PLANT GROWTH REGULATORS IN POPLAR CLONES DIFFERING IN RESISTANCE TO THE FUNGUS *CERATOCYSTIS FIMBRIATA* ELL. ET HALST

JADWIGA STOPIŃSKA

Department of Plant Physiology and Morphogenesis,
Institute of Biology, Copernicus University, Gagarina 9, 87-100 Toruń, Poland

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ABSTRACT

In poplar clones with different resistance to the fungus *Ceratocystis fimbriata* growth of shoots, intensity of transpiration and the level of endogenous growth regulators were determined. More resistant clones, *Populus 'Robusta'* and *P. 'PK-136-2'* (*P. nigra 'Italica'* × *P. laurifolia*) had more intensive growth of shoots, higher water content in leaves and a lower intensity of transpiration than the more susceptible clone, – *P. 'NE-42'* (*P. maximowiczii* × *P. trichocarpa*). The leaves of the more resistant clones contained more auxins (IAA) and cytokinins, especially zeatin, and less growth inhibitors (ABA) than those of the susceptible one. The level of plant growth regulators and/or the relations between them may be responsible for the different poplar resistance to *C. fimbriata*.

KEY WORDS: resistance, poplar clones, *Ceratocystis fimbriata*, auxins, cytokinins, growth inhibitors

INTRODUCTION

The resistance or susceptibility of plants to fungal infection may be influenced by biotic or abiotic factors such as temperature (Bhattacharyya and Ward 1987), carbon dioxide and oxygen levels (Arnold and Rahe 1977), Fraedrich and Tainer 1989), light intensity and photoperiod (Goosens and Vendrig 1982, Kurowski and Gudmestad 1990), water (Vannini and Scarascia Mugnozza 1991) and mineral elements (Ylimartimo 1991).

The resistance of plants to various stress factors, also to fungal diseases, depends also on their anatomical features and the presence of chemical substances, such as toxic phenols and organic acids as well as of nutrient or hormonal substances (Bidwell 1979).

There are many data which indicate that exogenously supplied growth regulators, such as auxins (Sinha and Wood 1967, Oka and Pimentel 1976), cytokinins (Edwards 1983, Liu and Bushnell 1986), the ratio of auxins to cytokinins (Haberlach et al. 1978, Kozłowska et al. 1988), abscisic acid (Ward et al. 1989, Dunn et al. 1990, Ersek et al. 1991), ethylene (Stahmann et al. 1966, Michniewicz et al. 1986) may increase or decrease the hypersensitive reaction, the degree of infection and susceptibility of tissues.

Some authors indicated that plants of different resistance or susceptibility to diseases contained also different levels of auxins (Artemenko et al. 1980, Seifers et al. 1985), cytokinins (Vizarova 1987, Vizarova et al. 1988), abscisic acid (Chigrin

et al. 1981). However, it must be stressed that data concerning this problem, especially in trees, are very scant.

In the investigations performed by Przybył (1984) it was found that poplar clones differed in their degree of resistance to the fungus *Ceratocystis fimbriata* and that the rate of the pathogen development was correlated with this feature (Przybył 1984a). The aim of the present study was to find out if poplar clones differing in their resistance to the fungus differ also in the intensity of growth, water and level of plant hormones before infection with this fungus.

MATERIAL AND METHODS

Three poplar clones were used: *Populus 'NE-42'* (*P. maximowiczii* × *P. trichocarpa*) – susceptible clone, *P. 'PK-136-2'* (*P. nigra 'Italica'* × *P. laurifolia*) – moderately resistant clone, and *P. 'Robusta-8341'* – resistant clone each showing different resistance to the pathogenic fungus, *Ceratocystis fimbriata*.

Pieces of one-year-old shoots after the start of rhizogenesis were transferred to a growth chamber with light intensity about 6.000 lux, a 16-hour day, a temperature of 25°C and 50% relative humidity. The plants were cultured in Hoagland solution with Arnon microelements at pH 5.6, diluted in the ratio 1:2. The solution was changed after 10 days. The determinations of growth parameters were performed after two months of culture for fifteen plants in each poplar clone. Other parameters measured were: fresh and dry matter of leaves and water content in these organs. The intensity of transpiration was determined by the water loss method after 24 hours in four replications with four plants in each.

