

## Recent Development of Augusta Disease in the Netherlands

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### Abstract

In the Netherlands there has been a shift in tulip bulb production on sandy soils towards heavy clays. These soils are sensitive to the occurrence and enhancement of Augusta disease. Clay soil was collected from a tulip production field with a history of Augusta. Part of the soil was sterilized by steaming. The clay soil was compared to sandy soil, which were both steamed and not steamed. Two tulip stocks were chosen, based on the observed infection rates in the field during the growing season. The one was heavily infected and the other had a very low disease incidence. Both lots were planted early in October 2002, on the selected soils. Observation in the following spring showed that the tulips with a low incidence stayed healthy on the steamed clay and on both steamed and not steamed sandy soil. The untreated clay resulted in a high infection. The highly infected tulips showed infection on all soils, but the severity was lower on the steamed soils and untreated sandy soil than on the not steamed clay soil.

Tulips planted before the middle of October are more susceptible to infection compared to tulips planted in the middle of November. If the precipitation in autumn is high and temperatures are relatively high, the disease is likely to show up in the following spring. This phenomenon might explain why the disease has a cyclic occurrence in time. This is still under investigation.

Application of chalk had no effect and the usage of *Pseudomonas* sp. to control Augusta was not conclusive so far.

### INTRODUCTION

Augusta disease in tulips is caused by an infectious virus/fungal complex of *Tobacco necrosis virus* (TNV) and its vector *Olpidium brassicae* (Van Slogteren and Visscher, 1967). The disease is named after its occurrence in tulip 'Queen Augusta' in 1928. The disease occurs worldwide where tulips are in production. The host range of the fungus includes many commonly found weeds such as annual blue grass, annual sowthistle, chickweed, dandelion, shepherd's purse and others. Some of these *Olpidium brassicae* hosts are also hosts of TNV such as annual sowthistle, barley, chickweed and shepard's purse (Brunt et al., 1996). On occasion Augusta has been found on other bulbous crops such as hyacinth and lily (Derks et al., 2003). It is not a major problem in these ornamental bulbs. An attempt to artificially infect hyacinth with infectious tulip debris failed.

Symptoms of the disease in tulips are diverse. A distinction is made based on the appearance of Augusta symptoms and the time it appears. One refers to early Augusta and late Augusta. Early Augusta appears in the early spring and the plants are small and show necrotic spots and malformations as curling, stunting and premature dying of the plants and necrosis of the bulbs. Late Augusta shows upon the leaves as oval to round spots or necrotic to brown-necrotic stripes. The tulip has a regular appearance. The symptoms appear around flowering or later in the growing season. On the flower the symptoms appear as stripes along the veins of the petal. Sometimes pitting areas are visible on the bulbs during storage. Damage caused by Augusta disease can be severe in the Netherlands. Disease incidence occurs up to 60% in fields in some years and can be up to 20 % in the flower forcing (Bijman et al., 2003a). The high disease incidence in tulips in the spring and summers of 2001 and 2002 justified the reinvestigation of factors

determining Augusta occurrence.

## MATERIALS AND METHODS

The occurrence of Augusta disease was determined by comparison of tulip cultivar, planting date, soil type and (infectious) plant debris such as roots, tunics, etc. In the first experiment the tulips were free of Augusta and were size 9/10. Plastic pond baskets were filled with sandy soil from Lisse or filled with clay soil from an infectious field originating from Bobeldijk, centered in an Augusta sensitive region. In each basket 20 tulip bulbs were planted and thereafter dug in. As an additional control, one treatment of steamed clay from the same field was added to determine the contribution of the soil to the disease development and if the bulbs were actually disease free. Early planting was on October 1, 2002 and late planting was on November 11, 2002. Plant debris used in the experiment was obtained from a heavily infected stock and was added to the soil before planting. Samples of the planted bulbs stocks were tested for the presence of TNV. In the spring of 2003 the tulips were visually rated in the field and samples were run at planting and during the growing season using ELISA, to confirm infection. Two tulip cultivars were used being Angelique, known to be sensitive, and Inzell known as less sensitive to TNV. All treatments were replicated four times.

