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31-08-2006

THIS WORK WAS PARTLY FUNDED BY THE VOCBAS (VOLATILE ORGANIC COMPOUNDS IN THE BIOPSHERE_ATMOSPHERE SYSTEM) PROGRAM OF ESF (EUROPEAN SCIENCE FOUNDATION). I COMMIT MYSELF IN ACKNOWLEDGING THE CONTRIBUTION OF VOCBAS IN ALL PUBLICATIONS ARISING FROM THE ESF GRANT. I WILL TIMELY INFORM VOCBAS ABOUT PUBLICATION OF THE RESULT BY SENDING A LIST OF THE CONTRIBUTIONS AND COPIES OF ALL MATERIAL IN WHICH VOCBAS SUPPORT HAS BEEN ACKNOWLEDGED.

ABSTRACT
Botrytis blight caused by the fungus *Botrytis cinerea* is probably the most common disease of greenhouse-grown crops like tomato. Botrytis blight in tomato plants is mainly detected by visual inspection or destructive biochemical and molecular determinations. These methods are time consuming and not suitable for large sample sizes. In contrast we propose a fast and non-destructive detection method for plant diagnosis using volatiles as an early indicator of plant diseases. This report presents the variation in volatile production during mild and severe infection of tomato plants by the phytopathogenic fungus *B. cinerea.* Volatile emission from tomato plants before and after inoculation with *B. cinerea* were analyzed using on-line gas chromatography coupled to mass spectrometry. The emission was monitored from 2 to 72 hours after inoculation/exposure with a time resolution of 1 hour. The multivariate data was subjected to principal component analysis for fast interpretation of the variation between mild and severe infection symptoms. In addition a statistical test was performed to search for significant differences in headspace composition between the period before and after inoculation. Results show that there are no significant different compounds between headspace composition before and after inoculation when binning the data from mild and severe infected plants. This implies that the severity of infection has a significant effect on the main emissions.

CONTENTS

1 INTRODUCTION

It is interesting to note that an abundant list of scientific papers is available on emissions of volatile organic compounds (VOCs) from pathogen inoculated agricultural products after harvest. The emission of VOCs from these products has been studied since late 70's with the intent of developing a tool for early and non-destructive disease detection in storage systems. Onion bulbs, apple, carrots, potato, mangoes and grain are products that have been screened for pathogens using dedicated gas analysers (Costello et al. 2003; Lui et al. 2005; Ouellette et al. 1990; Prithiviraj et al. 2004; Varns et al. 1979; Vikram et al. 2006). In these experiments several disease discriminatory compounds have been identified and discriminant analysis models have been developed for early detection and discrimination of post-harvest diseases. However, these studies were undertaken at lab-scale using small containers and validation under practical conditions is still required.

Moraes (2004) first suggested the detection of infected plants at an early stage based on volatiles released by the diseased plants. In the same year, Holopainen (2004) concluded that, "Non-destructive metabolic profiling of VOC emissions is a promising tool for quickly detecting the physiological status of crop plants as well as for identifying the initial phase of pathogen and herbivore infections". To the best of our knowledge, so far, (Baratto et al. 2005) were the first to report experiments on plant health monitoring based on detection of volatile compounds. Their results indicate that a gas sensor is able do detect the onset of plant stress due to insect damage and herbicide spraying.

To our knowledge there is hardly any literature on chemical analysis of volatiles from plants after inoculation with a pathogen. Only few articles report changes in headspace after inoculation with a pathogen. Croft (1993) analysed the volatile products of bean leaves during a response to the plant pathogenic bacterium *Pseudomonoas syringae* with the objective to check if these volatile compounds possess antimicrobial activity. Doughty (1996) investigated the ability of a fungus to trigger the degradation of glucosinolates during infection by comparing amounts of their volatile catabolites. Shulaev (1997) inoculated tobacco plants with the tobacco mosaic virus and proved that the emission changed. Shulaev (1997) proved that the emanating volatiles might function as an airborne signal which activates disease resistance and the expression of defence-related genes in neighbour plants and in the healthy tissues of the infected plants. Deng (2004) investigated the same response to virus inoculated tomato leaves. Cardoza (2002) studied the influence of simultaneous attack of insects and phytopathogens on the production of volatiles. Huang (2003) examined the role of signalling pathways in volatile induction during pathogen attack. However no link is established to the opportunity of early disease detection for plants.

An extensive literature survey shows that pathogen induced volatile formation is scarce. On the other hand there is abundant information on the induction and release of VOC from numerous plants species before and after plant-insect interactions. Emissions of volatiles from plant-insect interactions are studied commonly because

plant volatiles are of great importance for host recognition by many pest insects. In addition, plant volatiles function as indirect defence by attracting natural enemies of the insect herbivores (Turlings et al. 1990). Also tomato plants have been widely used as model for volatile analysis after insect induced stress. This type of stress on tomato plants resulted in the formation of a wide array of volatile compounds, such as methyl jasmonate, methyl salicylate, green leafy volatiles and isoprenoids (Ament et al. 2004; Dicke et al. 1998; Maes et al. 2003). In contrast no studies have been done concerning the changes in volatile profile of tomato plants after inoculation with a pathogen. The objective of this research was to determine the variation among volatile profiles induced by *Botrytis cinerea* infection of tomato plants. In previous experiments we analysed the headspace of tomato plants after a mild Botrytis infection (Jansen 2006). For this report we undertook replicate measurements to determine the variation among volatile emission after infection.

2 MATERIALS AND METHODS

2.1 Plant material

Seeds of tomato plants (*Lycopersicon esculentum* Mill) of the cultivar Moneymaker were germinated in a commercial mixture of soil, peat and compost (Pikiererde, Plantaflor, Vechta, Germany). After germination the plants were cultivated individually in standard substrate (Einheitserde, type ED 73) under the same conditions. The plants were grown in a chamber at 20℃ with light from fluorescent lights (300–400 µmol m^{-2} s^{-1}). The light/dark condition used was 12 h light/12 h dark. Eight- to 10-weeks-old plants were used in all experiments. Description of the experiments performed in August – September 2006 are summarized in Table 1.

