

ETHYLENE PRODUCTION IN TULIP BULBS POTENTIALLY SUSCEPTIBLE AND RESISTANT TO GUM FORMATION AFTER INFECTION BY *FUSARIUM OXYSPORUM* F. SP. *TULIPAE*

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Abstract

Relationship between ethylene production and development of fusariosis caused by two isolates of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs of cultivars susceptible and resistant to gum formation was studied under laboratory conditions. Fusariosis occurred on all bulbs of the nine cultivars used. Bulbs of three cultivars only ('Apeldoorn', 'Fringed Elegance', 'Prominence') produced gums after infection with both *F.ox.t.* isolates, while bulbs of six other cultivars ('Cassini', 'Christmas Marvel', 'Couleur Cardinal', 'Kees Nelis', 'Monte Carlo', 'White Dream') did not form gums.

Bulbs of all the cultivars produced different amounts of ethylene after inoculation with *F. oxysporum* f. sp. *tulipae*. The highest level of ethylene was produced by 'White Dream' bulbs, which did not form gums after infection. No relationship was found between ethylene production and induction of gummosis in infected bulbs of the cultivars in question.

Key words: *Fusarium oxysporum* f. sp. *tulipae*, cultivars, tulip, gum, ethylene

Introduction

Tulip bulb rot is caused by *Fusarium oxysporum* f. sp. *tulipae* (*F.ox.t.*). This is the most severe disease and one of the main problems during storage, breeding and cultivation in the urban greenery and gardens. Tulip bulbs infected with *F. oxysporum* f. sp. *tulipae* produced considerable quantities of ethylene, which inhibited the development of healthy bulbs and growth of tulips. Tulip bulbs with progressing fusariosis produced high amount of ethylene in some cultivars, including

'Apeldoorn', 'Aureola', 'Bartigon', 'Diplomate', 'Enterprise', 'Madame Lefebvre' and 'Makassar', and could cause gum formation both in healthy and in infected bulbs, when they were being stored together (Bergman 1965, Kamerbeek et al. 1971, De Hertogh et al. 1980). Most tulip cultivars including 'Albury', 'Bellona', 'Leen van der Mark', 'Monte Carlo', 'Red Champion', 'Rose Copland' and 'White Sail' do not produce gums when they are infected with *F.ox.t.*, even with large amounts of ethylene (De Hertogh et al. 1980). Symptoms of gummosis are observed on bulbs after their digging out but they can also appear in soil at the end of vegetation period if ethylene producing fungi are present. Substantial gum production was also observed in healthy, uninfected tulip bulbs treated with ethephon (2-chloroethanophosphonic acid; Saniewski 1980). Tulip gums are mainly polysaccharides composed of xylose, arabinose and uronic acid (Saniewski et al. 2000).

Several tulip bulbs infected by *F.ox.t.* can be the source of ethylene sufficient to cause gummosis in a few hundred bulbs even.

The aim of this study was to find relation between ethylene production on one hand, and *Fusarium* bulb rot caused by two isolates of *F.ox.t.* in susceptible and resistant cultivars on the other hand, and gum formation in bulbs.

Material and methods

Two strongly pathogenic isolates of *F. oxysporum* f. sp. *tulipae* (*F.ox.t.* 218 and *F.ox.t.* 242) were used. The studies on healthy bulbs were conducted in July using cultivars from Group Triumph – 'Cassini', 'Couleur Cardinal', 'Kees Nelis', 'Prominence', 'White Dream', from Group Single Early – 'Christmas Marvel', from Group Double Early – 'Monte Carlo', from Group Darwin hybrid – 'Apeldoorn' and from Group Crispa – 'Fringed Elegance'.

After removing scales, bulbs were surface disinfected in 50% ethanol for 5 min and then rinsed three times in sterile distilled water. Afterwards the bulbs were inoculated with 5 mm diameter discs of the pathogen mycelium, placed on slightly damaged epidermis near to the basal plate.

Tulip bulbs were placed individually in 200 cm³ glass vials and sealed tightly. After 8, 16 and 28 days of incubation 1 ml gas samples were withdrawn and ethylene levels were determined with Hewlett Packard 5890 gas chromatograph. Jars were ventilated for 1 h after gas sampling. Ethylene level change was determined for four bulbs, two replications of each bulb.

