Gummosis in Tulip (*Tulipa gesneriana* L.): Focus on Hormonal Regulation and Carbohydrate Metabolism

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ABSTRACT

This mini-review describes the promoting effects of *Fusarium oxysporum* f.sp. *tulipae*, ethylene and jasmonates on gum induction and its accumulation in tulips (*Tulipa gesneriana* L.). Tulip bulbs infected by *F. oxysporum* f.sp. *tulipae* have shown to produce considerable quantities of ethylene enough to induce gummosis in diseased and/or healthy bulbs of some cultivars. Morphological and histological disease symptoms induced by *F. oxysporum* f.sp. *tulipae* are mostly gummosis, and metabolic activities considered to be regulated by ethylene (i.e. respiration, inhibition of flower bud formation) in tulip bulbs are affected. Interestingly, methyl jasmonate (MeJA) exogenously applied as a lanolin paste also induced gums in tulip bulbs, but also in stem and basal part of leaves. It should be mentioned that under natural conditions for normal growth, gums are not formed in stems and leaves. The simultaneous application of ethylene with MeJA greatly accelerates gum formation in the bulb, stems and leaves compared to the application of MeJA alone. MeJA and jasmonic acid (JA) were successfully identified in stems and the possible mechanism of jasmonates to induce gummosis in tulips is relevant to their effects on sugar metabolism. Not only the content but also the composition of tulip gum polysaccharides has been determined and been shown to consist of glucuronoarabinoxylan (GlcN: Ara: Xyl = 1: 2: 3) with an average molecular weight of ca. 700 kDa. The induction and production of gums are suggested to be regulated by a signal network of jasmonates and ethylene, especially by cross-signals between them.

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INTRODUCTION

Gums are complexes of different substances but the most important constituents are polysaccharides (Boothby 1983). Induction and formation of gums designated as gummosis are found throughout the plant kingdom (especially in plants of the Rosaceae), and gummosis is induced by biotic and abiotic environmental factors such as bacterial and fungal infection, insect attack, mechanical and chemical injury, water stress, and other environmental stressors in some plant species.

Infection of *Fusarium oxysporum* f.sp. *tulipae* has been shown to induce gummosis in diseased and/or healthy bulbs of some tulip cultivars (Kamerbeek *et al*. 1971; Kamerbeek and de Munk 1976). All these factors are considered to act via ethylene produced in plant tissues. Ethylene or ethylene-releasing compounds (i.e. ethephon; 2-chloroethylphosphonic acid) also stimulate gum formation in stone-fruit trees and in fruits of the Rosaceae family, including

apricot, cherry, ornamental Japanese cherry, peach, plum and almond (Boothby 1983). These results suggest that ethylene is a common factor involved in the induction of gummosis (Boothby 1983).

Jasmonic acid (JA), methyl jasmonate (MeJA) and their derivatives (referred to as jasmonates) are widely distributed in the plant kingdom, showing various biological activities in regulating plant growth and development (Ueda and Kato 1980; Saniewski 1995; Creelman and Mullet 1997; Murofushi *et al*. 1999; Saniewski *et al*. 1999). As well as ethylene, it is well known that biotic and abiotic stressors cause a rapid increase in the level of endogenous jasmonates, mainly JA and MeJA (Saniewski 1997). Jasmonates exogenously applied to intact plants or to plant tissues also stimulate ethylene production. From this point of view, jasmonates are also possible key factors in the induction of gummosis in plants. This idea is true. Saniewski *et al*. (2000, 2006) have already shown that MeJA substantially induces gummosis in cherries, plums, peaches, apricots and tulips.
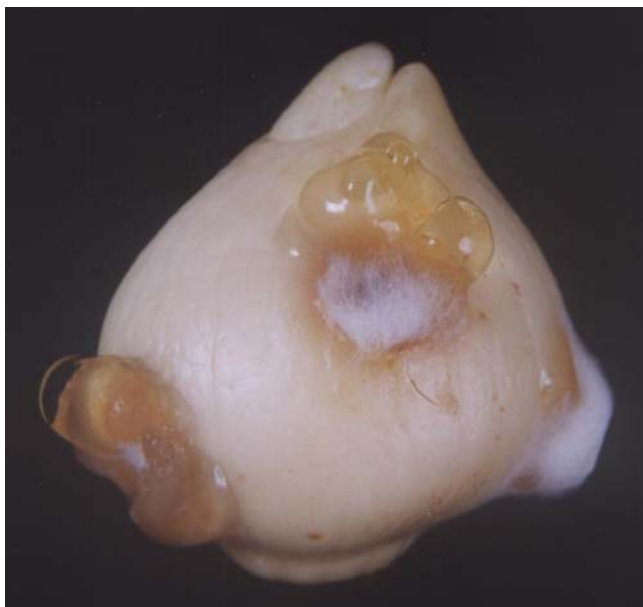
Invited Mini-Review

Fig. 1 Gummosis in tulip bulb (cv. 'Apeldoorn') induced by *Fusarium oxysporum* f. sp. *tulipae* after artificial infection; mycelium growth of the pathogen can be seen on the extruded gums before gums were solidified. Inoculation with the pathogen on the bulb was made at the end of July and bulbs were kept in laboratory conditions. Picture was made on 10 days after treatment.