Table 1: Experiments August – September 2006

Experiment	Threatment	Start	End	Inoculation
1	*B. cinerea* inoculation	21-08-2006, 14:00	25-08-2006, 10:50	22-02-2006, 16:12
2	*B. cinerea* inoculation	25-08-2006, 11:00	28-08-2006, 13:45	25-02-2006, 15:05
3	Control	07-09-2004, 11:41	10-09-2004, 10:24	-
4	Control	29-02-2006, 13:45	28-02-2006, 13:45	-

2.2 Leaf inoculation

Botrytis cinerea strain (B0510) was cultured on malt extract agar (CM0059, Oxoid, Bastingstoke, England) at 25±1 ^{o}C in Petri dishes with notches in the dark by covering with black plastic foil. Prior to pouring, the agar was sterilized in an autoclave at 121 ^{o}C for 10 min. The agar was inoculated in a laminar flow hood with a sterilized Pasteur pipette containing a small droplet of spore suspension. After four days the cultures were exposed for 24h to near-UV light to stimulate sporulation. One to two weeks after near-UV stimulation, conidia were harvested from sporulating mycelium by washing with 10 mL sterile water, containing 0.05% Tween 20 (Merck-Schuchardt, Hohenbrunn, Germany). The conidia were washed three times by centrifuging (3 min, 800 rpm, 20 ^{o}C) and resuspended in 10 mL sterile water to get rid of the Tween. The spores were finally resuspended in an inoculum buffer. This inoculum buffer consisted of 50 mL filter sterilized water supplemented with 0.6 gr. potato medium (Duchefa Biochemie bv, Haarlem, The Netherlands). The concentration of spores in the suspension was counted and adjusted to 1 ×10^{6} spores ml^{-1}. This suspension was pre-incubated for one to two hours, with occasional shaking by hand. The leaves of four tomato plants were inoculated on the ventral leaf surface using a micro-sprayer. Each plant was sprayed inside the chamber with 25 ml of the spore suspension.

2.3 Plant chamber

The glass chamber used for the experiments has been described by (Beauchamp et al. 2005). In short, the glass chamber with a volume of or 1450 L, was mounted in a temperature controlled housing. This chamber was supplied with several connections to introduce temperature- and light intensity sensors and to connect the tubings for gas-phase analysis and air supply. These tubings were either made of Teflon or glass in order to minimize wall losses. The glass chamber was placed under a flux of

photosynthetic active radiation amounted to 360 µmol · m^{-2} · s^{-1} at mid-canopy height. Filters (OptoChem, type IR3) that reflect wavelengths between 750 and 1050 nm were used as heat shields to avoid overheating of the plants by infrared radiation. Ambient air was purified by an adsorptive drying device (Zander, KEA 70) and a palladium catalyst. The air flow through the chambers was kept constant by mass flow controllers. Between 20 and 200 L · min^{-1} of air were led through the chambers. A Teflon fan was used for homogeneous mixing of the chamber air. The dew point of the air at chamber inlet was reduced to about –18 ^{o}C by the adsorptive drying device. To increase the relative humidity of the air in the chamber required in the first hours after inoculation, water vapor was added by diverting a part of the inflowing air in a bypass mode through a water containing glass vessel. After 10-16 hours, the relative humidity was reduced to 30% RH. This reduction is required to prevent water in the thermal desorption trap of the gas chromatography – mass spectrometry.

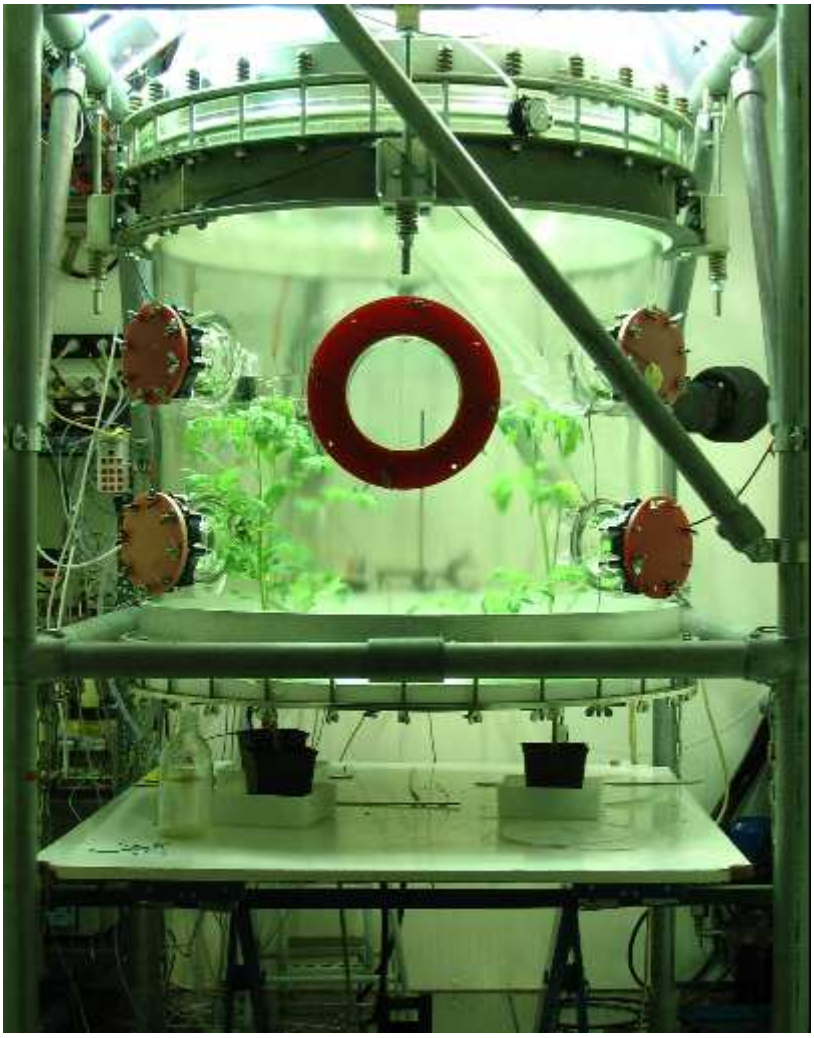

Figure 1: Glass chamber for headspace collection.

2.4 Gas chromatograpy – mass spectrometry

A detailed description of the GC-MS system used for VOC analysis was given by (Heiden et al. 1999). In short, samples for VOC analysis were collected on-line on solid sorbents (Tenax TA/Carbotrap). The thermal adsorption/desorption system (Gerstel online TDS G) was connected to a cooled injection system (Gerstel, KAS 3) where samples were cryofocused before injection into a GC-MS system (HP5890 Series II – HP5972A). The sample time was 40 minutes and the time resolution of one analytical run about 60 minutes. A BPX-5 column (SGE, 50 m × 0.22 mm × 1 µm) was used for separation. Peaks in the samples were identified by comparing the mass spectra with mass spectra libraries i.e. Wiley mass spectral library and NIST library.

