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A new method of standardized inocculation of rooted poplar cuttings by the fungus *Chondroplea populea* (Sacc.) Kleb. (*Dothichiza populea* Sacc. et Briard.) in order to check their resistance

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In recent years considerable advances were made in the studies on the resistance of trees to diseases. In these studies poplars have a special position. The introduction into cultivation of new poplar varieties requires that they be first tested for resistance to fungal diseases that can be dangerous. *Chondroplea populea* (Sacc.) Kleb. = *Dothichiza populea* Sacc. et Briard is one such disease.

By experimentally inocculating poplars with spores or mycelium of this fungus the resistance of different varieties has often been tested. In each case however different methods of artificial inocculation have been employed and the results obtained are basically restricted to the specific conditions of each experiment.

The further development of studies on the resistance of poplars to diseases requires a standardization of the basic experimental methods. For these reasons in this paper a detailed description is given of a new method of artificial inocculation of poplars by a mycelium of *Chondroplea populea*. This method could readily be used in further studies of this type.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse in 1968 on rooted cuttings of 13 poplar varieties: *Populus 'Hybr. 277', P. 'Hybr. 194', P. 'Hybr. 275', P. 'Geneva', P. 'Oxford', P. 'Kórnik 6', P. 'Marilandica', P. 'Robusta', P. 'Regenerata Grandis', P. 'Serotina', P. 'Gerlica', P. 'Sarce Rouge', P. 'I — 214'*.

The cuttings have been collected in January from a clone archive. They were 20 cm long with a diameter of 7 to 10 mm. The cuttings with the sealed with paraffine wax have been stored for two months in a specially prepared, sterile soil pit. In March the cuttings were planted to a depth of 1/3 of their length into pots with sterile nursery soil. Left in this state the cuttings rooted within two months and from their apical

Table 1

Results of the measurements made presented as averages over 15 cuttings for each of the poplar varieties

No.	Poplar variety	Length of the black stain on the pith in long. section in mm			Length of terminal green shoot in mm	Root dry weight in g	Diameter of cuttings in mm
		Min.	Max.	Average			
1.	*P. 'Hybr. 277'*	20.0	50.0	35.7	272.0	0.67	8.1
2.	*P. 'Hybr. 194'*	20.0	50.0	31.0	324.7	0.65	8.4
3.	*P. 'Hybr. 275'*	40.0	80.0	58.3	412.0	0.47	9.0
4.	*P. 'Geneva'*	45.0	105.0	62.0	302.0	0.59	8.2
5.	*P. 'Oxford'*	35.0	80.0	55.7	350.7	0.35	8.3
6.	*P. 'Kórnik 6'*	15.0	40.0	28.2	298.0	0.69	9.5
7.	*P. 'Marilandica'*	20.0	100.0	59.3	188.0	0.68	7.3
8.	*P. 'Robusta'*	35.0	120.0	65.3	247.3	0.47	8.1
9.	*P. 'Regenerata Grandis'*	10.0	90.0	45.3	163.3	0.66	7.4
10.	*P. 'Serotina'*	40.0	160.0	80.3	198.7	0.57	5.6
11.	*P. 'Gerlica'*	30.0	135.0	75.7	247.7	0.88	9.1
12.	*P. 'Sarce Rouge'*	40.0	130.0	80.7	278,7	1.00	9.1
13.	*P. 'I-214'*	0	65.0	34.0	310.7	1.03	9.8

buds two green shoots developed with well formed leaves. Each of the studied thirteen varieties has been represented by 15 well rooted individuals. A detailed description of the method in which the cuttings were prepared for studies of poplar resistance to the fungus *Chondroplea populea* has been published in a paper by S i w e c k i 1969.

The fungus has been isolated on Petri dishes with an agar-brewers wort medium on the 12th of October 1967 from diseased cuttings in a greenhouse (S i w e c k i 1969). A pure culture of the fungus has been stored in a thermostat at a temperature of 22—24°C on agar-brewer's wort slants. In order to maintain the biological activity of the pathogen, a pure culture has been reinocculated onto new slants on 1st of December 1967, the 9th of February 1968 and the 19th of February 1968.

