

## Studies on the resistance of poplar hybrids to *Aplanobacterium populi* (Smith) strain Ridé.

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In recent years considerable development of studies on the selection and resistance of poplars to serious fungal and bacterial diseases is observable. In a tabular listing of such studies presented by Gerhold (1969) for the Second World Consultation on Forest Tree Breeding (Washington, 7—16 August 1969) poplars have the most prominent position among the broadleaf species.

Simultaneously in many countries after a closer investigation of the poplar canker caused by the bacterium *Aplanobacterium populi* (Smith) Ridé interest in this serious disease increased. The disease leads to the formation of a canker, which develops on a stem and on branches of poplars susceptible to the disease. Necrosis of the bark develops already one year after infection. Infection of resistant clones of poplars results in a complete healing over of the wound without symptoms of the canker. The disease is particularly serious for trees over 10 years old. It is commonly considered as one of the more serious diseases of poplars in central and western Europe. The most effective method of controlling the disease is by cultivation of resistant poplar varieties.

In the years 1968—69 in the Institute of Dendrology and Kórnik Arboretum of the Polish Academy of Sciences and in the Institute of Microbiology of the Warsaw University, the bacterial canker of poplars has been intensively studied (Danilewicz, Siwecki 1970a, 1970b; Danilewicz, Janowska, Siwecki 1970).

The aim of the studies reported here was to test the resistance of poplar hybrids to *Aplanobacterium populi* (Smith) following artificial inoculation with a strain of the disease obtained from Dr. M. Ridé, France.

### MATERIALS AND METHODS

**The experimental material.** Two experiments on artificial inoculation have been conducted in 1968 and 1969 in the greenhouses of the Institute of Dendrology and Kórnik Arboretum of the Polish Aca-

Table 1

List of poplars used in the 1968 experiment\*

No.	Cultivar	Hybrid	Origin and year of introduction in Kórnik	No. in the Kórnik Arboretum inventory
1	2	3	4	5
		Section <i>AIGEIOS</i> DUBY		
1	<i>P. 'Gelrica'</i>	<i>'Marilandica' × 'Serotina'</i>	Arnhem 1938 (Holandia)	8310
2	<i>P. 'I-214'</i>		Casale Monferrato 1959 (Włochy)	11246
3	<i>P. 'Marilandica'</i>	<i>nigra × 'Serotina'</i>	Puławy (Polska) 1950	3048
4	<i>P. 'Regenerata Grandis'</i>	<i>nigra × 'Serotina'</i>	Weener (Niemcy) 1925	2539
5	<i>P. 'Robusta'</i>	<i>angulata 'Cordata' × nigra plant.</i>	L. Spaath (Niemcy) 1925	3395
6	<i>P. 'Serotina'</i>	<i>nigra × deltoides</i>	Arboretum Kórnik 1955	385
7	<i>P. 'Sarce rouge'</i>	<i>nigra × deltoides</i>	Arnhem 1938 (Holandia)	8346
		Section <i>TACAMA-</i> <i>HACA</i> SPACH.		
8	<i>P. 'Geneva'</i>	<i>maximowiczii × berol.</i>	Morton Arb. USA 1938	7838
9	<i>P. 'Hybr. 194'</i>	„	„	7816
10	<i>P. 'Hybr. 275'</i>	<i>maximowiczii × trichocarpa</i>	„	7828
11	<i>P. 'Hybr. 277'</i>	<i>maximowiczii × berol.</i>	„	7829
12	<i>P. 'Kórnik 6'*</i>	<i>maximowiczii × trichocarpa</i>	Arboretum 1950 Kórnik	—
13	<i>P. 'Oxford'</i>	<i>maximowiczii × berol.</i>	Morton Arb. (USA) 1938	7840

\* The cultivar P. Kórnik 6 is a clone from the hybrid progeny *P. maximowiczii × P. trichocarpa* obtained from controlled pollinations in Kórnik in 1950. The progeny was numbered PK-14 and the clone 14—59 (Bugata and Stecki 1961, Stecki 1967).