This review describes the physiological effects of *Fusarium oxysporum* f.sp. *tulipae*, ethylene and MeJA on induction of gummosis and gum accumulation in tulips.

MORPHOLOGICAL AND HISTOLOGICAL DISEASE SYMPTOMS INDUCED BY *FUSARIUM OXYSPORUM* f.sp. *TULIPAE*

Morphological and histological studies in tulip bulbs cv. 'Apeldoorn' infected by *Fusarium oxysporum* f.sp. *tulipae* were intensively carried out in Poland by Saniewska *et al.* (2004). These studies revealed that *F. oxysporum* f.sp. *tulipae* infects tulip bulbs at all stages in their development. Although *F. oxysporum* f.sp. *tulipae* is a soil pathogen attacking tulip bulbs, all other organs of tulip shoots can also be infected with no specific preference (Saniewska and Wach 2003b). However, gummosis occurs exclusively in infected bulbs and such symptoms have not been found around infected areas on other tulip organs (**Fig. 1**).

Gummosis induced by the pathogen appears in small bulbs collected during forcing, during flowering outdoors, directly after lifting as well as during and after flower bud formation, up to the end of October. When a mycelium disk (5 mm in diameter) of *Fusarium oxysporum* f.sp. *tulipae* was applied to the surface of tulip bulbs, much higher gum production in artificially inoculated bulbs was observed up to the end of July, followed by a gradual disappearance of this process. Excreted gums are visible on the surface of scales 3-4 days after inoculation with *F. oxysporum* f.sp. *tulipae*. In early November, uncooled bulbs with progressing fusariosis show only traces of gum induction, which finally ceases in the middle of December. In the case of cooled bulbs, gum does not form in response to the pathogen (Saniewska *et al.* 2004). The reason why the response of tulip bulbs cv. 'Apeldoorn' by gum formation after infection with *F. oxysporum* f.sp. *tulipae* disappears gradually after lifting is unclear, but some metabolic changes during flower bud formation including some senescence processes of scales may be involved.

Pathogen-induced gums in tulip bulbs first form red colored structures, and some time later, the entire gum turns pinkish-red with mycelia growing within. The presence of pathogen was also detected in gums induced in the scales of the bulb, mostly in parenchyma, but not in those adjacent to the infected part of the scale. Microscopic analysis showed that gum production around the infected area in a scale started as soon as 48 h after inoculation with spores. Gum was also found within intercellular spaces distant (uninfected) to the infected tissues. Occurrence of gummosis and its expansion within the parenchyma of bulb scales brought about changes such as thickening of cell wall in the vicinity of the gum formation area, frequent injuries and degradation of cell walls, cell plasmolysis, gradual degradation of starch grains and enlargement of intercellular spaces. The latter was related to a loss of wall continuity frequently observed in parenchyma cells (Saniewska *et al.* 2004). The gums are formed mainly on the surface of the outer bulb scale, in certain layers below the epidermis. When gum production increases too much, the epidermis bursts, resulting in extrusion of gums and the color of gums gradually becomes bistered.

Histological studies were conducted on tulip stems during different stages of gum formation induced by MeJA applied exogenously with ACC, as a source of ethylene, and compared to control stems (Saniewski and Dyki 1997). Transversal and longitudinal sections of stems were stained with periodic acid-Schiff's reagent (PAS), ruthenium red, safranin-fast green, and Sudan III. The dissolution of the cell wall of the epidermis, cortex and pith cells was observed and finally gum ducts of different sizes were shown to form. The space of cell walls was impregnated with gum. The degradation products of the cell wall and protoplasts seem partially to contribute to the production of gum exudates. The destructive processes of the cell wall were not observed in the vascular bundles occurring in the pith part of the stem.

