no. May 07

RESEARCH NEWSLETTER

This Flower Bulb Research Program Newsletter is published by Anthos, Royal Trade Association for Nurserystock and Flowerbulbs in cooperation with Dr. Bill Miller of Cornell University.

Fusarium, Tulips and Ethylene: Not as Simple as You Thought

William B. Miller
Cornell University

Summary
Ethylene evolution is a well-known result of *Fusarium* infection of tulip bulbs. We inoculated bulbs of 36 cultivars with a liquid *Fusarium* suspension (isolated from Dutch-grown bulbs), and held at 21C. Ethylene production was monitored by gas chromatography. It was found that *Fusarium* produced very different levels of ethylene, depending on the cultivar. By 19 days, inoculated 'Furand' and 'Nashville' produced ethylene at ca. 800 ul/bulb/day. Approximately 40% of the cultivars produced ethylene at rates greater than 150 ul/bulb/day, and 11% of them produced less than 5 ul/bulb/day. Thus, not all tulip cultivars support ethylene production by *Fusarium*, and some produce much more than "expected". Potentially, high-ethylene producing tulips could be stored separately from other cultivars, or increased ventilation should be maintained during storage or transportation. Knowledge of cultivar variation might also be useful in breeding programs.

While always a threat, in the last 5-6 years, *Fusarium* has emerged as a persistent and expensive problem in many tulip production regions of the world. Although *Fusarium* is "always" present in the tulip sector, its emergence as a larger problem may have a number of causes including a) changes in farming practices (e.g., larger farms, with borderline capacity for drying and ventilation), b) new bulb handling equipment (e.g., "peeling" machines), c) regulatory changes affecting fungicide availability, d) buildup of spore and inoculum in the soil, and e) the appearance of one or more "new" *Fusarium* strains that may be more resistant to fungicides and/or more aggressive in their infection and spread. Whether or not any of the above factors have been adequately proven is an open question.

In addition to direct injury, the *Fusarium* fungus is known to produce ethylene. Work done at the Bulb Research Centre many years ago first established this link and described the entire process of kernrot. Kernrot is the death, at an early stage, of the flower bud. It's initial cause is *Fusarium*-produced ethylene retarding the growth of the developing flower petals, such that the anthers are exposed at the top of the young flower bud. Mites of various species (mainly *Tyrophagus*) can then enter the bulb and feed on the young anthers and bud, causing complete loss of the flower.

Although the phenomenon of *Fusarium*-induced ethylene formation in tulips is well known, relatively little work has been conducted in this area in the last three decades. In the 1970's, Swart and Kamerbeek found that *Fusarium* isolates pathogenic on tulips produce at least 2,000 times more ethylene than nonpathogenic species or forms, and that ethylene production requires oxygen. The rate of ethylene production by infected tulips is also temperature dependent, and is maximal at 21ºC. At this temperature, infected 'White Sail' tulip bulbs produce ca. 150 ul/bulb/day, a value that appears to be the basis for industry standards for tulip ventilation requirements. Temperatures above or below 21°C reduce ethylene production.

Regardless if its source, ethylene has many negative effects on tulip bulbs, including flower abortion (any time between G-stage and anthesis the following spring), reduced shoot growth, reduced or slower rooting, production of "twisted" appearance and root hairs, enhanced bulblet production in the field ("splitting"), polysaccharide eruption from the bulb's cells (gummosis), and increased respiration.

The main technique used by industry to remove ethylene from the atmosphere surrounding bulbs is forced ventilation, the goal being to keep ethylene levels surrounding the bulbs at 0.1 ppm (ul/L) or less. Ethylene accumulation during transportation in temperature-controlled shipping containers is of special concern, and the maximal ventilation rate (>100 m^3/h) is less than optimal for complete removal of ethylene. The ventilation required is

obviously affected by the degree of *Fusarium* infection in the cargo, and the rate of ethylene production by the fungus. As will be seen below, the rate of ethylene production by *Fusarium* bulbs is not constant, and varies tremendously by cultivar.

The work reported in this newsletter was begun in 2003-2004, and was inspired by our Dutch intern that year, Martijn Verlouw of Creil. From a tulip-growing family, Martijn was interested in *Fusarium* and asked the simple question: "Do all tulip cultivars "produce" the same amount of ethylene when infected with *Fusarium*"? We can now answer that question with a resounding "No!"