Table 2

List of poplars used in the 1969 experiment

No.	Cultivar	Hybrid	Origin and year of introduction in Kórník	No. in the Kórník Arboretum inventory
1	2	3	4	5
1	<i>P. 'Androscoggin'</i>	<i>maximowiczii</i> × <i>trichocarpa</i>	Morton Arb. USA 1938	7815
2	<i>P. 'Geneva'</i>	<i>maximowiczii</i> × <i>berol.</i>	„	7838
3	<i>P. 'Hybr. 194'</i>	„	„	7816
4	<i>P. 'Hybr. 275'</i>	<i>maximowiczii</i> × <i>trichocarpa</i>	„	7828
5	<i>P. 'Hybr. 277'</i>	<i>maximowiczii</i> × <i>berol.</i>	„	7829
6	<i>P. 'Hybr. 280'</i>	„	„	7830
7	<i>P. 'Hybr. 282'</i>	<i>angulata</i> × <i>berol.</i>	„	7831
8	<i>P. 'Hybr. 283'</i>	„	„	7832
9	<i>P. 'Oxford'</i>	<i>maximowiczii</i> × <i>berol.</i>	„	7840
10	<i>P. 'Rochester'</i>	<i>maximowiczii</i> × <i>nigra</i> plant.	„	7841

demy of Sciences. For each of the experiments the material was obtained in the form of cuttings from a clone archive. In the 1968 experiment cuttings of 13 poplar varieties representing section Aigeiros and section Tacamahaca have been used (Table 1).

For the 1969 experiment cuttings from 10 poplar varieties have been used all from section Tacamahaca, brought to Kórník Arboretum from Morton Arboretum, USA in 1938 (Table 2).

Table 3

Dates on which the main experimental treatments were performed

Place and year of experiment	Sequence of treatments				
	Cutting and earting of cuttings	Plating of cuttings in pots	Multiplication of <i>A. populi</i> culture	Artificial inoculation	Termination of exp. and scoring of results
1	2	3	4	5	6
Greenhouse 1968	27.I.—31.I.	18.III.	9.V.	11.V.—12.V.	26.VII.
Greenhouse 1969	10.II.—13.II.	6.III.	10.V.	12.V.—14.V.	25.VII.

The cuttings have been rooted in pots by the method described earlier (Siwecki 1969). The dates on which the various functions were performed are presented in table 3.

**Lay-out of the experiment.** In the 1968 experiment each of the poplar varieties mentioned in table 1 has been represented by 36 well rooted cuttings that have been artificially inoculated and the same number of uninoculated control cuttings.

In the 1969 experiment each of the 10 studied poplar varieties (Table 2) has been represented by a different number of cuttings ranging from 4 to 11, for both the inoculated and the control variables, all well rooted in pots.

Both the experiments were given a random arrangement in the greenhouse, so that each cutting constituted one experimental replicate. This arrangement has permitted the use of an analysis of variance and a Duncan test (Snedecor 1956) in the interpretation of results.

**Preparation of the inoculate.** A pure strain of the bacterium *A. populi* used in the experiments has been obtained directly from Dr. M. Ridé in France on the 3rd of February 1967. This pathogen has been isolated by Dr. Ridé from a cankerous tissue of *Populus* 'Serotina' in April 1966 (Ridé 1967). After the strain was obtained its virulence was tested by infecting rooted 1, 2 and 3 year old plants of *Populus* 'Bachelierie'. The occurrence of characteristic swelling and slime at the point of infection (under buds or on the bud scar on which the bacterial material was applied with a brush) was recorded as symptomatic evidence that Ridé strain has maintained its virulence.

For this studies on the resistance of poplars the bacterial strain was stored on agar slants at a temperature of  $+4^{\circ}\text{C}$ . The strain was stored and multiplied on a fluid medium or on a solidified one with 1.5% agar. It contained 1% glucose, 0.5% bacto peptone Difco, 0.5% yeast extract Difco and distilled water, it had a  $\text{pH} = 7.2$ , and was sterilized at  $3/4$  atmospheres for 15 minutes. Composition of the medium was suggested by Ridé (1967). The strain was incubated at a temperature of  $22-25^{\circ}\text{C}$ . A bacterial culture in the form of a dense slime has been used for the experimental inoculations.