Saniewska and Wach (2003a) studied the development of fusariosis caused by *Fusarium oxysporum* f.sp. *tulipae* on potentially susceptible ('Apeldoorn', 'Prominence') and resistant ('Cassini', 'Couleur Cardinal', 'Kees Neils', 'White Dream', 'White Sail', 'Christmas Marvel') tulip cultivars to gum formation in bulbs under laboratory conditions. After the inoculation of *F. oxysporum* f.sp. *tulipae* mycelium to tulip bulbs, the development of fusariosis took place in all tulip cultivars although the smallest infection was observed on the bulbs of cvs. 'Cassini', 'Couleur Cardinal', 'Kees Neils' and 'White Sail'. In the case of cvs. 'Christmas Marvel', 'Monte Carlo' and 'White Dream', the development of fusariosis was much larger. In infected bulbs of cvs. 'Apeldoorn' and 'Prominence', strong and moderate gummosis were observed, respectively, but the development of the disease in both cultivars was almost similar. Thus, the development of fusariosis on bulbs of different tulip cultivars is not strictly connected with the induction of gummosis. It is an open question as to why only some tulip cultivars showed a strong gummosis response to ethylene treatment (e.g. cvs. 'Apeldoorn' and 'Enterprise'), whereas bulbs of other cultivars (e.g. cvs. 'Red Champion' and 'White Sail') hardly showed any response to ethylene, i.e. lack of gummosis (Kamerbeek *et al.* 1971; **Table 1**). It is possible that the lack of gummosis in bulbs of some tulip cultivars infected with *F. oxysporum* f.sp. *tulipae* can be connected with differential ability of tissues of different cultivars to bind ethylene to receptors but it is not dependent on the degree of infection by the pathogen. This also possibly depends on other factors such as the mode of pathogen infection, location of infection, dose of spore application, developmental stage of the bulb and physiological age of the tulip plant/bulb.

The physiological role of gums in plants is not clearly understood. It has been believed that gums have a function limiting the spread of fungal and bacterial pathogens and insects by isolating the infected and infested tissues from healthy ones (Boothby 1983). In light microscopic analysis of cross-sections of gums induced in tulip bulbs after inoculation with *Fusarium oxysporum* f.sp. *tulipae*, the presence of pathogen hyphae and spores was observed in gums neighbouring the infected scale (Saniewska *et al.* 2004).

Table 1 Cultivars of tulip susceptible and resistant for gummosis after inoculation with *Fusarium oxysporum* f.sp. *tulipae* or treated with exogenous ethylene.

Cultivar	Induction of gummosis*	References
Apeldoorn	+ (E)	Kamerbeek *et al.* 1971
Enterprise	+ (E)	Kamerbeek *et al.* 1971
Bartigon	+ (E)	Kamerbeek *et al.* 1971
Madame Lefeber	+ (E)	Kamerbeek *et al.* 1971
Diplomate	+ (E)	de Hertogh *et al.* 1980
Golden Melody	+ (I)	de Hertogh *et al.* 1980
Makassar	+ (E)	de Hertogh *et al.* 1980
Paul Richter**	+ (I)	de Hertogh *et al.* 1980
Aureola	+ (E)	de Hertogh *et al.* 1980
Elmus	+ (I)	de Hertogh *et al.* 1980
Robinea	+ (E)	de Hertogh *et al.* 1980
Yellow Present	+ (I)	de Hertogh *et al.* 1980
Prominence***	+ (E)	Saniewska and Wach 2003a
White Sail	-	Kamerbeek *et al.* 1971
Red Champion	-	Kamerbeek *et al.* 1971
Rose Coplan	-	Kamerbeek and de Munk 1976
Leen van der Mark	-	de Wild *et al.* 2002
Albury	-	de Hertogh *et al.* 1980
Bellona	-	de Hertogh *et al.* 1980
Bing Crosby	-	de Hertogh *et al.* 1980
Gander	-	de Hertogh *et al.* 1980
Kees Nelis	-	de Hertogh *et al.* 1980
Monte Carlo	-	de Hertogh *et al.* 1980
Most Miles	-	de Hertogh *et al.* 1980
Olaf	-	de Hertogh *et al.* 1980
Orient Express	-	de Hertogh *et al.* 1980
Prominence***	-	de Hertogh *et al.* 1980
Apricot Beauty	-	de Hertogh *et al.* 1980
Cassini	-	de Hertogh *et al.* 1980
Christmas Marvel	-	de Hertogh *et al.* 1980
Couleur Cardinal	-	de Hertogh *et al.* 1980
Mirjoran	-	de Hertogh *et al.* 1980
Palestrina	-	de Hertogh *et al.* 1980
Peerless Pink	-	de Hertogh *et al.* 1980
Paul Richter**	-	de Hertogh *et al.* 1980
Princess Irene	-	de Hertogh *et al.* 1980

* (+) = presence, (-) = absence, (E) = external gummosis, (I) = internal gummosis.
** In forcing season 1976/77 - internal gummosis was induced by ethephon but in forcing season 1977/78 - totally no gummosis.
*** In 1980 - no gummosis after ethephon treatment but in 2003 - moderate gummosis after inoculation with *Fusarium oxysporum* f.sp. *tulipae*.
In references of Kamerbeek *et al.* (1971), Kamerbeek and de Munk (1976) and Saniewska and Wach (2003a), tulip bulbs of these cultivars were inoculated with *Fusarium oxysporum* f.sp. *tulipae*. In references of de Hertogh *et al.* (1980) and de Wild *et al.* (2002), ethephon was only used for testing of gummosis in bulbs of these cultivars.