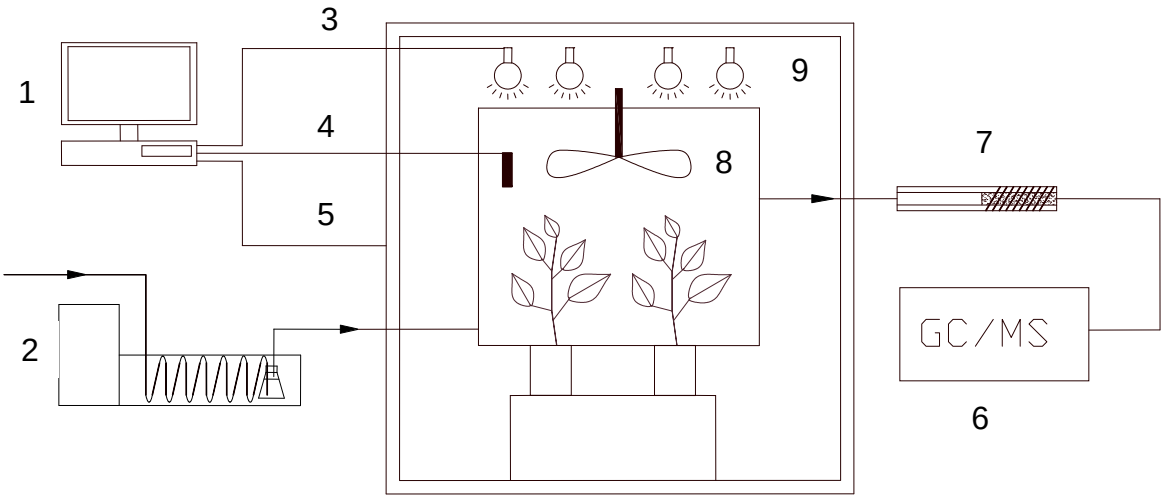

Figure 2: Schematic overview of the sample setup: (1) Personal computer for (3) light control, (4) temperature measurements and (5) temperature control. (2) humidifier, (6) GC/MS, (7) Thermal desorption system (8) rotor and (9) lights.

2.5 Data analysis

2.5.1 Data pre-processing

Several methods are described in literature for the pre-processing of chromatographic profiles. This pre-processing step is generally applied to improve the statistical analysis of the data. In this report, the MetAlign software package is used. This toolbox for pre-processing of the chromatographic data is extensively described (Vorst et al. 2005; Vos et al. 2005). It is useful for smoothing, filtering, normalisation, baseline correction and alignment of chromatographic data. Parameters of MetAlign were set according to the specific chromatographic conditions used in the experiment. Hereafter, MetAlign started the pre-processing of GC-MS data by performing the following steps:

1. Data smoothing by digital filters related to the average peak width
2. Estimation and storage of local noise as a function of retention time and mass peaks
3. Baseline correction of mass peaks and introduction of a threshold to realise noise reduction
4. Scaling and calculation and storage of peak maximum amplitudes
5. Between chromatogram alignment

2.5.2 Exploratory analysis

Principal component analysis (PCA) is an appropriate technique for the exploratory analysis of the GC-MS datasets because it is able to detect subtle changes in multivariate datasets. The PCA model tries to explain the covariance structure of multivariate data by means of a lower dimensional subspace that explains maximum variation in the data. The dimension of this subspace is defined by the number of principal components selected. These principal components are linear combinations for the original variables, and often allow for an interpretation and a better understanding of the different sources of variation. PCA was carried out in the MATLAB v. 7.0 environment (MathWorks Inc., Natick, Ma, USA), using the routines of PLS Toolbox 4.0 (Eigenvector Technologies, Manson, USA).

2.5.3 Quantitative analysis

Manual interpretation of chromatographic data is time consuming and often requires subjective decisions (Hansen et al. 2005). Therefore we also try to identify discriminatory compounds in and automated way. Before the GC/MS data is subjected to automated data interpretation, this data is pre-processed. Such pre-processing is generally applied to improve the statistical analysis of GC/MS data. Several software programs are described in literature for the pre-processing of chromatographic profiles (Broeckling et al. 2006; Katajamaa et al. 2005). For this report, the MetAlign software package is used. This toolbox for pre-processing of the chromatographic data is extensively described (Vorst et al. 2005). It is useful for smoothing, filtering, normalisation, baseline correction and alignment of chromatographic data (Tikunov et al. 2005). In order to perform these procedures in an acceptable manner, setting had to be optimised. The optimal settings were determined on the basis of empirical observations of the GC/MS performance characteristics and results of previous experiments (Laothawornkitkul et al. 2005). These settings were entered as default settings in the program (Table 2).

Table 2: MetAlign settings

Setting	Value
Retention begin	1000
Retention end	10000
Maximum amplitude	6000000
Peak slope factor	0.5
Peak threshold factor	5
Average peak width at half height	5
Scaling	Scale on marker peak (m/z 57 at scan nr. 4444)
Initial peak search criteria : maximum shift	35
Pre-align processing	Iterative
Maximum shift per 100 scans	20

After pre-processing, the proportion of abundance of identified peaks was subjected to a non parametric Wilcoxon test ($p = 0.05$) by means of procedure SIGNRANK in Matlab (version 7, MathWorks Inc., Natick, Ma, USA). Molecular fragments that were situated within a maximal deviation in retention time of 6 scans were considered to belong to the mass spectrum of one and the same compound.

3 RESULTS

3.1 Climate chamber

Tomato plants were put in the chamber during the light phase, approximately 1 day before inoculation. During this day, the plants were allowed to adapt to the chamber climate. After this period the plants were spray inoculated inside the chamber. Directly after inoculation the humidity was increased and the lights were manually switched of. No light and the increase in humidity was required to favour the start of the infection process. Approximately 16 hours after inoculation (HAI) the lights were manually switched on. Then an automatic day/night regime of 12h light / 12h dark was applied. Headspace measurements took place until 70 HAI. During each experiment, light, temperature and dew point were recorded with a time resolution of 4 minutes. Typical data from the climate chamber for one experimental run can be seen in Figure 3. Additional climatic data can be seen in appendix D.