In order to reproduce the pure culture of the fungus the standard malt extract agar Difco medium has been employed. Thus as the first stage the fungus was transplanted from the agar brewer's wort slants onto a Petri dish with the malt agar medium on the 19th of February 1968.

During about two weeks of incubation in a thermostat at a temperature of 22—24°C the fungus has grown over the whole surface of the Petri dish. Then the mycelium was washed with a sterile solution of 0,01% Tween 80 (Polyoxyethylene sorbitan monooleate), suspended in a physiological salt solution and transplanted on the 20th of April 1968 onto a fluid regeneration medium, which consited of a 10% aqueous

solution of malt extract. Both the standard agar and the fluid media have been sterilized for the cultures in an autoclave at 0,75 atmospheres for 15 min. The transplanted mycelial suspension was incubated statically in 100 ml of medium in 500 ml Erlenmayer flasks at a temperature of 22° for 14 days. After this time on the 7th of May the medium was decanted and the mycelium was trice washed with a physiological salt solution in a homogenizer "Universal laboratory aid, type 309, Unipan, Made in Poland". The motor has been gradually fed with a voltage of 50 V later increased to 100 V (source is 220 V). The homogenization time was related to the amount of the mycelium. The contents of 10 Ehrlenmayer flasks after incubation for 14 days was homogenized for 5 minutes. The homogenized mycelium was centrifuged in a centrifuge with a cooling device, at 4°C and 4500 g for 20 minutes. The mycelium was washed trice, and at each washing the mycelium was suspended in a physiological salt solution.

Table 2

Analysis of variance table for the length of the black stain on the pith of inocculated cuttings of the studied poplar varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	194	149266.2	769.4	—
Varieties	12	61729.2	5144.1	10.3**
Blocks	14	3638.2	259.8	0.5
Residual	168	83898.8	499.4	

The centrifuged mycelium, homogenized and washed from the medium and its metabolic products has been transplanted into a sterile container and stored at a temperature of 4° C. Before the artificial inocculation with the mycelium, its viability was tested by transplanting onto several Petri dishes with a 4.5% malt agar medium.

The method of inocculation

The poplar cuttings rooted in pots have been inocculated on the 11th of May by placing the homogenized mycelium with the help of a scalpel directly onto a wound immediately after it was formed by the cutting off of the lower green shoot. This has permitted an immediate contact of the mycelium with a fresh wound which has increased the likelihood of infection of the cuttings.

Within a month after inocculation the cuttings have undergone a se-

vere infection. On the termination of the experiment 4th July 1968 the degree of infection of the cuttings has been estimated on the basis of the length of the black stain on the pinth as seen in a longitudinal section. Simultaneously the length of the terminal green shoots has measured and the dry weight of the roots of each cutting was recorded. The results, are presented in Table 1 as averages over 15 cuttings for each of the poplar varieties used in the experiment. The results were treated statistically by the method of the variance analysis, the Duncan test and the regression analysis (Snedecor 1956).

RESULTS

The method of artificial inocculation of poplar cuttings with a homogenized mycelium that was described here is very simple in use. This is of particular importance in the studies on resistance of poplars that are being conducted in laboratory conditions and in the field. The mycelium of the pathogen when homogenized has the consistency of a paste. It is well washed from the medium and metabolic products, is viable for a long time and when stored at 4°C it does not dry out. Depending on the purpose of the conducted studies the mycelium can be placed onto the inoculated plant in defined quantities by the use of calibrated droppers (or with a sterile scalpel).

In the conducted experiment the degree of cuttings infection as measured by the length of the black stain on a longitudinal section was different for the different poplar varieties tested. As can be seen from table 2 the differences in the degree of infection of the individual varieties were statistically proven on the 0.99 level of significance. There were no significant differences between blocks (replicates).

In order to select from the studied poplar varieties those that are most resistant and those most susceptible to infection by the fungus *Chondroplea populea*, the statistical analysis was completed by the Duncan test (Snedecor 1956). Results of this test are presented in table 3, in which the varietal names are substituted by their numbers as shown in column 1 of table 1.