The times at which the bacterial cultures were multiplied and the poplars inoculated for both the experiments are indicated in table 3 columns 4 and 5.

**Methods of artificial inoculation.** Artificial inoculation of the poplars with a strain of *A. populi* has been conducted in the greenhouse on the dates indicated in table 3 column 5 maintaining as far as possible sterile conditions.

In the 1968 experiment the culture of the inoculate has been picked up from agar slants with the help of a sterile brush penicil and then applied directly onto the whole surface of the fresh wound caused by the

removal of the lowest green shoot from the rooted cutting (Fig. 1, 5A). The infected place was covered with a disc of sterile 2% agar and fastened with dressing plaster. The agar prevented the quick drying out of the inoculum. From one agar slant 6 cuttings were inoculated. In uninoculated control cuttings, the lowest green shoot was also removed and the wound

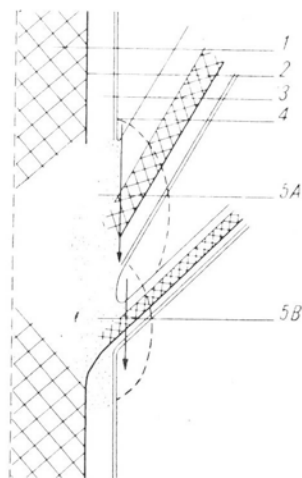


Fig. 1. Longitudinal section through a node on a one year old shoot of poplar (a cutting)

1 — xylem, 2 — cambium, 3 — phloem and bark, 4 — cork, 5A — lateral shoot gap, 5B — supporting leaf gap. The arrows indicate the place where bacterial culture was applied. The stippled area indicates the range of infection caused by the inoculation by the methods of 1968 and 1969. Swelling of the cankerous tissue has been indicated by a broken line.

formed was covered as before with an agar disc and fastened with the dressing plaster. The green, fast growing upper shoot with leaves, that developed on the cuttings, both inoculated and controls, was left to grow freely.

In the 1969 experiment the rooted cuttings of the studied poplars have been inoculated by the method of "the fresh leaf scar" (Anonym 1966; Lange 1968). In this method, the inoculum was applied with a brush penicil into a fresh leaf scar formed by the removal of the leaf supporting the lowest green shoot (Fig. 1, 5B). After inoculation the lower shoot has been reduced to a length of about 5 cm. The uninoculated control cuttings were treated in the same manner. Similarly as in the 1968 experiment the upper fast growing green shoot has been left intact.

**Evaluation of the infected cuttings.** When the experiment was terminated on the dates indicated in table 3, column 6, the degree to which each of the cuttings was affected was estimated according to a two point scale:

- 1° — no symptoms of infection (healthy cuttings).
- 2° — symptoms of infection observable (sick cuttings).

The symptoms of the disease as observed in the place of infection were as follows: a changed, light colour of bast, a large wound, necrosis and an excessive growth of pith tissue in the region of the stem gap and supporting leaf gap.

Uninoculated cuttings, as well as the inoculated ones in which symptoms of the disease were not observed have been cut longitudinally through the stem. In these cuttings no evidence of the disease has been observed.

**Statistical analysis.** The results obtained in the two experiments on the infection of cuttings (Tables 4 and 6) have been subjected to an analysis of variance. The analyses were performed on angular values obtained following a transformation according to the Freeman-Tukey tables as published by Mosteller and Youtz (1961). The control cuttings, none of which showed symptoms of infection, were not included in the analyses.

In the 1968 experiment each of the varieties was represented by 36 cuttings, which has enabled us to perform the variance analysis directly on the angular values obtained from transformation using a theoretical value for the error term. Since in the 1969 experiment the number of inoculated cuttings per cultivar varied an analysis of variance with unequal sub-class numbers had to be performed.

In order to evaluate the differences in the degree of infection more accurately the Duncan test was used and its result is presented in figures 2 and 3.

Also in the 1968 experiment analyses of variance have indicated significant differences for 3 growth characters between the inoculated and the uninoculated cuttings of 4 of the studied cultivars (Table 5).