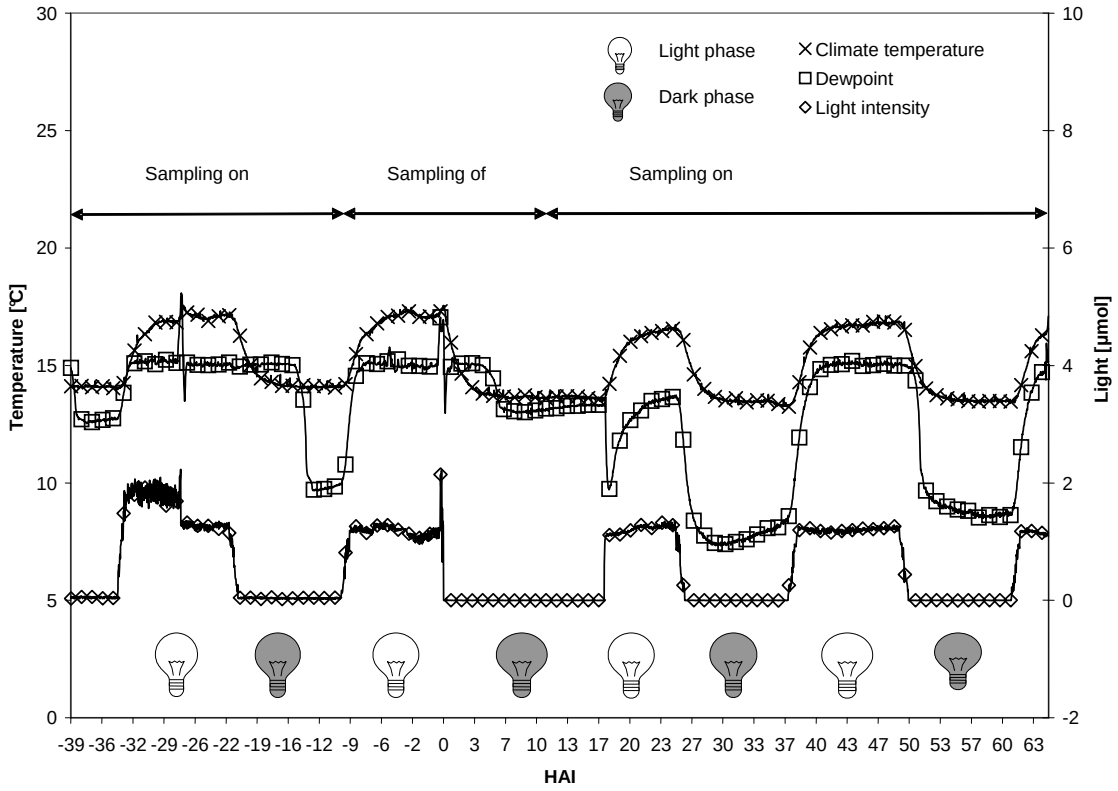

Figure 3: Typical data from the chamber: -26 HAI: Plants putted in chamber. -10 HAI: Increase of humidity. 0 HAI: Inoculation of plants. 10 HAI: Removal of humidifier. 70 HAI: Removal of plants Total period: 80 hours, 09.03.2006 00:01 till 13.03.2006 08:57.

Not only chamber temperature but also leaf temperature was measured during the experiments. Also the airflow through the chamber was measured using digital mass flow controllers. Results of these measurements are displayed in Figure 4. Leaf temperature, airflow and the total leaf area can be used to calculate the flux of VOC's. Flux calculations are not part of this report due to limitations in time available.

