Effect of bark washing from *Populus* 'Robusta' on the course of artificial inoculation of some poplar hybrids by the fungus *Dothichiza populea* Sacc. et Briard.

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The ease with which poplars can be cross-fertilized, their relatively quick selection procedures and the possibility of their vegetative propagation are the main reasons why these trees are so commonly cultivated for the production of wood. It is also for these reasons that poplars represent an important research subject on the problem of resistance to diseases.

In the last years breeding and selection of poplars in many parts of Europe is dependent increasingly commonly on the development of studies on the resistance of these plants to their main diseases (Doneubauer 1964; Gerhold, Schreiner et al. 1966; Schreiner 1963). In these studies stress is being placed on the natural resistance of poplars, which is being inherited by progeny following generative reproduction (Gerhold, Schreiner et al. 1966; Gorlenko 1962; Schreiner 1963).

The various studies conducted by Bier (1966), over many years have demonstrated that the occurrence on a tree bark of a saprophytic microflora can sometimes be one of the factors in the mechanism of the trees resistance to diseases. In view of these results it was considered of interest to conduct an experiment in laboratory conditions on the influence of unidentified bark saprophytes from a healthy *Populus* 'Robusta' on the course of infection of selected poplar hybrids by the fungus *Dothichiza populea*.

A detailed recognition of the role bark saprophytes play in defending a tree from pathogens can be of significance to studies on the biological methods of disease control.

The results presented in this paper concern an experiment, conducted in the Institute of Dendrology and Kórnik Arboretum of the Polish Academy of Sciences, on the resistance of selected poplar hybrids obtained in Kórnik to artificial inoculation by *Dothichiza populea* Sacc. et Briard.

MATERIALS AND METHODS

In the experiment artificial infection of cuttings has been performed by a pure culture of *Dothichiza populea*.* Simultaneously with the infection sterilization of the cuttings with alcohol has been tried and also the influence of an unidentified collection of bark saprophytes, present in a washing of the bark of a growing tree *P. 'Robusta'*, on the resistance of the cuttings to artificial inoculation has been tested. Cuttings from four poplar progenies have been used in the experiment:

PK-136 *P. nigra* 'Italica' $\times$ *P. laurifolia*

PK-137 *P. nigra* 'Italica' $\times$ *P. nigra*

PK-127 *P. maximowiczii* $\times$ *P. laurifolia*

PK-124 *P. maximowiczii* $\times$ *P. nigra*

Each progeny was represented in the experiment by 6 clones. A detailed description of the way which the individual clones have been selected as well as the method of obtaining the experimental material has been published in the paper by Siwecki and Giertych (1965).

Cuttings for the experiment have been obtained from a clone archive established in January of 1964. In January 1965 cuttings 20 cm long and 7–15 mm in diameter were cut. The apex of each cutting was individually sealed with paraffine wax. In this condition the cuttings were stored in the ground in sterilized (by heat) sand. The experiment has been conducted in a phytotron chamber in April 1965. In order to have sufficient data for a statistical treatment of the results two replicates have been employed and each of the 24 clones (6 from each of the four progenies) was represented by 36 cuttings over all the experimental variables.

Cuttings were infected by a pure culture of the fungus *Dothichiza populea* cultivated on Petri dishes on a brewer's wort medium (17 g of agar, 250 ml of wort, distilled water to make up to 1 liter, pH of medium = 6). The inoculate 8 $\times$ 8 mm in area, has been cut out by a form-knife on the Petri dish together with the medium and placed directly on the bark of the cutting fixing it with a transparent piece of sello-tape (photo 1).

The bark washing containing the unidentified bark saprophytes has been obtained (2 liters in all) from the bark of an 8-year old tree of *P. 'Robusta'* growing in our collection. It was collected one day before establishment of the experiment by washing the bark of the tree at 60 to 130 cm above the ground with a stream of distilled water under pressure from a rubber pump. Until used in the experiment it was stored at room temperature. Before the dipping of the poplar cuttings in the washing it was thoroughly mixed.

On half of the cuttings (432) artificial inoculation has been performed by the mycelium of *Dothichiza populea*, and the following experimental variants were employed:

*) A pure culture of the fungus has been obtained by the author from dr Cz. Kozłowska from the Phytopathology Department of the Forest Research Institute in Warsaw, for which the author expresses his sincere gratitude.

1. Inoculation of cuttings directly on the bark following extraction from the sand (without treatment with the bark washings).