Saniewska (2002a, 2002b) studied the effect of gums induced by *F. oxysporum* f.sp. *tulipae* in tulip bulbs on the mycelium growth and development of the pathogen *in vitro*. The addition of gums at a concentration of 5 mg·cm^{-3} to Czapek-Dox-Agar (CzDA), Malt-Extract-Agar (MEA) and Potato-Dextrose-Agar (PDA) greatly stimulated mycelium growth of *F. oxysporum* f.sp. *tulipae* and sporulation of the pathogen. The surface of mycelia of the pathogen after 7 days of culturing on CzDA, MEA and PDA supplemented with gums was stimulated to spread six-, two- and three-fold respectively, compared to the control (the same media without gums). The effect of gums induced in tulip bulbs on *in vitro* growth and development of *formae speciales* of *F. oxysporum* nonpathogenic for tulip on CzDA medium was also investigated (Saniewska 2002c). The addition of gums at a concentration of 5 mg·cm^{-3} did not affect linear mycelial growth of *F. oxysporum* f.sp. *callistephi* and *F. oxysporum* f.sp. *narcissi*, but mycelia were more abundant; great felt-like aerial mycelial growth was observed. The addition of tulip gums to the medium greatly stimulated sporulation of the investigated *formae speciales* of *F. oxysporum*. On the basis of these results, tulip gums are at least not antifungal substances and they have an evident stimulatory effect on the mycelial growth of *F. oxysporum* f.sp. *tulipae*,

although finally the spread of the pathogen is limited.

Different kinds of oligosaccharides have been shown to function in plants as molecular signals (elicitors) that regulate growth, development and survival in the environment (Aldington *et al.* 1991; Côte and Hahn 1994; Ebel and Mithöfer 1998). It is possible that a polysaccharide of tulip gums, which is a glucuronoarabinoxylan (Saniewski *et al.* 2000), acts as an elicitor that regulates some processes connected or responsible for mycelial growth of *Fusarium oxysporum* f.sp. *tulipae*. At present the stimulatory role of the polysaccharide of tulip gums as a substrate on the mycelial growth of *F. oxysporum* f.sp. *tulipae* was not excluded, since tulip gums also contain many other unidentified compounds, which may have a stimulatory effect on mycelium growth of the pathogen.

Consequently, Saniewska *et al.* (2005) studied the effect of tulip gums on the secretion of some enzymes to the culture medium. *F. oxysporum* f.sp. *tulipae* grown on liquid Czapek-Dox-Broth medium containing sucrose (CzDB) did not secrete all analyzed enzymes to the medium. However, on CzDB medium supplemented with tulip gums *F. oxysporum* f.sp. *tulipae* clearly secreted β-1,4-glucosidase, α-1,4-galactosidase, β-xylosidase and polygalacturonase. Incubation of *F. oxysporum* f.sp. *tulipae* on mineral liquid CzDB (m-CzDB) caused secretion of β-1,4-glucosidase, α-1,4-galactosidase and polygalacturonase to the medium and supplementation of the medium with tulip gums greatly increased secretion of those enzymes and caused secretion of β-xylosidase. In all analyzed variants of used media after incubation of *F. oxysporum* f.sp. *tulipae* no activity was detected in the following enzymes: β-1,4-galactosidase, β-1,4-glucuronidase, α-1,4-xylosidase, α-1,4-arabinosidase, α-1,4-L-arabinosidase, β-L-arabinosidase, α-1,4-mannosidase, α-1,4-rhamnosidase and β-1,4-galacturonidase. Enzymatic adaptation is readily accomplished by *F. oxysporum* and facilitates growth and pathogenicity. Enzyme production is conservative, generally being formed as required; if not needed, such as when alternates are available, enzyme synthesis may be at very low level (Woltz and Jones 1981). Exposure to tulip gums as the carbon source activates enzymes or the enzyme-synthesizing mechanisms in *F. oxysporum* f.sp. *tulipae*.