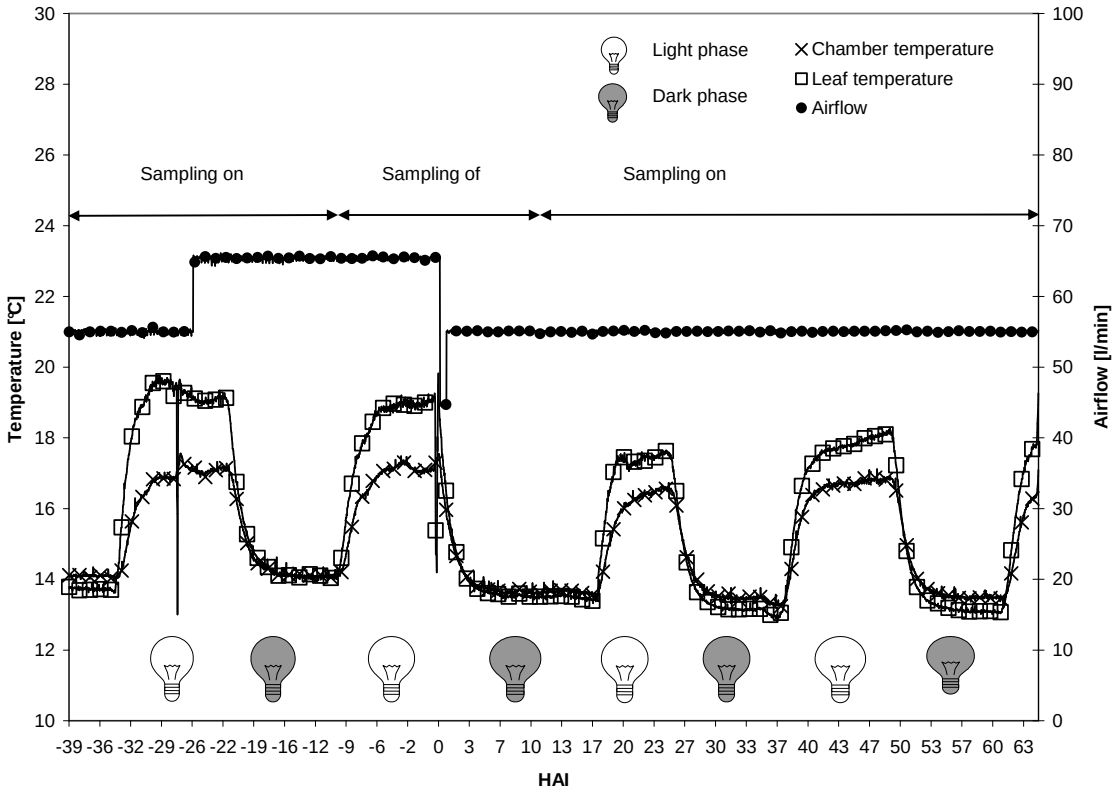

Figure 4: Typical data from the chamber

As can be seen from Figure 3 and Figure 4 , each experiment consists of 8 phases determined by inoculation requirements and the applied day/night rhythm. Phase 1 is the period in which the plants are placed in the chamber. The positioning of plants in the chamber takes place during the light phase. Chromatograms from this period are characterised by emissions of monoterpenes due to handling of plants. This handling causes damage of trichomes that store the monoterpenes such as beta phellandrene and alpha-terpinene. Phase 2 is the 1st dark period of the plants in the chamber. Due to absence of light, the stomata close. During this dark period, plants are able to recover from damage caused by the positioning of plants in the chamber. Decrease of stress and closing of stomata results in chromatograms characterised by the absence of intense peaks. Phase 3 is the light phase before inoculation of the plants. The plants are now fully recovered from stress as can be seen by the reduced emission of monoterpenes. At the end of phase 3, the plants are spray-inoculated with *B. cinerea*. The following phase 4 is the dark phase directly after inoculation. This period is characterised by high emissions of LOX products due to the damage of trichomes caused by spraying. The stomata are closing during this period 4.
Phase 5 is the first light period after inoculation. In general the first symptoms of stress emission are observed: increase of TMTT and MeSa emissions. In the subsequent dark phase (period 6), the TMTT and MeSa emission drop due to closing stomata. However, the TMTT and MeSa emission remain high compared to the emission of the plants in the dark phase before inoculation. Following light and dark phases emphasise this trend. The emissions of TMTT and MeSa increase during light (phase 7) and drop during the subsequent dark phase (phase 8). An overview of the phases during each experimental is displayed in Table 3.

Table 3: Phases during each experimental run.

Phase	**Period (HAI)[1]**		**Luminosity**
	From	**Till**	
1	-36	-22	Light phase
2	-21	-9	Dark phase
3	-8	0	Light phase
4	0	17	Dark phase
5	18	27	Light phase
6	28	38	Dark phase
7	39	52	Light phase
8	53	63	Dark phase

[1]HAI: Hours after inoculation

3.2 Lesion expansion

An essential question is whether we are able to measure differences in the volatile emissions before visible symptoms appear. Therefore we made visual observations during the experiments. The penetration of the host tissue by *Botrytis cinerea* can be divided in: Killing of the host tissue, primary lesion formation, and lesion expansion (Elad *et al.* 2004). At 12 hours after inoculation, no visible symptoms were observed. Small lesions were first observed at 24 hours after inoculation. The lesion sizes increased progressively thereafter. At the end of the experiments most of the leaves dried out partly. Unfortunately it was no option to take pictures from the leaves during the experiment because the wall thickness of the glass chamber prevented sharp pictures. A typical example of symptoms after the experiment is given in Figure 5.

Figure 5: Typical example of a leaf 76 hours after inoculation.

3.3 Data analysis

3.3.1 Data pre-processing

The first step in data pre-processing is selection of data in the chromatographic profile. Ignoring certain peaks in the chromatogram is often practiced due to knowledge of the system that causes the peaks. An example is shown in Figure 6 and Figure 7.

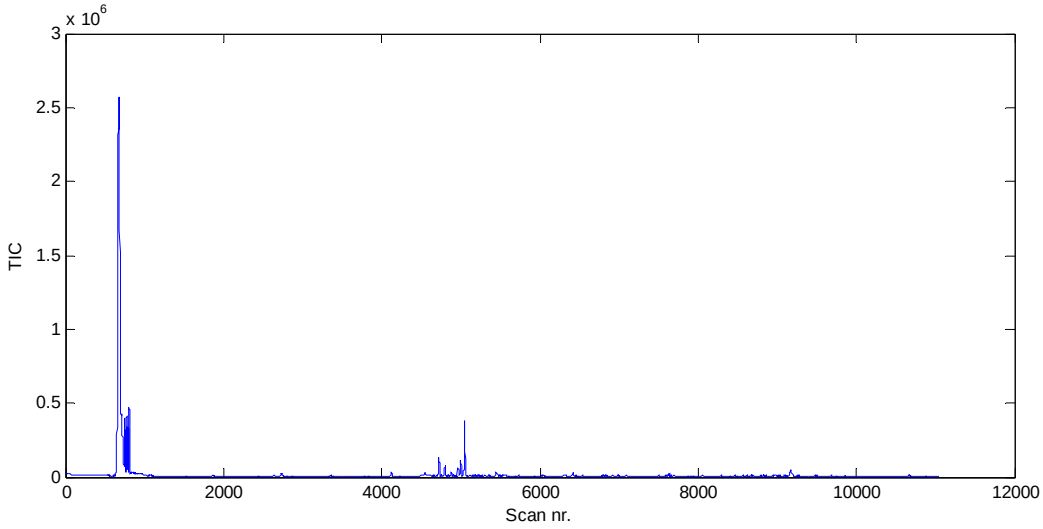

Figure 6: Example of a full chromatogram before pre-processing. File: 244T010.d.

The large peak at scan nr. 800 is caused by CO^2. This compound can not be quantified by the system and was therefore ignored. To standardize the length of each chromatogram, as required for pre-processing the data, the end of the chromatogram was defined at scan nr. 10.000. The final chromatogram consists of scan nr. 1000 till 10.000 as can be seen in Figure 7.

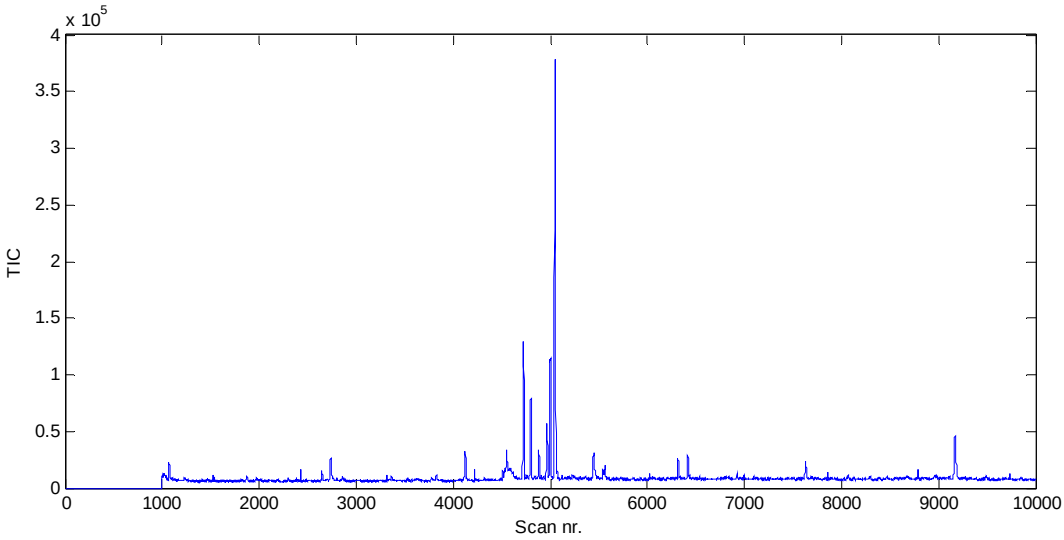

Figure 7: Example of chromatogram selection. File 244T010.d.

