	aggl. titre	dd	aggl. titre	dd	aggl. titre	dd	aggl. titre	dd	aggl. titre	dd
88	512	+[1]	512	+	1024	–	1024	+	1024	+	1024	+	256	–
115	1024	+	512	+	512	–	1024	–	1024	+	1024	+	256	–
102	1024	+	1024	+	1024	–	1024	–	1024	+	1024	+	256	–
91	16	w	–	–	512	w	1024	+	1024	+	64	–	–	–
119	1024	+	512	+	1024	–	1024	+	1024	+	1024	+	256	+
120	1024	+	512	+	1024	–	1024	+	1024	+	1024	+	128	+
121	1024	w	1024	+	1024	–	1024	+	1024	+	1024	+	128	–
122	512	+	512	+	1024	–	512	+	1024	+	256	+	128	+
124	256	+	–	+	1024	–	1024	+	1024	+	64	+	64	+
125	–	–	–	+	1024	–	1024	+	1024	+	–	–	32	–
126	256	w	64	+	512	–	1024	+	1024	+	256	+	64	–
128	512	+	256	+	1024	–	1024	+	1024	+	256	+	256	+
129	512	+	256	+	1024	–	1024	+	1024	+	256	+	512	+

[1] – = no reaction; + = clearly visible precipitation bands; w = hardly visible precipitation bands.

The serological experiments demonstrated that most isolates cross-reacted with the various antisera, both in the agglutination and in the double diffusion tests (Table 5, Fig. 2). In the diffusion test antiserum 91 gave a weak positive reaction only with its homologue antigens, while in the agglutination a positive reaction was obtained with various isolates (Table 5). This indicates that antiserum 91 is mainly directed against insoluble antigens and has less activity against soluble antigens. None of the antisera used were sufficiently specific to detect exclusively the species from which the antiserum had been prepared. The problem of specificity could not be resolved satisfactorily by means of cross-absorption, as the titres of the absorbed antisera fell to such low levels that they could no longer be used for routine serodiagnosis.

Discussion

Table 1 demonstrates, that 11 out of 28 plants showed typical disease symptoms after inoculation, while on the 14 control plants no symptoms developed. A statistical analysis of table 1 using Wilcoxon's two-sample test (cf. Sokal and Rohlf, 1969) revealed, that the difference of symptom expression between the inoculated plants and the controls was significant ($P = 0.02$). As the pathogen that had been inoculated was re-isolated from the diseased plant tissue, it is concluded that this *Xanthomonas* is the cause of the bark necrosis on poplar.

The results of the cross-inoculations (Table 2) demonstrate that in *X. campestris* pathovars occur which are adapted to one host (e.g. the *X. campestris* pathovars from *Brassica* and *Pelargonium* sp.) whereas others cause symptoms on more than one host as in the case of *X. campestris* from poplar. The fact that *S. dasyclada* was attacked by the poplar isolates after artificial inoculation suggests that *Salix* is also a host of this pathogen. However, to date no attack on *Salix* by *X. campestris* has been found in nature.

The re-isolations from plants that did not react show that although no symptoms developed the bacteria survived for a number of months in some non-hosts (Table 3). It should, however, be pointed out that this result was obtained after artificial inoculation; it remains to be seen whether such bacterial populations occur in nature and play a role in the survival or dissemination of the pathogen. Our antisera could be used to identify the genus *Xanthomonas,* but were insufficiently specific to distinguish species, subspecies or pathovars. Consequently, identification is best obtained through combined techniques. On poplar, two Xanthomonads occur which can easily be distinguished in culture: *X. populi* does not produce pigment on nutrient dextrose agar, nor does it grow on nutrient agar when dextrose is lacking or at 30 °C, whereas *X. campestris* produces pronounced yellow colonies on nutrient agar and grows excellently at 30 °C.

As to the taxonomical position of the organism causing bark necrosis in poplar, it is obvious from the cultural, physiological and serological characteristics that this organism is a xanthomonad and belongs to the species *X. campestris* (Pammel) Dowson, following the nomenclature in Bergey's Manuel of Determinative Bacteriology (1974). It differs in many aspects from the two subspecies of *X. populi,* but only differs from the other *X. campestris* isolates in its reaction to salicin. *X. campestris* from poplar, however, also differs in pathogenicity, as shown by the cross-inoculations (Table 2). It is therefore suggested that it be designated a pathovar of *X.*

campestris, according to the nomenclature proposed by Dye et al. (1980). As present knowledge indicates that the main host of this organism is poplar, it it proposed to name this newly described organism *Xanthomonas campestris* pv. *populi.*

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Samenvatting

Xanthomonas campestris pv. populi, de oorzaak van bastnecrose bij populier

Uit een oppervlakkige bastnecrose bij jonge populieren werd massaal een bepaalde bacterie geïsoleerd. Met deze bacterie werden gezonde populieren in het veld geïnoculeerd via verwonding van de bast. Als gevolg van de inoculaties ontwikkelden zich bij ongeveer 40% van de geïnoculeerde bomen hetzelfde type bastnecrosen, terwijl bij de controleplanten geen enkele reactie optrad. Uit de kunstmatig verkregen necrosen werd dezelfde bacterie geïsoleerd.

Identificatie met biochemische en serologische methoden toonde aan dat de bacterie *Xanthomonas campestris* was.

Vervolgens werden in de kas kruisinoculaties uitgevoerd met verschillende xanthomonaden op populier, wilg, kool en geranium. De *X. campestris* isolaten uit populier tastten behalve populier ook wilg aan. De andere gebruikte stammen waren waardplant-specifiek, al bleven sommige ervan minstens acht maanden in leven in een niet-waardplant, evenwel zonder symptomen te veroorzaken. Geconcludeerd wordt, dat de bastnecrosen zijn veroorzaakt door een nog niet beschreven pathovar van *X. campestris.* Voorgesteld wordt om deze bacterie *Xanthomonas campestris* pv. *populi* te noemen.

References

Bergey's Manual of determinative bacteriology, 1974, 8th ed. The Williams & Wilkins Company, Baltimore.

Dye, D.W. 1958. A taxonomic study of the genus *Xanthomonas.* Thesis, Univ. Edinburgh.

Dye, D.W., 1962. The inadequacy of the usual determinative tests for identification of *Xanthomonas* spp. New Z. Jl. Sci. 5: 393-461.

Dye, D.W., et al., 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Rev. of Plant Pathol. 59: 153-168.

Gray, P.H.H., 1926. A method of staining bacterial flagella. J. Bact. 12: 273-274.

Kam, M. de, 1980. Three Xanthomonads on poplar and willow in the Netherlands. Proc. FAO/IUFRO Symp. on Resistance mechanisms in poplar, Poland p. 169-171.

Pelczar, M.J. Jr. et al., 1957. Manual of microbiological methods by the Society of American Bacteriologists. Mc-Graw-Hill Book Cy, New York, Toronto, London.

Sokal, R.R. & Rohlf, F.J. 1969. In: Biometry. Freeman and Cy., San Francisco, p. 391-393.