During 28 days of incubation the development of fusariosis and gum formation induced by the pathogen was observed. Observations of disease symptoms development and gum formation were performed on 40 bulbs of each cultivar. The experiments were carried out in two series, 30 days apart. Uninoculated healthy bulbs with damaged epidermis were treated as a control.

Development of necrotic spots on inoculated bulbs' scales was the measure of tulip cultivars susceptibility to either of the tested *F.ox.t.* isolates.

The data were subjected to analysis of variance and t-Duncan's multiple range test ($\alpha = 0.05\%$ of significance level) was used for means separation.

Results and discussion

Infection of tulip bulbs by two isolates of *F. oxysporum* f. sp. *tulipae* (*F.ox.t.* 218 and *F.ox.t.* 242) resulted in development of *Fusarium* rot (Table 1). The slowest spread of disease was observed on cultivars 'Cassini' and 'Kees Nelis'. On their bulbs gum formation was not observed after inoculation with either isolate in question. On bulbs of 'Christmas Marvel', 'Couleur Cardinal', 'Monte Carlo' and 'White Dream' induction of gummosis was not observed but development of *Fusarium* bulb rot was noticed. On inoculated 'Prominence' bulbs an intense development of gummosis, as compared to the 'Apeldoorn' and 'Fringed Elegance' bulbs, was noticed.

In literature there is a hypothesis that gum formation could be a plant defense response to infection (Boothby 1983). Saniewska (2002) demonstrated that gums induced in tulip bulbs by *F. oxysporum* f. sp. *tulipae* are *de facto* stimulating not only growth and development of the pathogen, but also effect the *in vitro* growth and development of *F. oxysporum* formae speciales which are nonpathogenic to tulip, and by which gum induction process in host tissues has never been revealed.

Gum production may result from infection of bulbs by pathogens or from insect damage in many other species of plants, e.g. from Rosaceae family: plum, cherry, apricot, peach or almond (Boothby 1983).

It is known that *F. oxysporum* f. sp. *tulipae* under *in vitro* conditions can produce much more (up to several thousand times) ethylene than other formae speciales (Swart and Kamerbeek 1976, 1977).

Table 1

Development of fusariosis and induction of gummosis on bulbs of some tulip cultivars after inoculation with two isolates of *Fusarium oxysporum* f. sp. *tulipae*: *F.ox.t.* 218 and *F.ox.t.* 242

Group	Cultivar	Length of necrosis after 28 days of incubation (mm)		Depth of necrosis after 28 days of incubation (mm)		Presence of gum	
		<i>F.ox.t.</i> 218	<i>F.ox.t.</i> 242	<i>F.ox.t.</i> 218	<i>F.ox.t.</i> 242	<i>F.ox.t.</i> 218	<i>F.ox.t.</i> 242
Darwin hybrid	'Apeldoorn'	25.9 e	26.0 d	10.2 d	12.5 c	+++	+++
Single Early	'Christmas Marvel'	20.4 c	20.0 b	8.3 c	9.5 b	-	-
Double Early	'Monte Carlo'	19.7 b	19.0 b	9.5 d	10.0 b	-	-
Triumph	'Cassini'	15.7 a	16.4 a	6.5 a	7.6 a	-	-
Triumph	'Couleur Cardinal'	20.4 c	21.3 c	7.8 b	10.0 b	-	-
Triumph	'Kees Nelis'	15.4 a	15.9 a	6.2 a	7.2 a	-	-
Triumph	'Prominence'	20.5 c	25.0 d	7.8 b	9.4 b	++	++
Triumph	'White Dream'	18.7 b	20.7 b	7.7 b	10.6 b	-	-
Crispa	'Fringed Elegance'	23.7 d	24.0 d	11.0 e	10.0 b	+++	+++

Mean in columns followed by the same letter do not differ significantly at 5% level (Duncan's multiple range test).

"-" - no gums, "++" - medium gummosis, "+++ - large gummosis.