## RESULTS

Results of the experiment performed in 1968 are presented in table 4. Value of the coefficient  $F$  is significant at 99% level, which indicates that individual poplar varieties differed to a large extent between each other in the degree of susceptibility to the disease. These differences have been compared by the Duncan test, the result of which is presented in fig. 2. As can be seen from fig. 2. statistically significant differences in the susceptibility of poplar varieties have been considerable and the varieties can be allocated into 3 groups. Least affected and therefore most resistant were the varieties *P. 'I-214'*, *P. 'Marilandica'*, *P. 'Regenerata Grandis'*, *P. 'Gelrica'*, *P. 'Robusta'* and *P. 'Sarce rouge'*. In the group of medium susceptibility can be included the following varieties: *P. 'Hybr. 277'*, *P. 'Serotina'*, *P. 'Hybr. 194'* and *P. 'Geneva'*. Most seriously affected and therefore least resistant were the varieties *P. 'Hybr. 275'*, *P. 'Oxford'* and

Table 4

Results of the infection of cuttings artificially inoculated in the 1968 experiment

No.	Cultivar	Total no. of cuttings	No. of cuttings		Angular values
			healthy scale 1°	affected scale 2°	
1	2	3	4	5	6
1	<i>P. 'Gelrica'</i>	36	27	9	30.44
2	<i>P. 'I-214'</i>	36	34	2	14.99
3	<i>P. 'Marilandica'</i>	36	31	5	22.66
4	<i>P. 'Regenerata Grandis'</i>	36	27	9	30.44
5	<i>P. 'Robusta'</i>	36	25	11	33.88
6	<i>P. 'Serotina'</i>	36	13	23	52.84
7	<i>P. 'Sarce rouge'</i>	36	25	11	33.88
8	<i>P. 'Geneva'</i>	36	8	28	61.37
9	<i>P. 'Hybr. 194'</i>	36	13	23	52.84
10	<i>P. 'Hybr. 275'</i>	36	1	35	78.55
11	<i>P. 'Hybr. 277'</i>	36	16	20	48.10
12	<i>P. 'Kórník 6'</i>	36	0	36	85.27
13	<i>P. 'Oxford'</i>	36	0	36	85.27

Table 5

Results of variance analyses for three growth characters when comparing inoculated and control (uninoculated) cuttings in the 1968 experiment, separately for each cultivar\*

No.	Cultivar	Value of coefficient F		
		Character I Shoot length increment in cm.	Character II Diameter of cutting in cm.	Character III Dry weight of roots in g.
1	<i>P. 'Gerlica'</i>	0.62	1.08	0.72
2	<i>P. 'I-214'</i>	2.26*	1.16	0.62
3	<i>P. 'Marilandica'</i>	1.08	1.30	0.77
4	<i>P. 'Regenerata Grandis'</i>	1.83	3.55*	1.43
5	<i>P. 'Robusta'</i>	0.31	1.55	0.23
6	<i>P. 'Serotina'</i>	1.71	0.92	0.78
7	<i>P. 'Sarce rouge'</i>	1.46	1.57	0.97
8	<i>P. 'Geneva'</i>	2.88*	0.18	0.01
9	<i>P. 'Hybr. 194'</i>	0.32	1.26	1.29
10	<i>P. 'Hybr. 175'</i>	1.68	0.25	0.45
11	<i>P. 'Hybr. 277'</i>	1.99	0.97	2.31*
12	<i>P. 'Kórník 6'</i>	1.14	1.68	0.21
13	<i>P. 'Oxford'</i>	0.95	0.73	1.80

*P. 'Kórnik 6'*. It can be generally assumed that the poplars belonging to section *Aigeiros* are relatively more resistant to the disease than poplars from section *Tacamahaca*.

In the evaluation of the material from the 1968 experiment in each of the 13 poplar varieties for the inoculated and control cuttings the following three growth characters have been considered:

Character I — Increment of shoots in length in mm from the 14th of May to the 26th of July.

Character II — Diameter of cuttings in cm.

Character III — Dry weight of roots in g.