Subsequent baseline correction and noise elimination results in Figure 8.

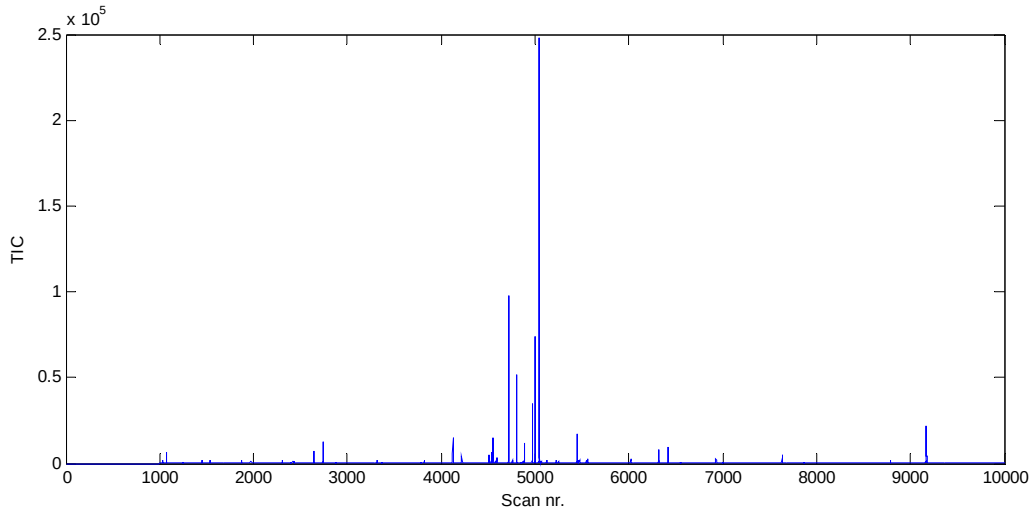

Figure 8: Example of chromatogram selection after baseline correction and noise elimination. File: 244T010.

The next step consists of alignment of shifted peaks. These small peak shifts are known to all chromatographers and is due to changes in the columns during use, minor changes in mobile phase composition, drift in the instrument, interaction between analytes etc. (Nielsen et al. 1998). An example of shift (differences in scans) can be seen in Figure 9.

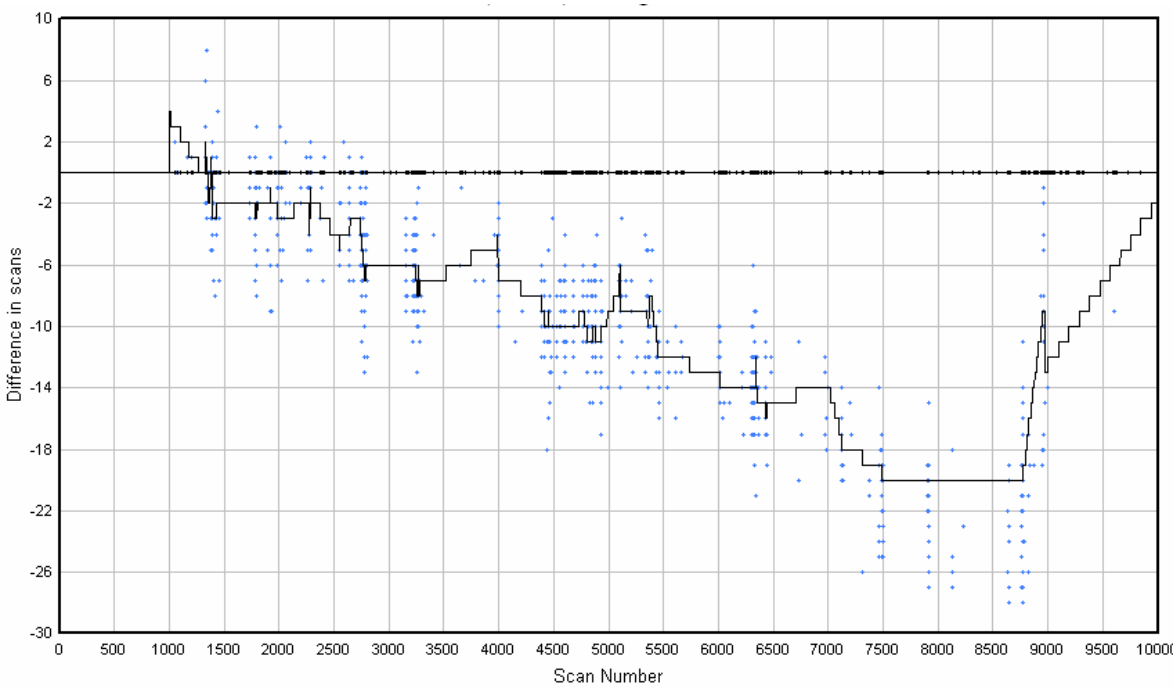

Figure 9: Typical example of the non-linear shift. Files: 354T084 and 354T085.

To demonstrate the aligning capability of pre-processing with Metalign, two arbitrary selected samples were chosen as an example. These two samples show significant difference in the retention time of a dominant peak before alignment. The phenomenon of drifted peaks and the result of alignment are illustrated in Figure 10. This figure represents the effect of pre-processing of two samples in a small part of the chromatogram (scan nr. 8200 – 9600). The figure gives a fine impression of aligning. Visual examination of the data confirmed the aligning capability of MetAlign.