Bulbs of all tulip cultivars in question after inoculation with two isolates of *F. oxysporum* f. sp. *tulipae* (*F.ox.t.* 218 and *F.ox.t.* 242) produced high amounts of ethylene (Fig. 1 A, B, C, D). Additionally, ethylene production by infected bulbs of all the cultivars and their reaction to gummosis were differentiated. The studied *F. oxysporum* isolates showed different activity in ethylene production. Bulbs of all cultivars produced more ethylene after inoculation with *F.ox.t.* 242. The highest amount of ethylene was produced by bulbs of 'White Dream' after inoculation with

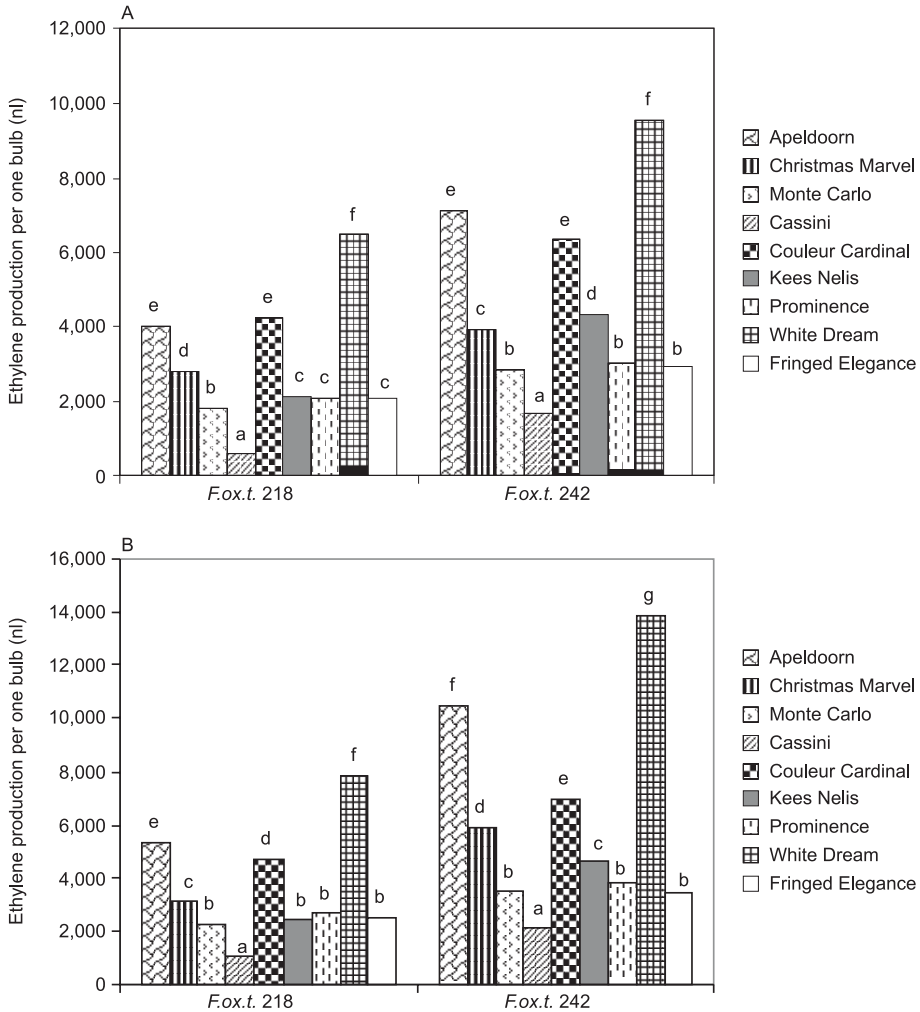


Fig. 1. Ethylene production in bulbs of some tulip cultivars after inoculation with two isolates of *Fusarium oxysporum* f. sp. *tulipae*: *F.ox.t.* 218 and *F.ox.t.* 242 after different time of incubation in closed jars; A – first measurement after 8 days of incubation, B – second measurement after 16 days of incubation, C – third measurement after 28 days of incubation, D – total production of ethylene after 28 days of incubation, sum from measurements A, B, C. Means for each isolate followed by the same letter do not differ significantly at 5% level (Duncan's multiple range test)