The value of the coefficient *F* for the three characters has been presented in table 5. As can be seen from table 5 statistically significant differences were obtained between the inoculated and uninoculated cuttings at 95% level of significance only in four of the poplar varieties. This was so in *P. 'I-214'*, the least affected cultivar (Table 4, Fig. 2) in which

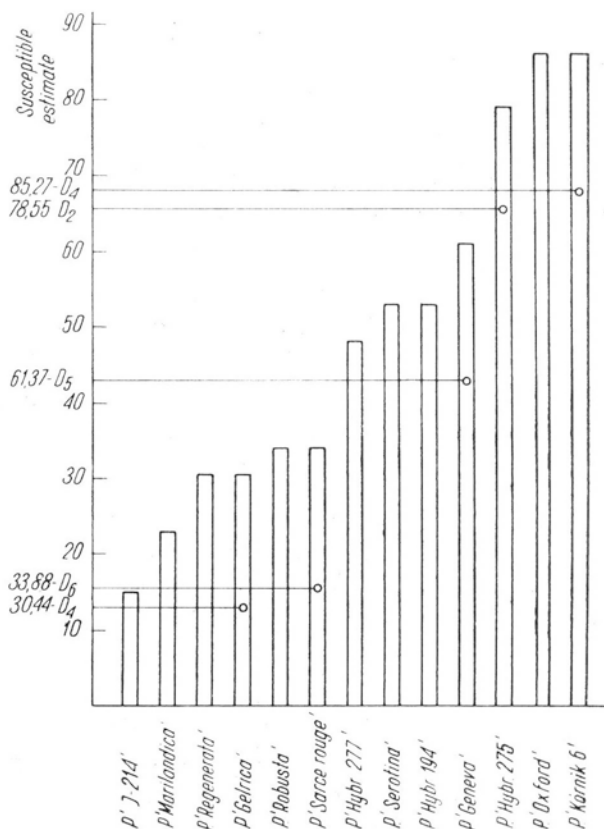


Fig. 2. Results of Duncan test on the susceptibility of 13 poplar cultivars to infection by *A. populi* in the 1968 experiment.



inoculation resulted in a considerable increase of the height growth increment compared with the controls. The increment was 202 mm following inoculation and only 172 mm on uninoculated cuttings.

On the other hand in *P. 'Geneva'* a considerable inhibition of extension growth was observed as a result of inoculation. Mean height of the shoots was 52 mm compared with the controls in which it was 96 mm. This was not confirmed in the 1969 experiment.

The diameter of cuttings differed significantly in *P. 'Regenerata Grandis'* in which the mean diameter in the inoculated variant was 0.77 cm while in the uninoculated controls the average diameter was 0.65 cm.

Also a statistically significant difference was observed between the inoculated and control cuttings of *P. 'Hybr. 277'* in the dry weight of roots. The mean value for the inoculated cuttings was 1.32g and for the controls 1.27 g. This was confirmed in the 1969 experiment.

For the remaining poplar varieties the differences between inoculated and control cuttings for the three growth characters have not been shown to be statistically significant.

Results of the 1969 experiment are presented in table 6. The value of coefficient F is significant at 99% level in this experiment, indicating that

Table 6

Results of the infection of cuttings artificially inoculated in the 1969 experiment

No.	Cultivar	Total no. of cuttings	No of cuttings		Angular values	
			healthy scale 1°	affected scale 2°	sums	averages
1	2	3	4	5	6	7
1	<i>P. 'Androskoggin'</i>	10	1	9	630.00	63.00
2	<i>P. 'Geneva'</i>	10	1	9	630.00	63.00
3	<i>P. 'Hybr. 194'</i>	11	7	4	427.50	38.86
4	<i>P. 'Hybr. 275'</i>	8	3	5	405.00	50.62
5	<i>P. 'Hybr. 277'</i>	9	4	5	427.50	47.50
6	<i>P. 'Hybr. 280'</i>	6	4	2	225.00	37.50
7	<i>P. 'Hybr. 282'</i>	4	4	0	90.00	22.50
8	<i>P. 'Hybr. 283'</i>	11	8	3	382.50	34.77
9	<i>P. 'Oxford'</i>	8	2	6	450.00	56.25
10	<i>P. 'Rochester'</i>	10	4	6	495.00	49.50

the poplar cultivars differed in the degree of susceptibility to *A. populi* infection. In order to evaluate the differences better the Duncan test was employed, the results of which are presented in figure 3.