Results of the disease ratings were converted to the fraction 'visually infected plants' and regression was conducted on transformed data, using the logit link, in a generalized linear model (GLM) to determine the effects of cultivar, planting time, tulip debris and soil type on the occurrence of Augusta disease in tulips. The fitted terms in the accumulated analysis of deviance are constant, block, planting time, soil type, plant debris, cultivar, interaction between planting time and plant debris and the interaction between cultivar and planting time.

The model had 79 degrees of freedom, with a binomial distribution and consists of 20 binomial totals. Factor block had 4 degrees of freedom and the other fitted terms have one degree of freedom. The residual factor had 69 degrees of freedom. The response variate was the percentage infected tulips.

In a second experiment tulips originating from known infested fields were planted on heavy clay. Part of the clay was steamed. In comparison sandy soil from Lisse was used.

Again two tulip cultivars were used. Bulbs from tulip 'Angelique' were heavily infected the year before and were collected from diseased plants. Tulip 'Lucky Strike' has a history of susceptibility but the disease had a very low incidence at the time of planting, based on field observation by the grower and negative results of bulbs sampled before planting. Bulb samples from each cultivar were tested, using ELISA, on the presence of TNV before planting. From each cultivar 20 bulbs were planted in a basket and each treatment was replicated four times. In the following spring the tulips were rated on visual Augusta symptoms. From doubtful plants, samples were taken to confirm the presence of TNV using ELISA. The soils were steamed to eliminate additional effects of primary infection expression due to the possible presence of *Olpidium brassicae*. This to obtain a clear view on the transmission of infection of infected bulbs to their offspring in time.

The application of *Pseudomonas* to control *Olpidium brassicae* was tested based on previous results on *Pythium* control in several bulbous crops (Boer et al., 2002). Chosen were isolate A and isolate C71, which both produce a bio-surfactant. Isolate C71 has performed better with iris and tulips, in controlling *Pythium*. Bacteria were applied at a cfu of  $10^7$ . The standard tulip 'Angelique' was used. There were two applications applied, drenching just before planting and soaking the bulbs for an hour before planting them in the baskets. The baskets were filled with clay originating from Bobeldijk and planted on 15 October 2002, considered to be an early planting. The experiment had 8 replications. In the spring of 2003 the tulips were rated on the presence of Augusta.

At growers request the usage of chalk and calcium cyanamide (Perlka\*) were

\* Perlka contains 19,8 % calcium cyanamide, and is produced by SKW Trostberg AG, Trostberg, Germany.

evaluated on their efficacy against *Olpidium brassicae*. Chalk application is used in the control of clubfoot in cruciferous crops worldwide, and the causal pathogen, *Plasmodiophora brassicae* (Wor.), and *Olpidium Brassicae* are both myxomycete fungus. The fungi have a similar disease cycle, causing infection of plant roots with zoospores and lack of mycelium formation. The trial consisted of three experiments. A field trial was laid out in cooperation with three growers and a growers association called WLTO. Three fields had strokes of chalk applied in the fall before planting. In the second experiment a grower's field had an application in strokes of calcium cyanamide at 500 kg/ha, applied in the fall, two weeks before planting. The intended application was 1000 kg/ ha. All grower fields were evaluated on incidence of Augusta during the growing season of 2003. In Lisse, a calcium cyanamide experiment was conducted in pond baskets to support field experiments. This in accordance to grower practices. The treatments were application of calcium cyanamide at 100g /m<sup>2</sup>, mixed throughout the soil, and mixed in the top 5 cm of the basket. The chemical was mixed in with moist clay soil from Bobeldijk, put in a bag and allowed to work out for two weeks prior to planting. Another treatment was the mixing in of calcium cyanamide (100g/m<sup>2</sup>) together with infected plant debris. The fifth treatment was the check, consisting of clay from Bobeldijk, without cyanamide. The experiments with calcium cyanamide were planted with tulips 'Lucky Strike'. The tulips were evaluated during the growing season of 2003 on the incidence of Augusta.

