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THE EFFECT OF GUMS INDUCED BY *Fusarium oxysporum* SCHLECHT. f. sp. *tulipae* APT. IN TULIP BULBS ON THE MYCELIUM GROWTH AND DEVELOPMENT OF THE PATHOGEN *in vitro*[1]

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Introduction

Gummosis is wide spread in taxonomically diverse plants and is affected by environmental factors such as fungal and bacterial pathogenesis, insect damage, mechanical and chemical injury, flooding and other stresses. All of these environmental factors that stimulate gum exudation have been shown to promote ethylene production in plants. Thus, ethylene may be a common factor involved in the induction of gummosis [BOOTHBY 1983]. Gums are a complex of different substances, but their most important constituent are polysaccharides of highly individual structure. Composition of gums polysaccharides shows variation between different species [BOOTHBY 1983]. It is well known that tulip bulbs infected by *Fusarium oxysporum* f. sp. *tulipae* can produce considerable quantities of ethylene, enough to cause gummosis in diseased and healthy bulbs stored in the same conditions. Gummosis in tulip bulbs can be easily induced in healthy bulbs by exogenously applied ethylene or ethylene-releasing compound, ethephon [KAMERBEEK, DE MUNK 1976]. Physiological role of gums in plants in unknown. It is belived that gums have a function in limiting the spread of fungal and bacterial pathogens by isolating the infected tissues [BOOTHBY 1983].

The aim of the present work was to study the effect of tulip gums on the *in vitro* growth of *F. oxysporum* f. sp. *tulipae* cultured on different media.

Material and methods

The gums induced by *Fusarium oxysporum* f. sp. *tulipae* in tulip bulbs (Photo 1), at final concentration 5 mg·cm^{-3} were dissolved in 5 cm^3 distilled and steri-

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lized water and added to Czapek-Dox-Agar (CzDA-Difco), Malt-Extract-Agar (MEA-Difco) and Potato-Dextrose-Agar (PDA-Merck) before sterilization. Five mm diam. plugs taken from 7-day-old culture of *Fusarium oxysporum* f. sp. *tulipae*, were placed in the middle of 90 mm Petri dishes containing above-mentioned media supplemented with gums. Control plates constituted the culture growing on CzDA, MEA and PDA without gums.

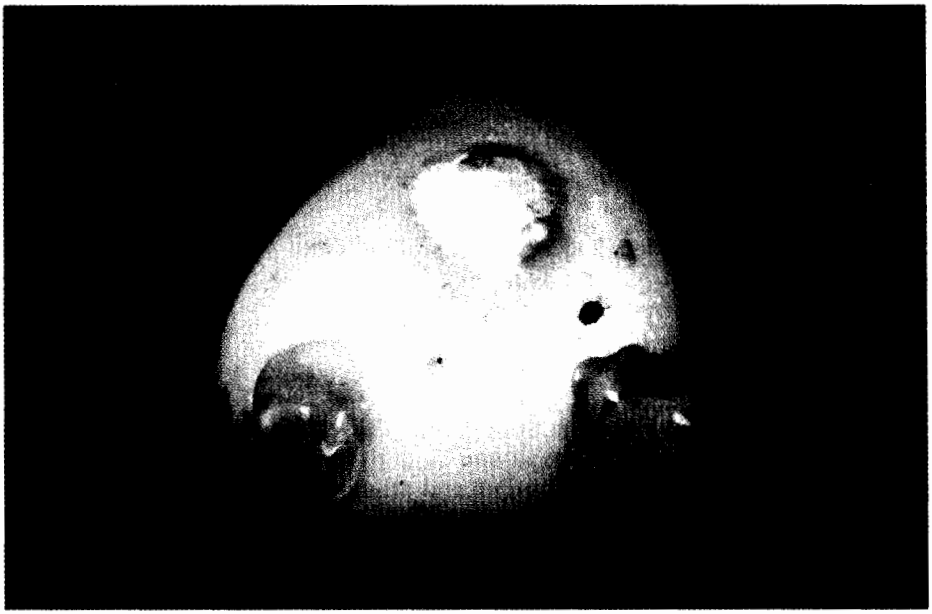


Photo 1. Gums induced in tulip bulbs by *Fusarium oxysporum* f. sp. *tulipae*
Fot. 1. Gumy indukowane w cebuli tulipana przez *Fusarium oxysporum* f. sp. *tulipae*

The diameter of *Fusarium oxysporum* f. sp. *tulipae* colony, was measured within an 7-day-incubation at 25°C in darkness.