2. Inoculation of cuttings following the sterilization of each one by alcohol (sterilized and without treatment with the bark washings).

3. Inoculation of cuttings first sterilized with alcohol (as in treatment 2) and then once dipped in the bark washings (sterilized and treated with the bark washings).

The remaining half of the cuttings (also 432) was treated as a control on which all the same treatments have been applied except the artificial inoculation. The

Photo 1. Artificial inoculation of cuttings with a mycelium of *Dothichiza populea* Sacc. et Briard.

functions associated with the inoculation of cuttings, sterilization and the dipping in the bark washing have been performed in a sterile chamber. Thus treated all the cuttings were dipped to 1/3 of their lengths in sterile soil in plastic boxes. In each box 18 cuttings were placed 3 each from the 6 clones of one progeny (photo 1). The plastic boxes were arranged in the chamber of a phytotron at a temperature of 23°–25°C, a relative humidity of 70–80% and a light intensity of 1500 lux. The plants were kept at a 16 hour photoperiod. To maintain a constant moisture of the soil in the boxes they were daily watered.

In order to objectively estimate infection of the cuttings only a two point scale has been accepted:

1. infected cuttings
2. uninfected cuttings.

In the first category dead cuttings (phloem and wood black in colour) have been placed. Leaf buds were totally dried and black. At the infection site on some cuttings numerous pycnidia of the fungus could be seen.

To the second category healthy cuttings were included. The bark had a normal

colour, and on a cut surface the phloem and wood could be seen to be normally green. Leaf buds were generally green, not dried at the site of infection and there were no pycnidia of the fungus.

RESULTS

The optimal conditions provided by the phytotron have resulted in an immediate rapid development of the mycelium of *Dothichiza populea* on the inoculated cuttings.

Within 2—5 days from the onset of the experiment leaf buds have opened on the cuttings. Hyphae of the mycelium (inoculate placed below buds, photo 1) have very quickly grown through the developing buds and bark to the phloem and wood of the cuttings. After one week on the cuttings from the susceptible progenies a blackening and falling off of the leaves resulted. On the cuttings dark stains could be seen extending up and down from the site of infection. At this stage of the experiment the inoculate was removed from the cuttings. Two weeks from the onset of the experiment differences in the intensity of infection became pronounced differentiating the various experimental variables. First to loose the leaves were the cuttings on which artificial inoculation was performed, with and without sterilization with alcohol (variants 1 and 2). The cuttings dipped in the bark washings (variant 3) only in rare instances became infected, while in most cases they remained healthy as in the controls.

After a month on the dead cuttings, particularly around the site of infection, pycnidia of *Dothichiza populea* have appeared. At this time the experiment was liquidated and the degree of infection was estimated for each cutting individually according to the accepted scale.

On plate I first clones from each progeny at all experimental variables are presented to exemplify the state of the cuttings at the end of the experiment.

From these observations the numbers of infected and uninfected cuttings were obtained for each of the experimental variables and for the two replicates.

DISCUSSION

On the basis of the results presented in Table 1 it can be concluded that there are differences in infection of poplars from the progenies employed to infection by *Dothichiza populea*. The lowest percentage of infected cuttings, following inoculation by the described method, has been observed in the hybrid progeny PK-137 *P. nigra* 'Italica' $\times$ *P. nigra* and the most infected progeny was PK-124 *P. maximowiczii* $\times$ *P. nigra* (table 1 variant 1).

Particularly low infection of the hybrid progenies has been observed in the 3rd variant of the experiment. Cuttings sterilized with alcohol and then once dipped in a washing from the bark of *Populus* 'Robusta' (table 1 variant 3) proved more resistant to the infection by *Dothichiza populea* than the cuttings sterilized with

Plate I

Plate I. Cuttings of all the experimental variables at the time of termination of the experiment:

A — unsterilized, no bark washings applied, inoculated; *B* — unsterilized, no bark washings applied, not inoculated; *C* — sterilized, no bark washings applied, inoculated; *D* — sterilized, no bark washings applied, not inoculated; *E* — sterilized, bark washings applied, inoculated; *F* — sterilized, bark washings applied, not inoculated.

alcohol only (table 1 variant 2) and those inoculated directly after removal from storage.

Results of the experiment appear to indicate that the unidentified factors, and among them presumably the saprophytic microorganisms, which were present in the bark washings from a healthy specimen of *Populus 'Robusta'* seem to inhibit the development of the fungus *Dothichiza populea*.