ETHYLENE AS A GUMMOSIS-INDUCING FACTOR IN TULIPS

Infection of *Fusarium oxysporum* Schlecht f.sp. *tulipae* Apt. to tulip bulbs brings an intensive production of ethylene and formation of gums in some cultivars (Kamerbeek *et al.* 1971; Kamerbeek and de Munk 1976). As proved, under *in vitro* conditions this pathogen can produce much more (even several thousand-fold) ethylene than other *formae speciales* of *F. oxysporum* and *Fusarium* species (Swart and Kamerbeek 1976, 1977). *F. oxysporum* f.sp. *tulipae* produces ethylene abundantly *in vitro* when it is grown in Pratt's liquid medium with glucose as the only organic substrate. This production starts after a lag phase of about 4 days, and peak production occurs when mycelium weight has reached its maximum value. The total production is also dependent on the oxygen concentration, but pure oxygen inhibits the total production by about 50% as compared with 21% oxygen. No relationship was found between ethylene production and mycelium growth (Swart and Kamerbeek 1977). 1-Aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ethylene in plants, was not detected in mycelial extracts, indicating that the ethylene biosynthetic pathway of *F. oxysporum* f.sp. *tulipae* differs from that in plants (Hottiger and Boller 1991). Hottiger and Boller (1991) suggest on the basis of their experimental work, that ethylene production in *F. oxysporum* f.sp. *tulipae* derived from arginine passes through glutamate/2-oxoglutarate.

During storage and shipping ethylene produced by *Fusarium oxysporum* f.sp. *tulipae* causes severe damage in tulip bulbs: an increase in respiration, gummosis, weight loss, inhibition of flower bud formation, flower abortion

(saleable sizes) and excessive splitting (planting stock) (Kamerbeek and de Munk 1976; de Wild *et al.* 2002; Gude and Dijkema 2005). de Wild *et al.* (2002) showed that treatment with 0.3 Pa ethylene for 2 days caused gummosis on 50% of the total number of bulbs of cv. 'Apeldoorn' known to be sensitive to gummosis. Pretreatment with 1-methylcyclopropene (1-MCP), an ethylene inhibitor, prevented ethylene-induced gummosis in cv. 'Apeldoorn', indicating that ethylene action has been directed via the ethylene receptor, since 1-MCP is known to block the ethylene receptor binding-site (Gude and Dijkema 2005). Gude and Dijkema (2005) tested the efficacy of 1-MCP in preventing the effects of ethylene in tulip bulbs under both laboratory and practical conditions and showed that the bulbs were fully protected from ethylene, including gummosis in cv. 'Apeldoorn', by treating them every 12 days for 24 h with 0.2 ppm 1-MCP. These results also suggest that ethylene action must have been directed via the ethylene receptor.

Miller *et al.* (2005) studied the biological source of ethylene produced by tulip bulbs infected by *Fusarium oxysporum* f.sp. *tulipae* and showed that all of the ethylene derives from the *Fusarium* mycelia since in the presence of *Fusarium*, heat-killed bulbs produced large quantities of ethylene. Heat-killed bulbs are no longer capable of metabolism as enzymes are denatured and membranes destroyed, however, the cellular contents used for fungal growth (amino acids, sugars and minerals) are fully available for the pathogen as the active defense systems of the bulbs are destroyed (Miller *et al.* 2005). Ethylene production from tulip bulbs with inoculation varies greatly with cultivar, and leads to speculation that the cultivars that support the greatest ethylene production contain a higher tissue concentration of arginine, the amino acid that probably directly supports ethylene biosynthesis (Hottiger and Boller 1991) or some other promoting factors (Miller *et al.* 2005). Miller *et al.* (2005) also suggested that alternatively, cultivars supporting lower ethylene levels may have greater levels of tulipaline-A.

The application of lanolin paste containing 5% ethephon to intact tulips around the stem, just below the flower bud, resulted in the formation of gum blisters at the basal end of the perianth leaves and the outflow of gums and hardening of the substance was achieved when the perianth base was wounded with a needle (de Munk and Saniewski 1989). Also the application of 1 or 5% ethephon in lanolin paste on the cut surface of the tulip stem after excision of the shoot below the basal leaf resulted in the extrusion of small amounts of gums from the wounded area within 4 days (de Munk and Saniewski 1989). These facts indicate that tulip organs other than bulbs are also capable of inducing gummosis in response to ethylene, although gummosis induced by the infection of *Fusarium* has not been found around infected areas on other tulip organs.