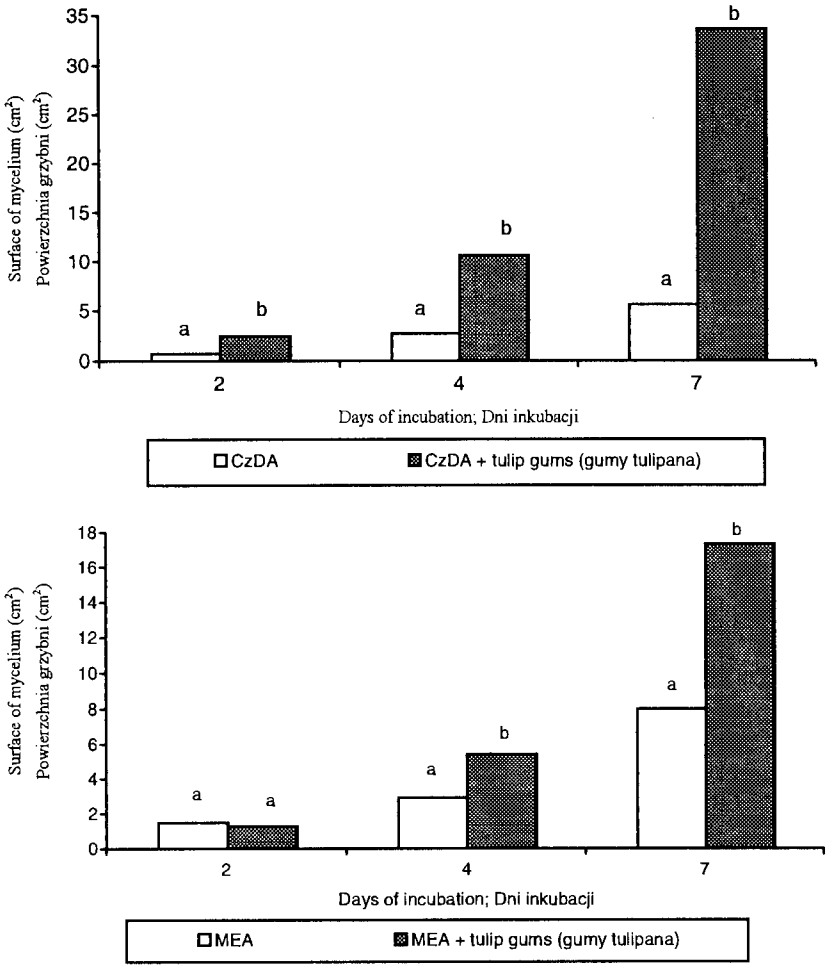
After six and thirteen days of mycelium incubation on CzDA medium and CzDA supplemented with gums, colonies of *Fusarium oxysporum* f. sp. *tulipae* were used for estimation of the effect of gums on sporulation of the pathogen. From mycelium colony of the pathogen, 6 cm^2 of colony fragments were cut out and transferred to clean Petri dishes containing 10 cm^3 sterilized water. These fragments of colony were smoothed by glass bagette for liberation of spores; after 30 min. spores were separated from mycelium using filter paper. Density of spores in 1 cm^3 of suspension was determined under microscope using Bürker's camera. From each of Petri dishes there were analyzed 4 fragments (6 cm^2) of mycelium.

Five dishes were used for each treatment and the experiment was repeated 3 times.

The data were subjected to an analysis of variance and Duncan's multiple range test at 5% of significance was used for means separation.