In the experiment plant growth regulators, auxins, cytokinins, and growth inhibitors were also isolated. The sample contained all leaves on the shoot in each poplar clone. Plant

List of abbreviations:

IAA – indole-3-acetic acid, NAA – 1-naphthalenacetic acid, 2,4-D – dichlorophenoxyacetic acid, ABA – abscisic acid

hormones were extracted from the samples with 80% methanol supplemented with BHT (2, 6-di-tert-buthyl-p-cresol) the antioxidant using 10 mg dm⁻³ at 5°C for 48 hrs. The methanol extract was evaporated at 35°C to water residue. For auxins and growth inhibitors determinations water residue was adjusted to pH 3.0 with 10% HCL and extracted three times with ethyl acetate and diethyl ether. The joined organic fractions were evaporated to dry residue, washed with 0.5 M phosphate buffer, pH 8.0 and applied to a column (1.0 x 9.0 cm) packed with Polyclar AT (polivinylpyrrolidone insoluble). The extracts were purified from phenols on columns washed out with 0.1 M phosphate buffer, pH 8.0 according to Gleen et al. (1972). The collected eluate was adjusted to pH 3.0 and extracted anew with ethyl acetate and diethyl ether. The combined organic fractions were evaporated to dry residue dissolved in 96% ethanol and fractionated on a column filled with Sephadex LH-20 (2.5 x 30 cm) according to Steen and Eliasson (1969). 70% ethanol with 0.001 M HCL as the eluate was collected in 10 cm³ fractions. Fractions corresponding to the localization of synthetic IAA (Fluka) or cis-trans ABA (Sigma) were evaporated to dryness and diluted in 100% methanol.

The cytokinins were isolated and purified from the water residue after evaporating methanol according to Hewett and Wareing (1973). The water fraction was adjusted to pH 2.5 and absorbed on an ion exchange Dowex 50WX 8H⁺ (50/100 mesh) column (v=50 cm³). The column was washed out with 1 vol. of 70% CH₃OH, 1.5 vol. of H₂O, 2 vol. of 2N NH₄OH and 5 vol. of 5N NH₄OH. The ammonia fractions were evaporated to dryness, dissolved in methanol and chromatographed on Wh 3MM paper in the solvent solution – isopropanol : ammonia : water = 10 : 1 : 1 v/v. To identify cytokinins in plant extracts zeatin (Sigma) was also chromatographed in the same solvent solution.

All plant growth regulators were analysed by biotests and by gas chromatography. The following biotests were used: for auxins – Avena section straight growth test (Audus 1959), for growth inhibitors – wheat coleoptile test (Rudnicki 1969), for cytokinins – soybean tissue test (Miller 1968).

Gas chromatography IAA and ABA

Fractions for gas chromatography were methylated with diazomethane after Schlenk and Gellerman (1960) and injected into Chromatron GChF 18.3-4 using a glass column (2.0 x 0.4 cm) packed with Gas Chrom Q (100/120 mesh), with liquid phase 5% SE-30. The column temperature was 190°C with injection and detector temp. (FID) of 300°C. N₂ was used as

carrier gas at a flow rate of 40 cm³ min. As control samples IAA (Fluka) and cis-trans ABA (Sigma) were used.

Zeatin and zeatin riboside

The cytokinins were determined as described in the previous paper (Stopińska 1991).

All experiments were performed in duplicate. The results were evaluated statistically by estimating significant differences (LSD) at P=0.05 or standard errors.

RESULTS AND DISCUSSION

The morphological characteristic of poplars and the systematic division of genus *Populus* were presented earlier by Bugała (1973).

The growth of poplar clones with different resistance to the fungus *Ceratocystis fimbriata* has been presented in Table 1. The clones – *P. 'PK-136-2'* and *P. 'Robusta'* had longer shoots with a larger number of leaves than *P. 'NE-42'*. The surface area, the fresh and dry matter of leaves and the water content in these organs were also greater in the more resistant clones, especially in *P. 'Robusta'*, than in the susceptible one, *P. 'NE-42'*. The leaves of these poplar clones differed also in transpiration intensity (Table 2). The most resistant clone, – *P. 'Robusta'*, had the lowest intensity of this process. These results confirm the earlier data for poplar shoots obtained by other authors (Borejsza-Wysocki and Krzywański 1989).