## RESULTS AND DISCUSSION

### Soil Type, Planting Date and Infectious Plant Debris

The factor soil type was significant and had no interactions with the other factors. Planting time, plant debris, and cultivar were also significant, showing significant interactions between planting time and plant debris and between planting time and cultivar. The prediction factor for clay is 0.53 and for sand is 0.07 (Table 1A). These results (Fig. 1) confirm earlier findings of high incidence of Augusta disease on clay soils (Asjes and Blom-Barnhoorn, 1996). Results again confirm that early planting leads to significantly higher infection by Augusta than late plantings (Fig. 2). The influence of infectious plant debris was stronger when the tulips were planted late with prediction factors of 0.13 when infectious plant debris was added compared to 0.01 when infectious plant debris was excluded (Table 1B). Infectious plant debris did not affect the infection when added to the early planting. The results are less pronounced than those found by Asjes and Blom-Barnhoorn (1996). 'Inzell' with a prediction factor of 0.12 is more sensitive to late planting than 'Angelique' with a prediction factor of 0.02. On both cultivars early planting resulted in high infections, but was not significantly different (Fig. 3). All other possible interactions between cultivar, planting time, tulip debris and soil types were not significant.

### Effect of Soil Type on Secondary Symptom Expression of Augusta

Tulip 'Angelique', originating from a high field infection in 2002, did not show any significant differences between treatments in 2003 (Table 2). The infections were for steamed sandy soil 94 %, steamed clay 96% and for infectious clay 100 %. Significant differences were obtained between treatments with tulip 'Lucky Strike' with a previously low infection. On the sandy soils infection was zero, on steamed clay 2 % and on infectious clay 85 %. The highly infected tulips showed infection on all soils, but the severity was lower on the steamed soils and untreated sandy soil than on the not steamed clay soil. These results confirm the influence of infectious clay as a convenient environment for disease occurrence (primary infections). Previous experiments showed that if you cultivate tulip stocks with high field infection on sandy soils, infection decreases in one season to a low level or disappears visually leading to cleaning up of an

infectious tulip stock (Asjes, 1994). The difference with our experiment with ‘Angelique’ is that in practice most infected bulbs are lost by visual inspection and selection, necrosis of infected bulbs, sorting after lifting of the bulbs, etc.

### **Biological Control of *Olpidium brassicae***

There was no significant difference between the treatments although numeric differences indicated an average decrease of 25% in Augusta incidence, when soaking the bulbs in a suspension of *Pseudomonas* isolate A for one hour just before planting. The variance was large, leading to insignificant difference between treatments. The young roots of tulips are the most susceptible to infection with TNV by zoospore penetration and it is possible that the *Pseudomonas* isolate A is capable of colonizing the roots and preventing zoospore penetration of the young rootlet with its production of bio-surfactant. As there are no chemical and biological options at this moment, growers requested the continuation of these experiments.

### **Application of Chalk and Calcium Cyanamide**

The application of both chalk and calcium cyanamide, were not effective in the control of Augusta disease. Moreover, it appeared that calcium cyanamide application resulted in crop damage (phytotoxicity). The application on heavy clay soils also limits the efficacy, due to the fact that the active ingredient is a gaseous substance. This experiment will not be continued.

### **Detection of *Olpidium brassicae* in Plant Roots, Soils and Water**

PCR primers were selected and were applicable for detecting *Olpidium brassicae* in plant roots. The obligate nature of the fungus makes the detection process complex. The detection in soils and water is still in the validation phase. We are currently actively involved in the sampling and confirmation trials to back up the application of PCR for the detection of *Olpidium brassicae* in soils and water. The detection of TNV with ELISA is satisfying.