JASMONATES AND GUMMOSIS IN TULIPS

Saniewski and Puchalski (1988) showed the successful induction of gummosis in the bulb, stem and basal part of the leaf in cvs. 'Apeldoorn' and 'Gudoshnik' following the application of MeJA (**Figs. 2, 3**). Tulip bulbs and stems applied with MeJA at a concentration of 1.0% in lanolin paste on different internodes extruded gums on the surface of bulbs and of stems mostly in the place of application and around after 5-7 days from treatment. MeJA applied on the basal part of the leaf (about 2 cm from the base of a leaf) of intact tulips caused strong yellowing of all parts of the leaves above the insertion point and extrusion of gum drops on the surface of the leaves near the place of treatment and these symptoms were well observed after 4-6 days after treatment. Treatments with MeJA in the upper part of the leaves did not induce gum formation. It is possible that the lower part of the leaves accumulates a higher level of carbohydrates functioning as a substrate for gum induction after MeJA application. It should be mentioned that under natural conditions of tulip growth, gums are not formed on

Fig. 2 Gum exuded on tulip bulb (cv. 'Apeldoorn') applied MeJA as lanolin paste at a concentration of 1.0% at the end of July. After the application, tulip bulbs were kept in a greenhouse under natural light conditions and at 20-25°C; picture was made 15 days after treatment.

Fig. 3 Gum exuded on tulip stem (cv. 'Apeldoorn') applied MeJA as lanolin paste at a concentration of 1.0% at the beginning of flowering time. After the application, tulips were kept in the greenhouse under natural light conditions and at 20-25°C; picture was taken 10 days after treatment.

the leaves and stem. However, it is possible to induce small amounts of gums in the wounded stems and in the base of the perianth in tulips under the influence of ethephon (de Munk and Saniewski 1989).

While analysis of cationic composition of ash from tulip gums induced by MeJA + ACC (source of ethylene) treatment in tulip stem showed a high content of calcium and potassium, and much smaller amounts of magnesium, iron, manganase, zinc, lead, copper and nickel, the majority of gum consisted of polysaccharides (Saniewski *et al*. 2000). Preliminary studies of the chemical composition of the polysaccharides in gums induced by MeJA in stems and bulbs of tulips showed that they were almost similar to gums induced by ethephon in bulbs, and they consisted mainly of arabinose, xylose, traces of mannose and glucose, and uronic acid(s) (Saniewski and Puchalski 1988; de Munk and Saniewski 1989). Tulip gums formed in leaves and the stem induced by MeJA + ACC treatment contained ca. 16% uronic acid, and the remaining neutral sugars consisted of arabinose (ca. 40%) and xylose (ca. 60%). These results suggest that tulip gums consist of glucuronoarabinoxylan (GlcN: Ara: Xyl = 1: 2: 3) with an average molecular weight of ca. 700 kDa (Saniewski *et al*. 2000; Skrzypek *et al*. 2005c). Composition of gum polysaccharide was quite different from that of matrix polysaccharide in the tulip cell wall, suggesting that gum is not merely derived from the degraded product of cell wall polysaccharides, but rather newly synthesized polysaccharides (Skrzypek *et al*. 2005c). However, the kind of carbohydrates that participate in gum formation induced by MeJA in the tulip bulb, stem and leaves and induced by ethephon in bulbs, and the biosynthesis pathway(s) of gum polysaccharides are still unknown.

JA and MeJA were successfully identified in the stem of tulips cv. 'Apeldoorn' using gas liquid chromatography-mass spectrometry (Skrzypek *et al*. 2005b). The total amounts of JA and MeJA designated as jasmonates in tulip stems were estimated at about 70-80 ng/g fresh weight, using deuterium-labeled jasmonates as internal standards. The application of ethephon only slightly affected the endogenous level of jasmonates in tulip stems (Skrzypek *et al*. 2005a, 2005c). In this experimental system ethephon had little effect on gummosis in tulip shoots.

MeJA has been found to affect fatty acid and sterol concentrations in the first internode of the tulip stem during the induction of gum production (Saniewski *et al*. 1992). MeJA greatly increased the concentrations of free and bound oleic and linoleic acids, but did not affect those of free and bound palmitic, stearic and linolenic acids. MeJA approximately doubled the concentration of stigmasterol, but did not influence those of campesterol and β-sitosterol. The changes in fatty acid and sterol concentration induced by MeJA in the tulip stem may be caused by promoted senescence and possibly are associated with the induction of gum formation.

MeJA also affected other metabolic processes in tulips, evidently stimulated polyphenol oxidase and peroxidase activities, decreased the content of starch and lowered the amylase activity in scales of tulip bulbs during the storage period (Saniewski and Czapski 1989), stimulated anthocyanin accumulation in the stem and leaves from uncooled and cooled bulbs, and slightly enhanced CO_2 evolution in sprouting bulbs independent of storage conditions (Saniewski *et al*. 1998a).