both isolates *F.ox.t.* 218 and *F.ox.t.* 242. After 28 days bulbs of ‘White Dream’ produced 20,069 nl per bulb and 32,986 nl per bulb when inoculated with isolates *F.ox.t.* 218 and *F.ox.t.* 242, respectively (Fig. 1 D). However, bulbs of that cultivar did not produce gums. The smallest amount of ethylene was produced by bulbs of ‘Cassini’: isolate *F.ox.t.* 218 – 2426 nl per bulb and twice as much – 5265 nl per bulb – isolate *F.ox.t.* 242 (Fig. 1 A, B, C, D).

Mechanism of ethylene production by bulbs of tulips infected with *F. oxysporum* f. sp. *tulipae* is not known.

Bulbs of all used cultivars which were not inoculated with *F. oxysporum* f. sp. *tulipae*, produced after epidermis damage vestigial amounts of ethylene (from 18.5 to 22 nl per bulb) or not registered amounts.

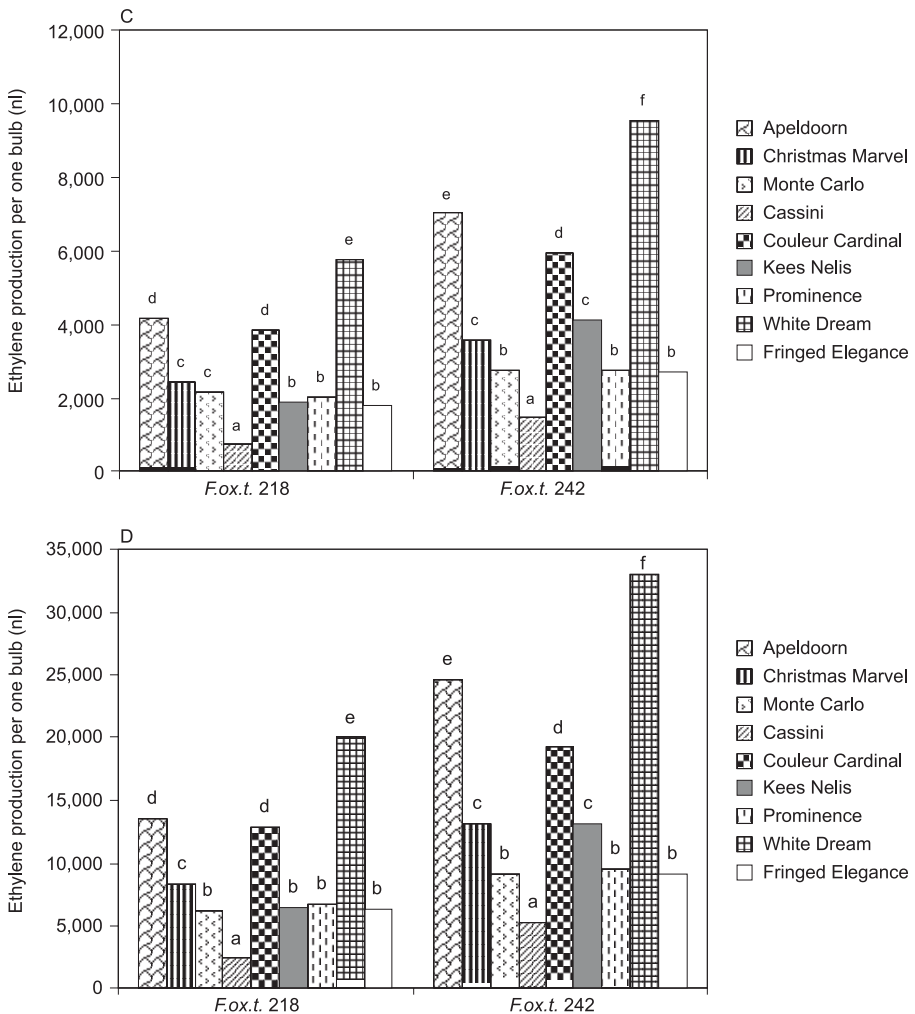


Fig. 1 – cont.