Table 1

Percentage of cuttings infected in the experimental variables 1, 2 and 3 following artificial inoculation with the fungus *Dythichiza populea* Sacc. et Briard

Progenies of hybrids	Percentage of infected cuttings		
	Direct inoculation	Inoculation following sterilization	Inoculation following sterilization and dipping in bark washings.
	1	2	3
PK-136 <i>P. nigra</i> 'Italica' × <i>P. laurifolia</i>	97%	58%	22%
PK-137 <i>P. nigra</i> 'Italica' × <i>P. nigra</i>	42%	64%	42%
PK-127 <i>P. maximowiczii</i> × <i>P. laurifolia</i>	83%	44%	28%
PK-124 <i>P. maximowiczii</i> × <i>P. nigra</i>	100%	69%	28%

It needs to be pointed out that the use of bark washings from *Populus 'Robusta'* has been the result of the author's observations on the resistance to diseases of poplars in our collections as well as the findings of the more recent research work on resistance in which *Populus 'Robusta'* is being considered as belonging to the group of poplars resistant to *D. populea*. (Doneubauer 1964; Gernold, Schreiner et al. 1966).

Summarizing the results it can be assumed that in the bark diseases of poplars, (to which *Dothichiza populea* belongs) an important factor of the plants resistance to these diseases lies in the bark washing obtainable for example by pouring water on the bark of a healthy specimen of *Populus 'Robusta'*. A knowledge of the composition and function of the microorganisms living saprophytically on the bark of poplars needs to be expanded before definite conclusion about their rôle could be made.

For valuable suggestions and discussions I express my sincere gratitude to dr M. Giertych.

SUMMARY

Cuttings from 4 hybrid progenies of poplars obtained in Kórnik from crosses between two mothers (*P. nigra* 'Italica' and *P. maximowiczii* Henry) and two fathers (*P. laurifolia* Ledeb. and *P. nigra* L.) have been artificially inoculated in the laboratory conditions by a mycelium of *Dothichiza populea*. Simultaneously with the inoculation a part of the cuttings was disinfected with

alcohol, and some also dipped in a bark washing from *P. 'Robusta'* growing in the collection of the Kórnik Arboretum. The washing contained unidentified saprophytes. The experiment was supplied with controls and replications. Results are given in Table 1.

The cuttings disinfected with alcohol, and then dipped in the bark washings from *P. 'Robusta'* (table 1, item 3) proved to be more resistant to infection by *D. populea* than cuttings disinfected with alcohol (table 1, item 2) or those inoculated immediately after extraction from the storage pit (table 1, item 1).

Results of the experiment indicate that unidentified factors (among them probably saprophytic microorganisms) present in a bark washing from a healthy *P. 'Robusta'* appear to have an inhibitive effect on the development of the fungus *D. populea*.

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Wpływ saprofitów korowych na odporność topoli przy porażeniu ich przez Dothichiza populea Sacc. et Briard.

Streszczenie

Zręzy z 4-ech rodów kórnickich mieszańców topoli będących potomstwem dwóch matek (*P. nigra 'Italica'* i *P. maximowiczii* Henry.) oraz dwóch ojców (*P. laurifolia* Ledeb. i *P. nigra* L.) zakażano sztucznie w warunkach laboratoryjnych grzybnią *Dothichiza populea*. Równocześnie przy zakażaniu stosowano dezynfekcję zręzków alkoholem, które następnie zanurzano w spłuczce z kory, otrzymanej z drzewa *P. 'Robusta'*, rosnącego w kolekcji Arboretum. Spłuczka zawierała nieokreślone saprofity. W doświadczeniu uwzględniono porównanie i kontrolę. Wyniki przedstawiono w tabeli 1.

Szczególnie słabe porażenie zręzków z poszczególnych rodów mieszańców zaznaczyło się w 3-cim wariancie doświadczenia. Zręzy dezynfekowane alkoholem, a następnie zanurzone w spłuczce z kory *P. 'Robusta'* (tab. 1 poz. 3) okazały się bardziej odporne na porażenie przez *D. populea* niż zręzy dezynfekowane alkoholem (tab. 1 poz. 2) i zręzy zakażone bezpośrednio po wyjęciu z dołownika (tab. 1 poz. 1).

Wyniki doświadczenia wskazują, że nieokreślone czynniki (wśród nich prawdopodobnie mikroorganizmy saprofityczne) zawarte w spłuczce z kory rosnącego, zdrowego drzewa *P. 'Robusta'* mogą, jak się wydaje, wpływać hamująco na rozwój grzyba *D. populea*.