Results and discussion

Addition of tulip gums at concentration of 5 mg·cm^{-3} to all used media; Czapek-Dox-Agar (CzDA), Malt-Extract-Agar (MEA) and Potato-Dextrose-Agar (PDA) greatly stimulated mycelium growth of *Fusarium oxysporum* f. sp. *tulipae*. Surface of mycelium of the pathogen after 7 days of culturing on used media was as follows (cm^2): PDA – 11.0, PDA + gums – 37.7; CzDA – 5.6, CzDA + gums – 33.6; MEA – 8.0, MEA + gums 17.3 (Fig. 1; Photo 2). Sporulation of mycelium of the pathogen on CzDA medium supplemented with tulip bulbs was stimulated about threefold compared to the control culture (Tab. 1). On the basis of these results it is clear that tulip gums are not antifungal substances but have an evident stimulatory effect on the mycelium growth and sporulation of *F. oxysporum* f. sp. *tulipae*. The mechanism of this kind of stimulatory action of tulip gums is still unknown.



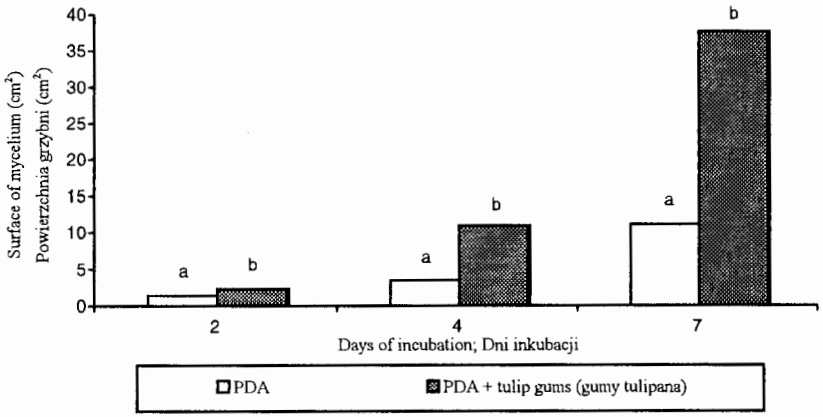


Fig. 1. Influence of tulip gums on the *in vitro* growth of *Fusarium oxysporum* f. sp. *tulipae* cultured on Czapek-Dox-Agar (CzDA), Malt-Extract-Agar (MEA) and Potato-Dextrose-Agar (PDA); all these used media were supplemented with tulip gums at concentration of 5 mg·cm^{-3}. In days of incubation, means followed by the same letter are not significantly different at P = 0.05 according to Duncan's test; values are calculated separately for each used media

Rys. 1. Wpływ gum tulipana na wzrost *in vitro Fusarium oxysporum* f. sp. *tulipae* na pożywce mineralnej Czapka zestalonej agarem (CzDA), agarowo-maltozowej (MEA) i agarowo-ziemniaczano-glukozowej (PDA); gumy tulipana dodano w ilości 5 mg·cm^{-3} pożywki. Średnie dla dni inkubacji oznaczone tą samą literą nie różnią się istotnie przy P = 0,05 (test Duncana); obliczeń różnic dokonano oddzielnie dla każdej pożywki

Table 1; Tabela 1

The effect of tulip gums on sporulation of *F. oxysporum* f. sp. *tulipae* on Czapek-Dox-Agar (CzDA)

Wpływ gum indukowanych w cebulach tulipana na zarodnikowanie *F. oxysporum* f. sp. *tulipae* na pożywce mineralnej Czapka zestalonej agarem (CzDA)

Medium Pożywka	Number of spores in cm³ of water solution from 6 cm² of colony fragment after days of incubation Liczba zarodników w cm³ zawiesiny pozyskanej z 6 cm² fragmentu kolonii grzybni po dniach inkubacji	
	6	13
CzDA (check; kontrola)	8.24 x 10^5 a	1.6 x 10^6 a
CzDA + 5 mg (gum)·cm^{-3}	9.92 x 10^5 a	5.2 x 10^6 b