TABLE 2. The intensity of transpiration of poplar clones with differing resistance to the fungus *C. fimbriata*

Clone	Intensity of transpiration (mg/dm ² /h)
<i>P. 'NE-42'</i>	0.78±0.03
<i>P. 'PK-136-2'</i>	0.75±0.07
<i>P. 'Robusta'</i>	0.65±0.03*

Explanations: as in Table 1.

In the leaves of the more resistant clones more auxins (IAA) and less growth inhibitors (ABA) were found than in the susceptible one, as estimated by the biological test (Figs 1, 2) as well as by gas chromatography (Fig. 3). The data in the literature concerning this problem are very scant. However, some authors indicate that auxin activity was higher in

TABLE 1. The growth of poplar clones with differing resistance to the fungus *C. fimbriata*

Clone	Length of shoot (cm)	Mean number of leaves	Mean per i leaf			
			Surface (dm ²)	Fresh matter (g)	Dry matter (g)	% H ₂ O
<i>P. 'NE-42'</i>	19.0	12.8	0.13	2.66±0.95	0.72±0.41	72.9
<i>P. 'PK-136-2'</i>	36.6*	24.7*	0.32*	4.29±1.08	1.09±0.40	74.6
<i>P. 'Robusta'</i>	25.9*	21.2*	0.46*	6.54±0.07	1.53±0.11*	76.6
LSD	2.7	1.4	0.01	–	–	–

Explanations: * – significant difference as compared to *P. 'NE-42'*

Fig. 1. Chromatographic analysis of auxins extracted from the leaves of poplar clones determined by biotest

Fig. 2. Chromatographic analysis of ABA-like inhibitors extracted from the leaves of poplar clones determined by biotest

Fig. 3. Total amount of IAA and ABA in the leaves of poplar clones determined by gas chromatography

the more resistant plant as wheat (Artemenko et al. 1980) or pine (Seifers et al. 1985) which are more resistant to the rust fungus. Contrary to these data in plants of *Medicago sativa* susceptible and nonsusceptible to *Verticillium albo-atrum* there was no difference in IAA content (Kratka and Kudela 1981). Other authors have pointed out that exogenously supplied auxins may change the susceptibility of plants to pathogens. It was found that NAA and IAA increased the resistance of tomato plants to *Fusarium oxysporum* (Corden and Dimond 1959) and to *Verticillium wilt* reducing also the fungus growth (Sinha and Wood 1967). 2, 4-D also increased the resistance of *Arachis hypogea* to *Phythium myriotylum* (Sinha and Wood 1967). According to Pegg (1976) the influence of auxins may be indirect through the stimulation of ethylene production, a very important factor in plant resistance reactions. It was found that more resistant poplar clones produced more ethylene than susceptible ones (Stopińska, unpublished data), which strongly inhibited the growth and development of *C. fimbriata* (Stopińska and Kuik 1991).

In this study the IAA to ABA ratio was found to be inverse. In the more resistant clones there was less ABA in leaves than in the susceptible plants. The high susceptibility of *P. 'NE-42'* to *C. fimbriata* may be associated with the stimulative effect of ABA on the growth and spore germination process of this pathogen (Stopińska and Michniewicz 1988). The same relationship in the content of ABA was found in wheat plants with different resistance to *Puccinia graminis* (Chigrin et al. 1981). Dunn et al. (1990), however, found that physiologically induced resistance of *Phaseolus vulgaris* to a compatible race of *Colletotrichum lindemuthianum* was associated

with increases in ABA content. Many workers underlined that exogenous treatment with abscisic acid increased the plant susceptibility e.g. of barley to *Erysiphe graminis* (Edwards 1983), of tobacco to *Peronospora tabacina* (Salt et al. 1986), or of soybeans to *Phytophthora megasperma* (Ward et al. 1989). Ersek et al. (1991) found that ABA inhibited the hypersensitive necrosis of potato tubers to elicitor from *Phytophthora infestans* but Henfling et al. (1980) demonstrated that potato tissues became susceptible to a noncompatible race of *Cladosporium* cucumber pathogen after ABA treatment. The studies of Henfling et al. suggest that ABA affects directly the plant reaction to pathogen, and not through the pathogen or its interaction with the host. The data obtained by Michniewicz et al. (1990) indicated that ABA stimulated disease development in wheat seedlings, and this reaction was associated with a decrease in ethylene level in leaves.