Procedures

The basic procedure was to wound bulbs and then inoculate using a suspension made from cultures of *Fusarium oxysporum* grown on potato dextrose agar. Ethylene production was then monitored over time. The *Fusarium* culture was obtained from *Fusarium*-infected bulbs originating in the Netherlands. Suspensions were prepared by homogenizing (in a blender) the fungal lawn from a 10-cm Petri dish with ca. 150 ml water.

Thirty-six cultivars were selected (Table 1). In late November, bulbs were wounded by piercing the bulb base (avoiding the root collar) 3 times with a plastic point to a depth of about 0.5 cm. The tunic, if present, was removed prior to wounding. Bulbs were inoculated by dipping the bottom half of the bulb into the fungal suspension. After inoculation, bulbs were placed into 0.5L jars and held at 21°C in the dark. Control bulbs were wounded, but not inoculated. To determine ethylene, bulbs were sealed in the jars for 1-2 hours. Then, a 1 ml air sample was removed with a syringe and injected into a gas chromatograph to measure the ethylene in the "headspace".

Ethylene production by the infected bulb system was determined at approximately 3-day intervals for 19 days. This experiment has been repeated several times with essentially similar results.

We also conducted an experiment to demonstrate that the fungus is the source of the ethylene…not the bulb. To do this, we killed tulip bulbs by heating them in a microwave until they "boiled". By so doing, the ability of the bulbs to 1) produce ethylene or 2) fight off *Fusarium* infection was destroyed. *Fusarium* was then added to these bulbs, and compared to ethylene production in inoculated, living bulbs.

What We Found

In a sense, tulip bulbs are merely the "food source" for fungal growth. In the 1970's it was shown by the LBO that tulip *Fusarium* strains produce ethylene when grown in pure culture. Ethylene produced by *Fusarium* infected bulbs could have two sources: the fungus or the bulb tissue itself. Heat-killed bulbs are no longer capable of metabolism as their enzymes are killed and membranes destroyed. However, the cellular contents used by the fungus (amino acids, sugars and minerals) are fully available for fungal growth as the active defense systems of the bulb are destroyed. In our experiments, ethylene production by heat-killed bulbs was negligible in the absence of *Fusarium*, and was very low in live, non-inoculated bulbs. In the presence of *Fusarium*, heat-killed bulbs produced large quantities of ethylene, and, for the cultivars 'Friso' and 'Prominence', the quantity produced was more than 3-fold greater than produced by inoculated, live bulbs. Thus, it is the fungus, and not the bulb, that produces the ethylene.

Tulip cultivars varied greatly in their ability to support ethylene production by *Fusarium oxysporum* (Table 1, Fig. 1). Of the cultivars tested, the highest ethylene producer was 'Nashville, which evolved more than 800 ul/bulb/day (nearly 1 ml!) on the 19th day after infection. This rate of ethylene evolution was ca. 300-fold greater than in wounded, non-inoculated bulbs and ca. 1,300-fold greater than the ethylene production rate of non-wounded, non-inoculated bulbs (the native ethylene production rate of non-inoculated and unwounded bulbs was less than 0.36 ul/bulb/day for any cultivar, and was usually 0.1-0.2 ul/bulb/day).

Cultivars also varied in the length of time from inoculation until the rapid increase in ethylene (Figs. 2 and 3). 'Furand' and 'Mary Belle' began massive ethylene production by day 8, where as 'Mondial' and 'Bright Parrott', a medium and low-producer, respectively, began ethylene production at days 12 and 14. In all cases, once ethylene production began, it continued to increase rapidly.

Ethylene production continued through at least 19 days, when 14 cultivars produced ethylene at rates >150 ul/bulb/day. The lag period from inoculation to the start of the logarithmic phase of ethylene production varied by cultivar from ca. 8 to 15 or more days. The changing ethylene production rates of the cultivars over time are illustrated by dividing them into three production categories – low, medium, and high (see Table 2). By the time

this experiment ended, maximal ethylene production rates of more than 800 ul/bulb/day were obtained for 'Nashville', and 7 other cultivars had more than 500 ul/bulb/day ('Furand', 790; 'Fusor', 740; 'Mary Belle', 690; 'Libretto', 660; 'Friso', 640; 'Prominence', 610 and 'Yonina', 570)(Table 1). In later experiments, we have shown that ethylene production continues for many days (at least 30-35 days), but ultimately it declines as the bulb dries out.

A comparison of the time course of ethylene production by the 6 highest control (wounded, but non-inoculated) cultivars showed that 'Louvre' produced the most wound-induced ethylene (10.8 ul/bulb/day), but other cultivars were substantially less than this (Table 3). It is possible that low levels of *Fusarium* might have existed on some of these control bulbs. In any case, the normal levels of ethylene produced by wounded bulbs is much less than with *Fusarium* infection.