### **Soil Activity and Agronomic Practices**

In the Netherlands there has been a shift in tulip bulb production on sandy soils towards heavy clays. *Olpidium brassicae* is spread by water and clay soils are more sensitive to standing water, thus enforcing the occurrence and establishment of the disease on these soil types. As a common practice, bulbs are grown on soils, which have been in pasture for a period of circa 6 years (Bijman et al., 2003b). Pastures are probably a favorable environment to enhance the spread of *Olpidium brassicae*. Aspects contributing to disease enhancement are the adequate availability of moisture, active decay of roots after tillage of the pasture, development of susceptible roots after planting tulip bulbs and the presence of many susceptible hosts for TNV and /or *Olpidium brassicae* in the pasture. Tulip debris such as tunics, roots, ‘bulblets’ and soil attached to the bulbs can spread Augusta. Some farmers used to return this (infectious) material to the soil before former pastures were planted with tulip bulbs. In the forcing of tulips, several growers recycle potting soil with plant debris, mixed in with fresh potting soil at planting (Bijman et al., 2003a). These unfavorable practices can result in occurrence and possible spread of Augusta or contribute to the reservoir of TNV and *Olpidium brassicae* in the soil. Spread of infection can be reduced if plant debris is exposed to minimum of 8 weeks at 40°C, or at 50°C for two weeks (Asjes and Blom-Barnhoorn, 2002). Weather conditions are important during autumn, winter and early spring. Certain conditions promote or reduce the release of zoospores from decaying roots and facilitate or prevent the active spread of the zoospores to the susceptible young tulip roots. Warm wet falls and springs promote in addition to late frosts in the spring, while dry and cold falls, winters and springs reduce the chance of infection. This might explain why the disease has a cyclic nature.

## Future Research

Currently we are examining the effect of soil structure and methods of land preparation in relationship to the occurrence of Augusta. Soil properties are in extent of this research. We are also focusing on the relationship between the decay of pasture which underwent tillage, the soil temperature and precipitation, release of *Olpidium brassicae* zoospores and development of emerging tulip roots. Aspects of biological control and potential fungicides are also under investigation but have a small scope. Expanding the lists of hosts for *Olpidium brassicae*, TNV or both is still current. Especially if you consider the fact that tulip growers move with their tulip production to other parts of the Netherlands, which are currently in use as arable farming or for vegetable production. The role of Augusta disease in the flower forcing is also currently under investigation. Important aspects are the hydro-culture and mixing in of old potting soil when potting soil is used. Also the possibility of infection during the season is important as the used bulbs are planted in field production and can contribute to the disease enhancement.

## CONCLUSIONS

Planting tulips early and on infectious clay soils lead to severe disease enhancement in certain years. To clean up an infected tulip stock it is advisable to do this on a sandy soil, but high bulb infection makes the practice less feasible. It is not clear yet how disease spreads to the offspring in case of stocks with late Augusta symptoms. Chalk application was not effective in controlling *Olpidium brassicae*.

## ACKNOWLEDGEMENTS

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## Tables

Table 1. Results prediction factors of the relationships between cultivar, planting time, tulip debris and soil type, Lisse, 2003.

1A. <u>Soil type</u>		
Clay	0.53a <sup>2</sup>	
Sand	0.07b	
1B. <u>Tulip debris* planting time</u>		
	Added to the soil	Without debris
Early <sup>1</sup>	0.56 c <sup>2</sup>	0.53 c
Late	0.13 b	0.01 a
1C. <u>Cultivar* planting time</u>		
	Angelique	Inzell
Early <sup>1</sup>	0.54 c <sup>2</sup>	0.56 c
Late	0.02 a	0.12 b

<sup>1</sup> Planting time: Early planting was on Oct. 1, 2002 and late planting was on Nov. 11, 2002

<sup>2</sup> Treatment means followed by the same letter do not differ significantly (p=0.05, Student's two-tailed *t*-test; number of replicas = 20)

Table 2. Effect of soil type on secondary symptom expression, as percentage bulbs with Augusta, Lisse, 2003.