INTERACTION OF ETHYLENE AND JASMONATES IN GUMMOSIS IN TULIPS

Consequently, the next question is whether or not ethylene is responsible for gum formation induced by MeJA in tulips. Saniewski and Wegrzynowicz-Lesiak (1994) showed that MeJA stimulated the evolution of ethylene and activity of ACC oxidase during gum induction in the basal part of tulip internodes, and that ACC application alone caused evolution of ethylene at a much higher level than by MeJA, but

did not induce gum formation. Also a high level of endogenous ethylene induced by exogenous auxins (IAA and NAA) in the tulip stem did not induce gummosis (Saniewski *et al*. 1990). These facts suggest that MeJA induces gum formation in the tulip stem in another way than only through stimulation of ethylene production (Saniewski and Wegrzynowicz-Lesiak 1994).

Further studies showed that gum formation in the stem and the basal part of the leaves induced by MeJA (1.0% w/w in lanolin) was strongly stimulated by the simultaneous application of 1 or 5 mM ACC (Saniewski and Wegrzynowicz-Lesiak 1995; Saniewski *et al*. 1998b). MeJA at a concentration of 0.1% did not induce gum, but together with ACC at a concentration of 1 or 5 mM induced it substantially. Moreover, MeJA induced gum formation in cut shoots of tulips 4 days after the treatment, especially in the basal part of the leaves, when it was applied as a solution at a concentration of 0.23 mM, or higher (Saniewski *et al*. 1998b).

α-Aminooxyacetic acid (AOA), a potent inhibitor of the conversion of *S*-adenosylmethionine (SAM) to ACC and $CoCl_2$, a potent inhibitor of ACC oxidase activity, did not affect gum production induced by MeJA in the tulip stem but partially lowered ethylene production induced by MeJA when measured 2 or 3 days after treatment, and on the fifth day after treatment did not inhibit ethylene evolution (Saniewski and Wegrzynowicz-Lesiak 1995; Saniewski *et al*. 1998b). MeJA also induced strong gummosis in all internodes of stems of cooled tulip bulbs whose growth was induced by IAA, after removal of the flower bud and all leaves (Saniewski 1989). Silver thiosulphate (STS), an inhibitor of ethylene action, applied to the first leaf sheath almost totally inhibited gum formation induced by MeJA, and STS applied alone did not cause morphological changes (Saniewski 1989). In this experimental system MeJA greatly stimulated ethylene production and ACC oxidase activity in the tulip stem, and STS inhibited ethylene production induced by MeJA and only partially inhibited ACC oxidase activity. It is possible that the inhibitory effect of STS on gum production induced by MeJA is connected with the inhibitory effect of STS on ethylene action and production (Saniewski 1989).

MeJA applied in the middle of the fourth internode partially inhibited stem growth induced by IAA applied in the place of the removed flower bud and after removal of all leaves in uncooled, derooted tulip bulbs of cvs. 'Apeldoorn' and 'Gudoshnik' (Saniewski *et al*. 2005). In the case of the cooled, derooted bulbs of both cultivars, treated in the same way as uncooled bulbs, MeJA inhibited only the fourth internode growth induced by IAA and induced gummosis in that internode (Saniewski *et al*. 2005). These results strongly suggest that MeJA is required for gum formation in tulip shoots, and that ethylene probably makes the tissues of shoots sensitive to MeJA (Saniewski *et al*. 1998b).

As was mentioned earlier, the application of MeJA together with ethylene greatly accelerated gum formation in the tulip stem in comparison to MeJA treatment alone. Ethephon applied simultaneously with MeJA greatly enhanced gum production in the tulip bulbs of cv. 'Apeldoorn' at

Table 2 Interaction of methyl jasmonate (MeJA) and ethephon (Eth) in gum production in tulip bulbs cv. 'Apeldoorn'.

Date of treatment	MeJA 1.0% (mg)		Eth. 1.0% (mg)		MeJA 1.0% + Eth. 1.0% (mg)	
	dark	light	dark	light	dark	light
August 21	807	-	249	-	1564	-
September 6	251	171	444	46	1429	1392
October 4	243	137	243	112	1290	1008
October 23	29	-	33	-	762	-
November 5	81	-	16	-	670	-
November 12	-	83	-	-	-	314