This experiment has been repeated several times in the last 2 years, with results that are similar to those above. In some cases, the total ethylene production levels are different, but the basic cultivar ranking holds true.

We have shown that ethylene production from living, inoculated bulbs varies greatly by cultivar, and leads to speculation on the reasons for this. Perhaps the cultivars that support the greatest ethylene production contain higher tissue concentrations of arginine, the amino acid that directly leads to fungal ethylene biosynthesis or some other promoting factor. Alternatively, cultivars supporting lower ethylene levels may have greater levels of tulipaline-A, or other inhibitors that might be present in the bulbs. Additional experiments are needed to follow these questions.

Conclusions

- The *Fusarium* fungus is the source of ethylene in infected tulip bulbs.
- Tulip cultivars vary enormously in their ability to support ethylene production when infected.
- Additional work with the most commonly grown cultivars should be done.
- Ventilation rates and/or storage protocols should be reviewed, as appropriate.

It is unknown whether ethylene production rates are closely related to the sensitivity of the cultivar to *Fusarium*.

Acknowledgements

In addition to ongoing support from Anthos, this work was also supported by the USDA/ARS as part of the Floriculture and Nursery Research Initiative. We are grateful to both organizations for this support. Holly Cirri, Martijn Verlouw and Karen Snover were instrumental in the conduct of this work.

Table 1. Ethylene production (ul/bulb/day) of 36 tulip cultivars 12 and 19 days after inoculation with a suspension of *Fusarium oxysporum* isolated from Dutch-grown tulip bulbs. Cultivars are sorted from highest to lowest ethylene production on day 19. Wounded, non-inoculated control values for Day 19 are also shown. All bulbs were wounded (3 wounds, each 0.5 cm deep) before inoculation.

Cultivar	Control	12 days	19 days
Nashville	2.8	384	833
Furand	0.4	483	793
Fusor	0.2	214	737
Mary Belle	1.2	439	693
Libretto	2.4	235	662
Friso	0.7	318	637
Prominence	0.5	131	615
Yonina	3.4	177	567
Annie Schilder	0.3	158	458
Yellow Present	0.4	122	347
Orange Princess	0.4	49	219
Pieter de Leur	1.5	59	207
Mondial	1.4	39	203
Synaeda Blue	3.2	54	200
Evita	2.2	36	148
Adamo	1.0	28	147
Kikomachi	2.0	58	145
Coloeur Cardinal	1.2	11	67
Laura Figi	0.6	19	65
Yellow Flight	0.4	6	55
Angelique	3.0	8	40
Jan van Nes	5.8	16	35
Sapporo	4.3	23	32
Strong Gold	1.5	7	29
Bright Parrott	0.5	3	25
World's Favourite	3.6	7	18
Sevilla	0.6	6	18
Louvre	10.8	11	17
Blue Ribbon	0.5	4	13
Varinas	0.9	3	10
The Mounties	1.2	2	10
Purple Flag	0.7	4	7
Wirosa	2.4	4	5
Pretty Woman	2.1	2	5
Calgary	1.0	3	4
Kees Nelis	0.3	2	3

Table 2. Categories of cultivar ethylene production (ul/bulb/day) for 8, 12, 15, and 19 days after inoculation.

Ethylene production (ul/bulb/day)	Days after inoculation			
	Day 8	Day 12	Day 15	Day 19
			Number of cultivars	
>150	0	1	8	14
5-150	11	20	19	18
<5	25	15	9	4

Table 3. Time course of ethylene production (ul/bulb/day) of wounded, non-inoculated tulip bulbs at 21C. Data are for the 6 highest ethylene-producing cultivars.

	Days after wounding				
Cultivar	1	8	12	15	19
Louvre	1.5	2.4	6.0	7.4	10.8
Jan van Nes	1.4	2.3	2.1	3.8	5.8
Sapporo	1.9	1.7	2.7	2.3	4.3
World's Favourite	2.9	2.7	2.8	2.7	3.6
Synaeda Blue	0.2	0.6	1.2	1.4	3.2
Angelique	1.0	1.2	1.5	1.9	3.0
30 remaining cultivars	0.5	0.7	0.8	0.9	1.1