The level of cytokinins in poplar plants differing in resistance to *C. fimbriata* has been presented in Figures 4-7. The more resistant clones, - *P. 'PK-136-2'* and *P. 'Robusta'*, contained also much larger total amounts of cytokinins, especially zeatin, in leaves, which was detected by the biological test (Fig. 4) as well as by gas chromatography (Figs 5, 7). No zeatin riboside was found in these organs (Fig. 6). Among seven cytokinins detected in mature leaves of *P. 'Robusta'* by Hewett and Wareing (1973) there were also two identified as zeatin and zeatin riboside-like cytokinins. A close correlation between the content of cytokinins and the resistance of plants to pathogens was found by other authors. Cultivars of wheat and barley more resistant to mildew contained also more cytokinins in the seeds (Vizarova and Vozar 1984) and leaves

Fig. 4. Chromatographic analysis of cytokinins extracted from the leaves of poplar clones determined by biotest

Fig. 5. Gas chromatography analysis of zeatin extracted from the leaves of poplar clones

Fig. 6. Gas chromatography analysis of zeatin riboside extracted from the leaves of poplar clones

Fig. 7. Total amount of zeatin in the leaves of poplar clones determined by gas chromatography

(Vizarova 1987). Cultivars of wheat and barley resistant to mildew (Vizarova 1987) and to stem rust (Vizarova et al. 1988) contained also higher levels of free zeatin and its derivatives during the whole ontogeny than susceptible plants. These results suggesting the importance of cytokinin viz. of free zeatin, for the plant's resistance to fungi are supported by the results of experiments in which zeatin strongly inhibited the growth of *Erysiphe graminis* (Vizarova 1987) or the sporulation and spore germination processes of *C. fimbriata* (Stopińska 1991). The role of cytokinins in plant resistance is also presented in papers concerning the treatment with exogenous cytokinins. It was found that kinetin increased the resistance of barley (Edwards 1983) and red raspberry callus tissue (Kozłowska et al. 1988) to fungi. Some authors indicated that cytokinins induced hypersensitive reaction of plants (Liu and Bushnell 1986), others, however, claimed that it eliminated this reaction (Haberlach et al. 1978). Kozłowska et al. (1988) suggested that plant susceptibility depended upon the ratio of auxins to cytokinins in the medium.

Taking all of these into consideration, the level of plant growth regulators and/or the relations between them may be responsible for different poplar resistance to *C. fimbriata*.

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REGULATORY WZROSTU W KLONACH TOPOLI O RÓŻNEJ ODPORNOŚCI NA GRZYB *CERATOCYSTIS FIMBRIATA* ELL. ET HALST.

STRESZCZENIE

W klonach topoli o różnej odporności na grzyb *Ceratocystis fimbriata* oznaczono wzrost pędów, intensywność transpiracji i poziom endogennych regulatorów wzrostu. bardziej odporne klony, *P. 'PK-136-2'* i *P. 'Robusta'*, cechowały się intensywniejszym wzrostem pędów, wyższą zawartością wody w liściach i niższą intensywnością transpiracji niż klon bardziej wrażliwy – *P. 'NE-42'*. Liście bardziej odpornych klonów zawierały więcej auksyn (IAA) i cytokinin, szczególnie zeatyny a mniej inhibitorów wzrostu (ABA) niż liście klonu wrażliwego. Poziom regulatorów wzrostu i/lub zależności pomiędzy nimi mogą stanowić o różnej odporności topoli na *C. fimbriata*.

SŁOWA KLUCZOWE: odporność, klony topoli, *Ceratocystis fimbriata*, auksyny, cytokininy, inhibitory wzrostu.