Means in columns followed by the same letters are not significantly different at P = 0.05 according to Duncan's test; Średnie w kolumnach oznaczone tą samą literą nie różnią się istotnie przy P = 0,05 (test Duncana)

It is well known that different kinds of oligosaccharides can function in plants as molecular signals (elicitors) that regulate growth, development and survival in the environment, through elicitation of various physiological and bio-

chemical processes [ALDINGTON et al. 1991; DARVIL et al. 1992; CÔTE, HAHN 1994; EBEL, MITHÖFER 1998].

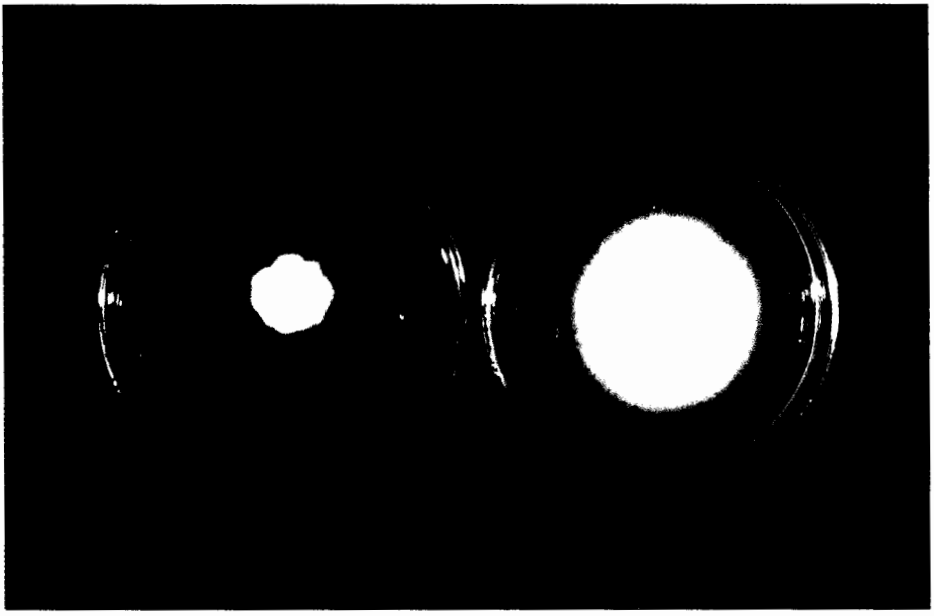


Photo 2. Influence of tulip gums on the *in vitro* growth of *Fusarium oxysporum* f. sp. *tulipae* cultured on Czapek-Dox-Agar (CzDA): on the left – control, CzDA only; on the right – CzDA supplemented with gums at a concentration of 5 mg·cm^{-3}

Fot. 2. Wpływ gum tulipana na wzrost *in vitro Fusarium oxysporum* f. sp. *tulipae* na pożywce mineralnej Czapka zestalonej agarem (CzDA): na lewo – kontrola, CzDA; na prawo – CzDA uzupełniona gumami tulipana w ilości 5 mg·cm^{-3} pożywki

It is possible that the polysaccharide of tulip gums, which is a glucuronoarabinoxylan, [SANIEWSKI et al. 2000] may act as an elicitor that regulates some processes connected or responsible for mycelium growth of *Fusarium oxysporum* f. sp. tulipae. At present, the stimulatory role of the polysaccharide of tulip gums as a substrate on the mycelium growth of *F. oxysporum* f. sp. *tulipae* cannot be excluded. Tulip gums contain also many other unidentified compounds, which may have a stimulatory effect on mycelium growth of the pathogen.

Literature

ALDINGTON S., MCDOUGALL J., FRY S.C. 1991. *Structure-activity relationship of biologically active oligosaccharides*. Plant, Cell and Environ. 14: 625–636.

BOOTHBY D. 1983. *Gummosis of stone-fruit trees and their fruits.* J. Sci. Food Agric.