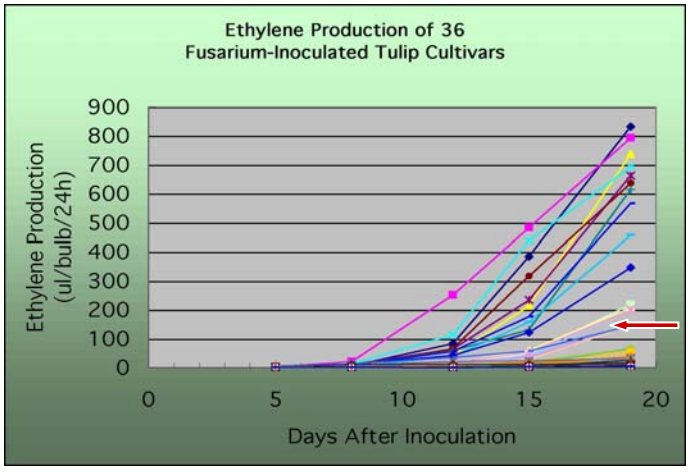

Figure 1. Time course of ethylene production by 36 tulip cultivars inoculated with *Fusarium oxysporum*. The red arrow indicates the 140 ul/bulb/day level, the maximum obtained in de Munk's 1970's work.

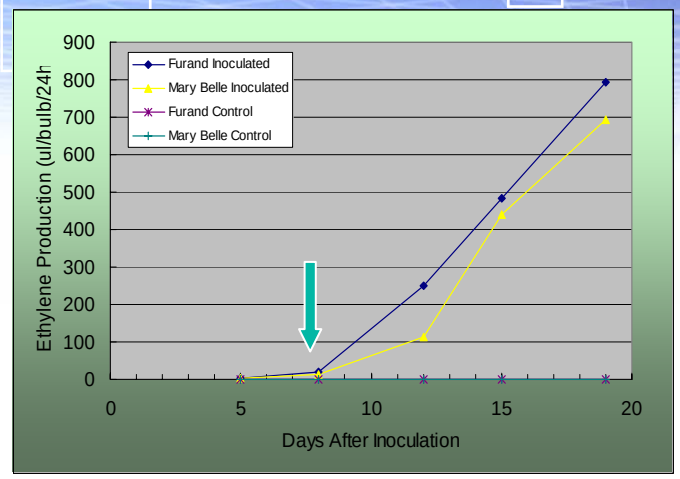

Figure 2. Time course of ethylene production by 'Furand' and 'Mary Belle', two of the highest-producing tulip cultivars following inoculation with *Fusarium oxysporum*. Non-inoculated controls are also shown. The arrow indicates the moment when ethylene production begins to increase.

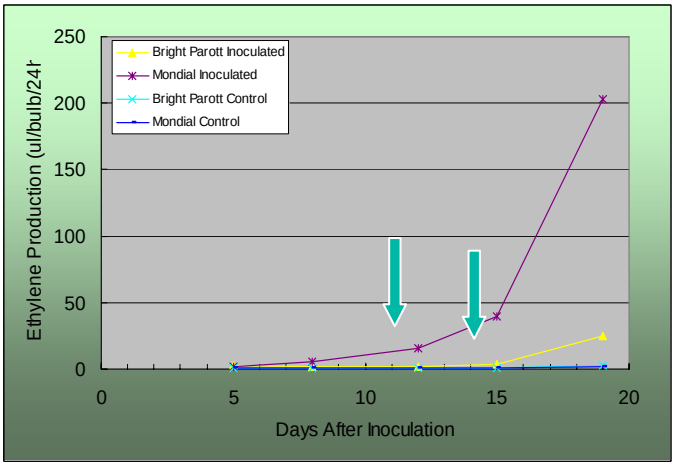

Figure 3. Time course of ethylene production by 'Mondial' and 'Bright Parrott', medium and low-producing cultivars, respectively, following inoculation with *Fusarium oxysporum*. Non-inoculated controls are also shown. The arrows indicate the moment when ethylene production

Flower Bulb Research Program — Cornell

Anthos — Koninklijke Handelsbond voor Boomkwekerij- en Bolproducten

Address:
Dept. of Horticulture
Cornell University
134 Plant Science Building
Ithaca, NY 14853
USA
Phone: + 1 0016072272780:
Fax: + 1 0016072559998:
wbm8@cornell.edu

Address:
Anthos
Weeresteinstraat 12
P.O Box 170
2180 AD Hillegom
Phone: +31 252 53 50 80
Fax: +31 252 53 50 88
secretariaat@anthos.org

The newsletter is distributed in North America bij the North American Flowerbulb Wholesalers' Assn, 2424 Hwy 72/221 E, Greenwood, SC 29666, email: nafwa1@aol.com.