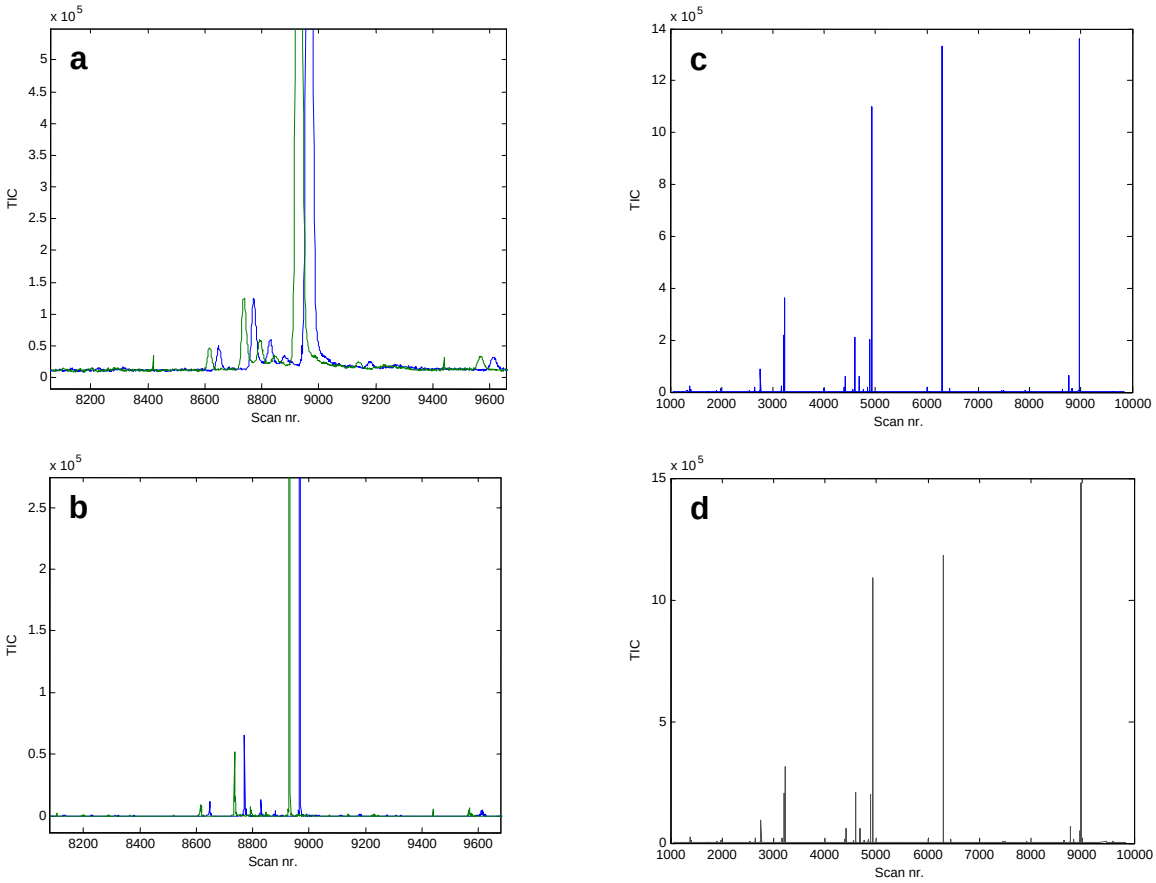

Figure 10: Shifting peaks (a) raw data, (b) data after baseline correction and noise elimination, (c+d) data after baseline correction, noise elimination and alignment. Files: 354T084 and 354T085.

3.3.2 Exploratory analysis

The chromatograms taken short after introducing the plants in the chamber were characterised by a high abundance of monoterpenes such as α-pinene, β-pinene, 2-carene, and β-phellandrene. These monoterpnes are known to be stored in the tomato leaf trichomes (Farag et al. 2002). Therefore we attribute the high abundances of monoterpenes to the damage of trichomes during insertion. To test this hypothesis, a separate experiment was made with control plants that were adapted to the chamber. These plants were touched at their trichomes. This resulted to the same strong monoterpene emissions as observed directly after introducing the plants into the chamber. During this experiment no significant emissions from products of the lipoxygenase (LOX) pathway were observed. Furthermore, no emissions of methyl esters, sesquiterpenes or homoterpenes were observed.

The emission of monoterpene emission after insertion of plants decreased within several hours. After approximately 8 – 15 hours, the emission of monoterpenes was reduced to a minimum. At that time the emission of monoterpenes had reached a steady-state level. Twenty to 24 hours after insertion of the plants, the humidity in the chamber was raised and the plants were spray inoculated. Trapping of wet air can cause technical problems to the GC/MS and therefore measurements were temporary paused for a few hours. Consequently no results are available for this period. However, after continuing with the measurements, 36 – 46 hours after inoculation, it was observed that the chromatograms were dominated by the abundance of LOX-products such as the C_6 compounds cis-3-hexenol, ethyl-2-hexenal, cis-3-hexenol-acetate and the C_5 compound 1-penten-3-ol. The biosynthetic pathway of these compounds by plants is well documented (Croft et al. 1993). During this pathway the applied stress leads to the formation of free fatty acids that activate the whole sequence of enzymes involved in most common C_5 and C_6 emission (Matsui 2006). This process is independent of the kind of stress. Emissions of LOX-products are therefore generally attributed to stress impacts on plants in cases where the stress exceeds a certain threshold (Heiden et al. 2003). We therefore assign the emissions of LOX-products to a non-specific and quick response of the plants to the infection of tomato plants to *B. cinerea*.

The emissions of LOX-products declined on a time scale of days. Approximately 2 days after inoculation the chromatograms were dominated by MeSA and the homoterpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). Such increased emissions of MeSa and TMTT was recently reported for tomato plants infested by spider mites (Kant et al. 2004). This can be explained as insect attack and pathogen infection both trigger oxidative reactions in the plant. Also, tomato plants have been widely used as model for volatile analysis after insect induced stress. This type of stress on tomato plants resulted in the formation of a wide array of volatile compounds, such as methyl jasmonate, methyl salicylate, green leafy volatiles and isoprenoids (Ament et al. 2004; Dicke et al. 1998; Maes et al. 2003).

Some typical examples of chromatograms recorded during the experiment are displayed in Figure 11. Figure 11a shows a chromatogram taken directly after inserting the plants in the chamber. Figure 11b is a chromatogram taken before inoculation and Figure 11c is taken after inoculation. Figure 11d is recorded near to the end of an experiment.