34: 1–7.

CÔTE F., HAHN M.G. 1994. *Oligosaccharins: structures and signal transduction*. Plant Mol. Biol. 26: 1379–1411.

DARVIL A., AUGUR C., BERGMANN C., CARLSON R.W., CHEONG J.-J., EBERHARD S., HAHN M.G., LO V.-M., MARFÀ V., MEYER B., MOHNEN D., O'NEILL M.A., SPIRO M.D., VAN HALBEEK H., YORK W.S., ALBERSHEIM P. 1992. *Oligosaccharides that regulate, development and defence responses in plants*. Glycobiology 2: 181–198.

EBEL J., MITHÖFER A. 1998. *Early events in the elicitation of plant defence*. Planta 206: 335–348.

KAMERBEEK G.A., DE MUNK W.J. 1976. *A review of ethylene effects in bulbous plants*. Scientia Hortic. 4: 101–115.

SANIEWSKI M., UEDA J., MIYAMOTO K., HOROBOWICZ M. 2000. *Gum induction by methyl jasmonate in tulip stem: Relevance to its chemical composition*. Acta Hortic. 515: 39–48.

Key words: tulip, gums, *Fusarium oxysporum* SCHLECHT. f. sp. *tulipae* APT., growth, development, *in vitro*

Summary

The effect of tulip gums on the *in vitro* growth of *Fusarium oxysporum* f. sp. *tulipae* cultured on Czapek-Dox-Agar (CzDA), Malt-Extract-Agar (MEA) and Potato-Dextrose-Agar (PDA) was investigated. Addition of gums at a concentration 5 mg·cm^{-3} to all used media greatly stimulated mycelium growth of *F. oxysporum* f. sp. *tulipae* and sporulation of the pathogen. Surface of mycelium of the pathogen after 7 days of culturing on CzDA, MEA and PDA supplemented with gums was stimulated respectively, sixfold, twice and threefold compared to the control (mentioned media without gums). On the basis of these results, it is clear that tulip gums are not antifungal substances, but have an evident stimulatory effect on the mycelium growth of *F. oxysporum* f. sp. *tulipae*.

WPŁYW GUM INDUKOWANYCH W CELULACH TULIPANA PRZEZ *Fusarium oxysporum* SCHLECHT. f. sp. *tulipae* APT. NA WZROST I ROZWÓJ TEGO PATOGENA W WARUNKACH *in vitro*

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Słowa kluczowe: tulipan, gumy, *Fusarium oxysporum* f. sp. *tulipae* SCHLET., wzrost, rozwój, *in vitro*

Streszczenie

W pracy przedstawiono wyniki badań nad wpływem gum indukowanych w cebulach tulipana przez *Fusarium oxysporum* f. sp. *tulipae*, na wzrost i rozwój

tego patogena, w warunkach *in vitro*, na pożywce agarowo-ziemniaczano-glukozowej (PDA), agarowo-maltozowej (MEA) i mineralnej Czapka zestalonej agarem (CzDA).

Dodatek gum w ilości 5 $mg \cdot cm^{-3}$ do wszystkich wymienionych pożywek, wpłynął silnie stymulująco, na wzrost i zarodnikowanie grzybni *F. oxysporum* f. sp. *tulipae*, w porównaniu do kultur kontrolnych patogena wzrastających na podłożach bez dodatku gum. Powierzchnia grzybni patogena, po 7 dniach inkubacji na badanych pożywkach z dodatkiem gum wzrosła: 6-krotnie na CzDA, 2-krotnie na MEA i 3-krotnie na PDA w porównaniu do kultur kontrolnych kultywowanych na wymienionych pożywkach bez dodatku gum.

Otrzymane wyniki pozwalają twierdzić, że gumy indukowane w cebulach tulipana przez *F. oxysporum* f. sp. *tulipae* nie są czynnikiem ograniczającym, ale przeciwnie, bardzo silnie stymulują wzrost i rozwój tego patogena.

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