Treatments	% tulips with Augusta
Heavy infected 'Angelique' bulbs on infectious clay	100
Heavy infected 'Angelique' bulbs on steamed clay	96
Heavy infected 'Angelique' bulbs on steamed sand	94
Slightly infected 'Lucky Strike' bulbs on infectious clay	85
Slightly infected 'Lucky Strike' bulbs on steamed clay	2
Slightly infected 'Lucky Strike' bulbs on steamed sand	0
LSD (p< 0.001)	6

## Figures

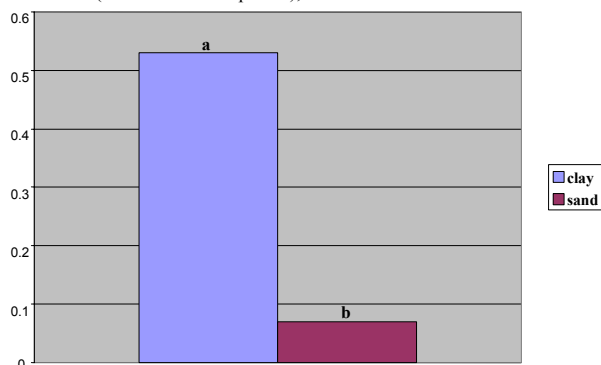


Fig. 1. Effect of soil type on the occurrence of Augusta disease in tulips (fraction infected plants); Lisse 2003.

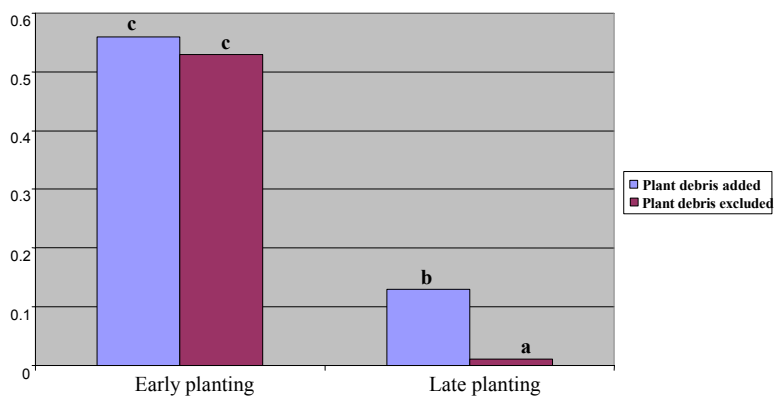


Fig. 2. Influence of planting time and plant debris on the occurrence of Augusta disease (Fraction infected plants), Lisse 2003.

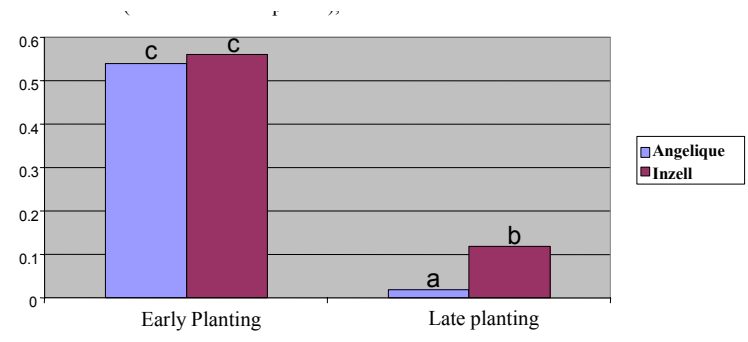


Fig. 3. Prediction values for the interaction between planting time and cultivar (fraction infected plants), Lisse 2003.