Miller et al. (2005) demonstrated that high production of ethylene in tulip bulbs infected with *F. oxysporum* f. sp. *tulipae* was associated with the mycelium, on the dead tissues of tulips.

It is possible that lack of gum formation in some cultivars infected with *F. oxysporum* f. sp. *tulipae* can be connected with differential ability of tissues to link ethylene to receptor places, but it does not depend on the degree of bulbs infection by the pathogen.

Conclusion

Formation of gums in tulip bulbs inoculated with *F. oxysporum* f. sp. *tulipae* is not connected with the amount of ethylene but depends on tulip cultivar.

Streszczenie

PRODUKCJA ETYLENU W CEBULACH TULIPANÓW ODMIAN POTENCJALNIE PODATNYCH I ODPORNÝCH NA TWORZENIE SIĘ GUM PO INFEKCJI PRZEZ *FUSARIUM OXYSPORUM* F. SP. *TULIPAE*

W warunkach laboratoryjnych badano zależności między produkcją etylenu i rozwojem fuzariozy spowodowanej przez dwa izolaty *Fusarium oxysporum* f. sp. *tulipae* (*F.ox.t.* 218 i *F.ox.t.* 242) na cebulach odmian potencjalnie podatnych i odpornych na tworzenie się gum. Na wszystkich dziewięciu badanych odmianach obserwowano rozwój fuzariozy. Cebule trzech odmian ('Apeldoorn', 'Fringed Elegance', 'Prominence') reagowały indukcją gumoży po zainfekowaniu przez oba izolaty *F.ox.t.*, a cebule innych sześciu badanych odmian ('Cassini', 'Christmas Marvel', 'Couleur Cardinal', 'Kees Nelis', 'Monte Carlo', 'White Dream') nie wytwarzały gum.

Cebule badanych odmian po inokulacji grzybnią *F. oxysporum* f. sp. *tulipae* produkowały zróżnicowane ilości etylenu. Największe ilości etylenu produkowały cebule odmiany 'White Dream', zarówno po inokulacji izolatem *F.ox.t.* 218, jak i po inokulacji izolatem *F.ox.t.* 242, ale odmiana ta nie reagowała indukcją gumoży w porażonych cebulach. Nie stwierdzono zależności pomiędzy produkcją etylenu a indukcją gumoży w zainfekowanych cebulach badanych odmian.

Literature

- Bergman B.H.H., 1965: Field infection of tulip bulbs by *Fusarium oxysporum*. Neth. J. Plant Pathol. 71: 129–135.
- Boothby D., 1983: Gummosis of stone fruit trees and their fruits. J. Sci. Food Agric. 34: 1–7.
- De Hertogh A.A., Dillely D.R., Blakely N., 1980: Response variation of tulip cultivars to exogenous ethylene. Acta Hort. 109: 205–210.

- Kamerbeek G.A., Verlind A.L., Schipper J.A., 1971: Gummosis of tulip bulbs caused by ethylene. *Acta Hortic.* 23: 167–171.
- Miller W.B., Verlouw M., Liou S.S., Cirri H.O., Watkins C.B., Snover-Clift K., 2005: Variation in *Fusarium*-induced ethylene production among tulip cultivars. *Acta Hortic.* 673: 229–235.
- Saniewska A., 2002: Wpływ gum indukowanych w cebulach tulipana przez *Fusarium oxysporum* Schlecht. f. sp. *tulipae* na wzrost i rozwój *in vitro* form specjalnych *Fusarium oxysporum* niepatogenicznych dla tulipana. *Zesz. Probl. Post. Nauk Roln.* 488: 773–778.
- Saniewski M., 1980: Rola etylenu we wzroście i rozwoju tulipanów. *Post. Nauk Roln.* 1: 37–48.
- 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.
- Swart A., Kamerbeek G.A., 1976: Different ethylene production *in vitro* by several species and formae speciales of *Fusarium*. *Neth. J. Plant Pathol.* 82: 81–84.
- Swart A., Kamerbeek G.A., 1977: Ethylene production and mycelium growth of tulip strains of *Fusarium oxysporum* as influenced by shaking of oxygen supply to the culture medium. *Physiol. Plant.* 39: 38–44.

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