Experiments were conducted in 2001. Tulip bulbs were kept in the dark or in the light. Gum production was determined one month after treatment.
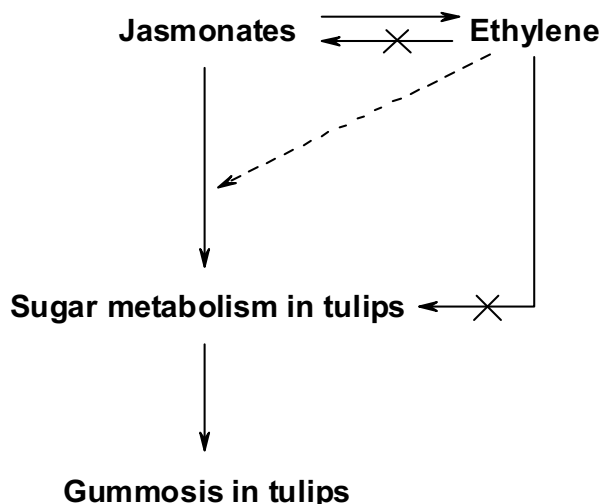- : not tested

Jasmonates ⟶ Ethylene

Sugar metabolism in tulips

Gummosis in tulips

Fig. 4 A possible mode of action of jasmonates and ethylene to induce gummosis in tulips in relation to sugar metabolism.

every date of treatment, starting from the beginning of July until the end of November (Saniewski *et al.* 2004). This kind of interaction was observed both in natural light conditions and in the dark (**Table 2**). The abilities of MeJA and ethephon to induce and produce gums decreased gradually from July to December. Slight gum formation was observed in the simultaneous application of MeJA and ethephon in November and December although no gums were found in the treatment of MeJA and ethephon alone at that time (Saniewski *et al.* 2004).

Skrzypek *et al.* (2005a, 2005c) suggested that the mode of action of jasmonates in inducing gums in tulip shoots is due to a change in sugar metabolism (**Fig. 4**). MeJA strongly decreased the total amount of soluble sugars in tulip stems even within 1 day after application, being ca. 50% of initial values 5 days after application, but ethephon did not. However, both MeJA and ethephon had almost no effect on the neutral sugar composition of soluble sugars, mainly consisting of glucose, mannose and xylose in the ratio of 20: 2: 1 and traces of arabinose. Both MeJA and ethephon applied exogenously stimulated senescence of tulip shoots shown by the loss of chlorophyll (Skrzypek *et al.* 2005c).

Simultaneous treatment of jasmonates and ethylene caused the synergistic induction of pathogenesis-related gene expression osmotin mRNA in tobacco seedlings (Xu *et al.* 1994), suggesting that the binding of ethylene to its receptor on the plasma membrane might sensitize MeJA receptors on the membrane. Emery and Reid (1996) also reported that the simultaneous application of MeJA and ACC caused a dramatic degradation of the cell membrane as indicated by an increase in conductivity in sunflower seedlings and neither the application of ACC alone nor that of MeJA with ethylene inhibitors evoked such an increase in conductivity. The relationships between jasmonates and ethylene in the regulation of some physiological processes in plants have been already reviewed by Saniewski *et al.* (1999, 2002, 2003).

The mechanism of the synergistic interaction between ethylene and jasmonates in gum induction and/or its production in tulips and other plants is unknown. Induction and production of gums have been suggested to be regulated by a signal network of jasmonates and ethylene, especially by cross-signals between them. It is possible that in the case of exogenously-applied ethylene or MeJA, the interaction between endogenous jasmonate and ethylene, and gene(s) expression responsible for gum biosynthesis takes place. Further research is needed to elucidate the physiological roles of ethylene and jasmonates in gum induction and formation.

CONCLUDING REMARKS

Plants are exposed to biotic and abiotic environmental stresses that influence their growth and productivity. Pathogen infection and insect invasion, mechanical wounding and other abiotic stresses are well known to be common factors inducing biosynthesis of ethylene and jasmonates in plants. On the other hand jasmonates have been reported to control ethylene biosynthesis in intact plants and their organs by stimulating the activities of ACC synthase and ACC oxidase. It is still an open question as to what is the role of jasmonates in controlling ethylene biosynthesis in plants infected by pathogens and attacked by insects, and in mechanically wounded tissues. Gums are induced in some plant species by infection, insect attack, mechanical and chemical injury, water stress, and other environmental stressors. In the case of tulip bulbs of some cultivars gum induction and production can be induced by infection by *Fusarium oxysporum* f.sp. *tulipae*, or the application alone of ethylene and MeJA to healthy bulbs. In the tulip stem and basal part of leaves gummosis can be induced by exogenous application of jasmonates, but not by treatment with ethylene alone. However, simultaneous application of ethylene and jasmonates substantially stimulates gum induction and production in bulbs, the stem and the basal part of leaves in tulips. It appears that jasmonates represent an integral part of the signal transduction chain between stress signal(s) and stress response(s), and interact with ethylene in gum induction and/or production and in many other physiological processes. The phenomenon of gummosis in tulips and other species induced by these compounds may be regulated by a signal network in which individual signals mediated by ethylene and jasmonates "cross-talk".

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