As can be seen from table 3 the most resistant poplars are the following four varieties: *P. 'Kórnik 6'* (6), *P. 'Hybr. 194'* (2), *P. 'I—214'* (13 and *P. 'Hybr. 277'* (1). From this group *P. 'Kórnik 6'* proved to be most resistant. This variety is a hybrid between *P. maximowiczii* × *P. trichocarpa* that has been obtained in Kórnik from a controlled pollination made in 1950. From this hybrid progeny (marked as PK — 14) clone 14—59 was selected out and named *'Kórnik 6'*. Detailed growth data and a description of this clone can be found in the papers of Bugała and Stecki 1961 and Stecki 1967. The origin of further three varieties from the

Table 3

The results of the Duncan test on the degree of infection of the studied poplar varieties

Variety no.	6	2	13	1	9	5	3	7	4	8	11	10	12
Length of black stain	28	31	34	36	45	56	58	59	62	65	76	80	81
Lines join varieties not differing significantly	(				)								
					(					)			
							(						)

group of resistant ones used in this experimental is well known. These varieties are commonly cultivated in Poland.

Resistance of *P. 'Regenerata Grandis'* (9) proved high but not as high as that for the 4 above mentioned poplars. It represents a variety intermediate between the resistant ones those of medium resistance (table 3). This median group includes also the varieties: *P. 'Oxford'* (5) *P. 'Hybr. 275'* (3), *P. 'Marilandica'* (7), *P. 'Geneva'* (4) and *P. 'Robusta'* (8).

Of the studied varieties the most susceptible ones to infection by *Chondroplea populea* were: *P. 'Gerlica'* (11), *P. 'Serotina'* (10) and *P. 'Sarce Rouge'* (12).

On the basis of regression analyses performed no corelation was observed between the length of the black stain and growth characteristics such as the length of the shoot ($r = -0.29$), dry weigth of roots ($r = -0.02$), diameter of cuttings ($r = -0.36$) and the volume of the shoots ($r = -0.34$)

DISCUSSION

As a result of the studies perfored it has to be stated that the infection of poplar cuttings with a mycelium of *Chondroplea populea* in the form of a paste can be used satisfactorily in further studies of this type.

Ot the thirteen poplar varieties tested in this experiment particular note may be made of the following in view their relatively high resistance to infection by such a serious disease as *Chondroplea populea: P. 'Kórnik 6', P. 'Hybr. 194', P. 'I—214'* and *P. 'Hybr. 277'*. Results of the resistance of these varieties (except *P. 'Kórnik 6'*) are consistent with the results of studies on the resistance of poplars conducted by Donaubauer (1964) and Magnani (1966).

It needs however to be stressed that the results of the experiment described here are only an indication for use in further studies on the resistance of poplar varieties cultivated in Poland to this serious fungal disease.

Future studies using this metod of artificial inocculation with the mycelium of *Chondroplea populea* in the form of a paste will show whether results reported here are of significance.

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Nowa metoda standardowego zakażenia przez grzyb Chondroplea populea (Sacc.) Kleb. = Dothichiza populea Sacc. et Briard ukorzenionych zrzezów topolowych w celu sprawdzenia ich odporności

Streszczenie

Opracowano nową metodę sprawdzania stopnia odporności topoli na groźną chorobę powodowaną przez grzyb *Chondroplea populea*. Próbę przeprowadzono w szklarni na ukorzenionych zrzezach trzynastu odmian topoli najczęściej uprawianych w Polsce. Inokulum stanowiła zhomogenizowana grzybnia patogena o konsystencji pasty, którą nakładano na ranę powstałą po odcięciu jednego z pędów, jakie rozwinęły się po zasadzeniu zrzezów w doniczkach.

Po okresie około jednego miesiąca od chwili zakażenia określono stopień porażenia zrzezów, mierząc długość czarnego przebarwienia, jakie powstało w rdzeniu w wyniku infekcji grzyba. Stopień odporności badanych topoli charakteryzowano średnią długością czarnego przebarwienia („czarnej plamy") na rdzeniu zrzezów. Wyniki opracowano statystycznie metodą analizy wariancyjnej, testu Duncana i analizy regresji.