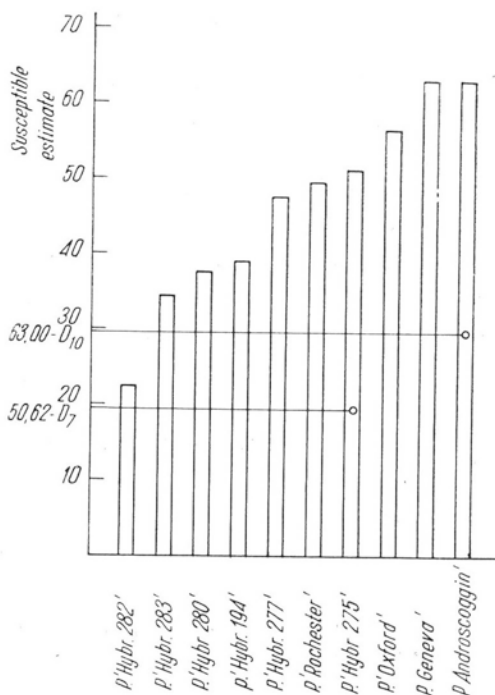


Fig. 3. Results of Duncan test on the susceptibility of 10 poplar cultivars to infection by *A. populi* in the 1969 experiment.

As can be seen from figure 3 a statistically significant difference in the infection by *A. populi* was observed between *P. 'Hybr. 282'* and the three cultivars *P. 'Oxford'*, *P. 'Geneva'* and *P. 'Androskoggin'*. The remaining varieties had intermediate values and did not differ significantly.

#### DISCUSSION AND CONCLUSIONS

The experiments conducted indicate that there exist differences between poplar cultivars in their susceptibility to infection by *A. populi*. The differences were relatively great, which generally agrees with results of earlier studies conducted by many authors and discussed in detail by Lange (1968).

Comparing the results obtained in 1968 (Fig. 2) with the information about ancestry of the cultivars (table 1, column 3) we believe that poplars from section *Tacamahaca* are more susceptible to infection by this pathogen than poplars from section *Aigeiros*. This is also indicated by the results obtained in 1969 in which the cultivars *P. 'Hybr. 283'* and *P. 'Hybr. 282'* (hybrids between *P. angulata* × *P. berolinensis* — see table 2 column 3) have been least affected (Fig. 3).

Four year studies of Burdekin (1969) in Great Britain also indicate that there is a greater resistance of the poplars from section *Aigeiros* to *A. populi* than of poplars from section *Tacamahaca*.

An important problem in studies on the resistance of poplars to *A. populi* is the use of a strain of the bacterium that would be characterized by high virulence. Studies on the metabolism of *A. populi* (Danilewicz and Siwecki 1970b) indicate that in future studies it will be necessary to obtain a virulent strain of the bacterium from only one colony or even only a part of a colony.

It is difficult to judge which of the two methods of inoculating poplar cuttings proved to be better one for the type of studies conducted. Both methods gave a distinct pathogenic effect in the form of increased growth of parenchymatous tissues, destruction of the bark and of the phloem.

It does however appear that the method of the "fresh leaf scar" (the 1969 experiment) in view of the fact that it harms the cutting less and therefore allows a less easy entry of pathogens as well as of saprophytic organisms to the wound proved to be the better one. With this method the penetration of the pathogen reaches not only the parenchyma of the leaf gap but also the bark and phloem all the way to the cambium. The direct inoculation of the parenchymatous tissue in the stem gap (the 1968 experiment) resulted in it becoming quickly affected by the pathogen frequently resulting in necrosis and excessive growth.

Since *A. populi* in the case of the method employed attacks primarily the parenchymatous tissue of the stem gap or supporting leaf gap, accurate anatomical study is needed of the vascular connections in the node that has been infected on the selected poplar cultivars. We have noted that poplars from section *Tacamahaca* develop thicker layers of tissues on the node which can also have an effect on the estimation of the pathogenic effect.

Analysing the growth characters (Table 5) in the conditions of the 1968 experiment only in four instances significant differences have been observed between the inoculated and uninoculated cuttings. It is interesting that the growth increment in length (character I) for the inoculated cuttings of *P. 'I-214'*, the least susceptible cultivar (Table 4 and Fig. 2) was greater than in the uninoculated controls. This result is opposite to the one obtained by Burdekin (1969) for two varieties, *P. 'Eugenei'* and *P. 'I-214'* where he has observed considerable inhibition of growth in length of one year old poplar transplants under the influence of infection with *A. populi*. In our experiment statistically significant inhibition of the growth of cuttings that have been inoculated in comparison with the uninoculated controls has been observed only for *P. 'Geneva'*, a cultivar from section *Tacamahaca* that was shown to be very susceptible to infection by the pathogen (Table 4 and Fig. 2).

The results obtained for the other two characters, diameter of cuttings (character II) and dry weight of roots (character III) are rather difficult to evaluate properly.

Results of the experiments conducted, when supplemented by data on natural resistance of trees growing in various plantation conditions may help in directing breeding programs for poplars resistant to the bacterial canker.

#### SUMMARY

Using two methods of experimentally inoculating poplar cuttings with *A. populi* (Smith) strain Ridé differences have been found in the susceptibility of the studied poplar cultivars. The generally recommended method of inoculation through a fresh leaf scar that has been used in the experiment conducted in 1969 proved more satisfactory than the method of inoculating a wound formed by the removal of a green lateral shoot (a in the 1968 experiment).

Under our experimental conditions poplars from section *Tacamahaca* proved to be less resistant than those from section *Aigeiros*. Inoculation of rooted cuttings of *P. 'I-214'*, the cultivar which proved to be most resistant to the pathogen, resulted in a significant increase in the growth increment in shoot length compared with the uninoculated controls. On the other hand in cultivar *P. 'Geneva'* which proved to be very susceptible to the pathogen the inoculation led to a significant decrease in height growth of the shoot compared with the control.

The results obtained, together with data on natural resistance of poplars growing in plantations in various field conditions, may help in directing breeding programs for poplars characterized by greater resistance to the bacterial canker.

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### *Badania odporności mieszańców topoli na porażenie Aplanobacterium populi* (Smith) szczep Ridé

#### Streszczenie

Przy zastosowaniu dwóch metod eksperymentalnego zakażenia w warunkach wykonywanych doświadczeń stwierdzono różnice w porażeniu badanych topoli przez *A. populi* (Smith) szczep Ridé. Zalecana powszechnie w badaniach nad odpornością topoli metoda zakażenia przez świeżą bliznę liściową zastosowana w doświadczeniu z 1969 roku, okazała się bardziej korzystna niż metoda zakażenia na ranę powstałą po odcięciu zielonego pędu bocznego (doświadczenie z 1968 roku).

W warunkach przeprowadzonych doświadczeń topole z sekcji balsamicznych (*Tacamahaca*) wykazały mniejszą odporność niż topole z sekcji czarnych (*Aigeiros*). Przy zakażeniu ukorzenionych zrzesów z *P. I-214'*, odmiany wykazującej najmniejsze porażenie patogenem, stwierdzono statystycznie istotne zwiększenie przyrostu pędów wierzchołkowych na długość w porównaniu z zrzesami niezakażonymi, kontrolnymi. Podczas gdy u odmiany *P. 'Geneva'* wykazującej silniejsze porażenie patogenem nastąpiło znaczne ograniczenie przyrostu pędu na długość zrzesów zakażonych.

Rezultaty wykonanych doświadczeń po uzupełnieniu badaniami odporności naturalnej topól rosnących na plantacjach w różnych środowiskach mogą wskazywać kierunki postępowania przy wyhodowaniu topoli charakteryzujących się zwiększoną odpornością na porażenie rakiem bakteryjnym.