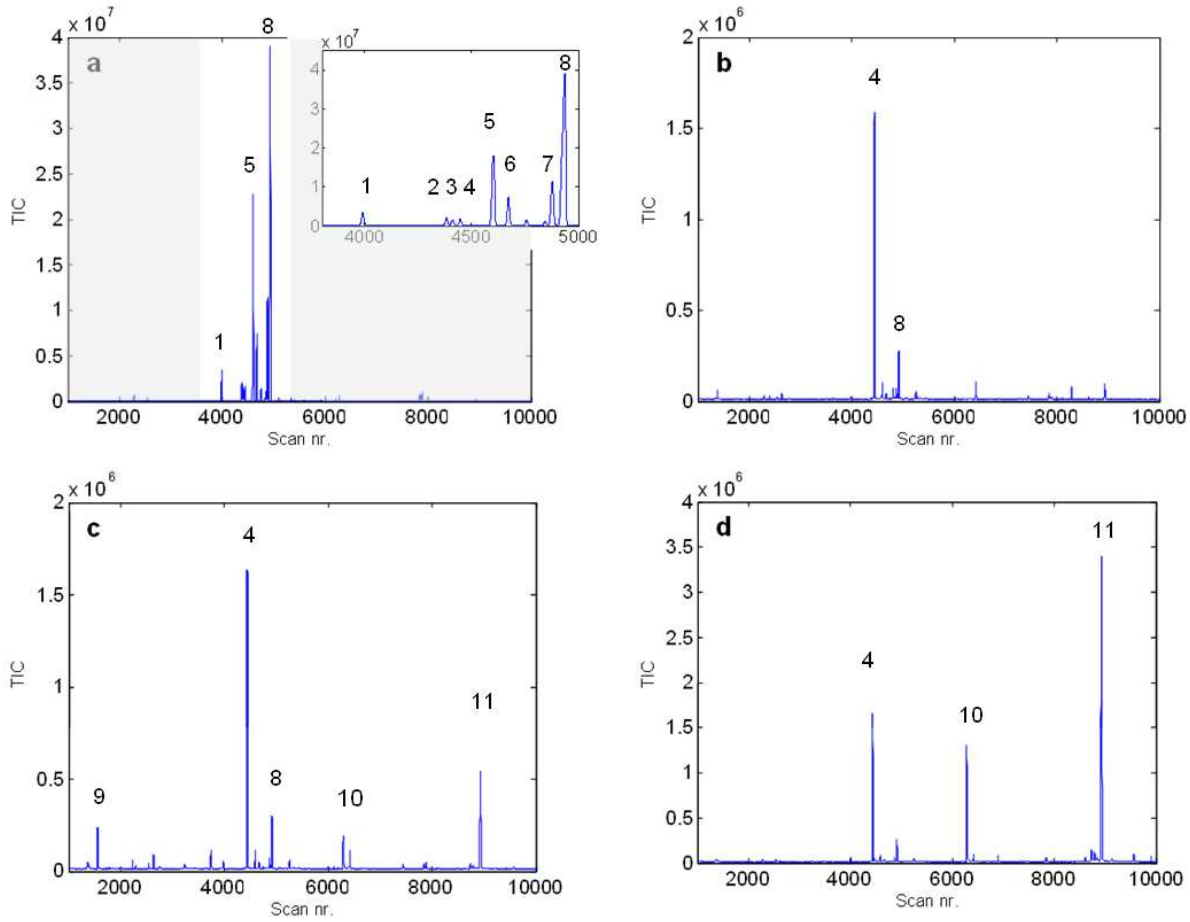

Figure 11: Typical chromatographic profiles from (a) phase 1; (b) phase 3; (c) phase 5; and (d) phase 7. (1): α-pinene; (2): p-cymol; (3): β-mycrene ; (4): decane (internal standard); (5): 2-carene; (6): α -phellandrene; (7): limonene; (8): β-phellandrene (9): impurity (column peak); (10) methyl salicylate; (11) TMTT. Note the different ranges on the y-axis. (TIC = total ion current)

For each chromatogram, most important peaks were manual integrated. An example for the result of manual integration and identification of typical compounds is displayed in Figure 12.

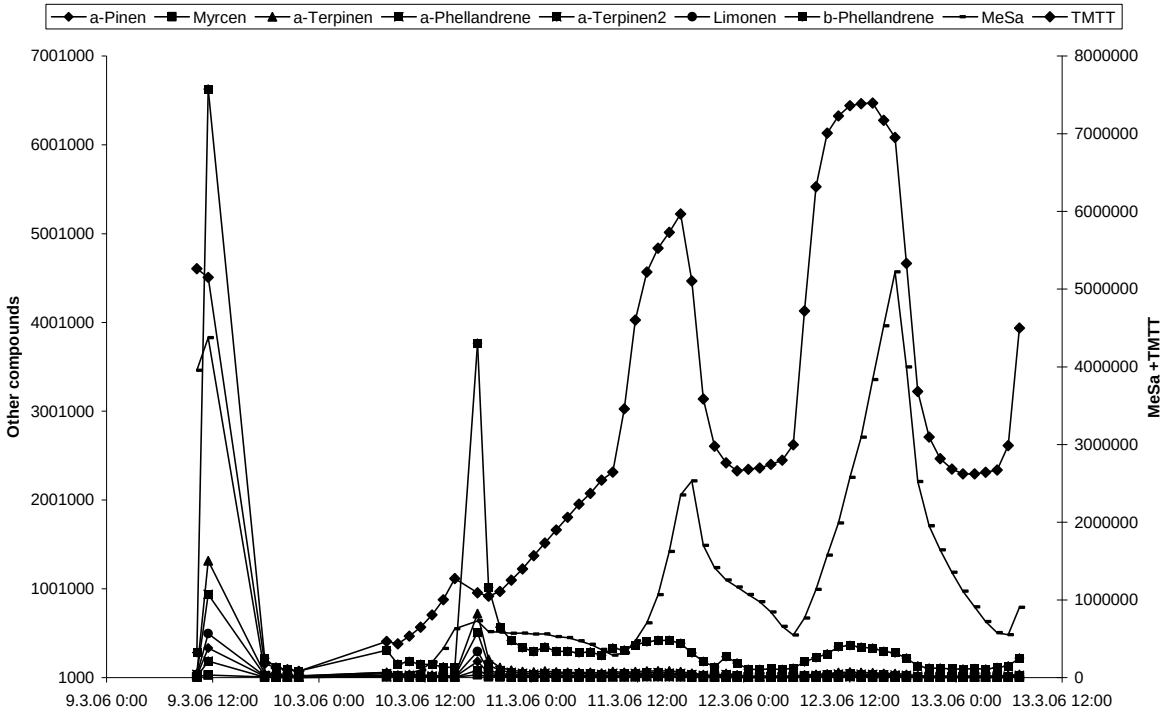

Figure 12: An example of the diurnal rhythm of volatile emission after *B. cinerea* infection.

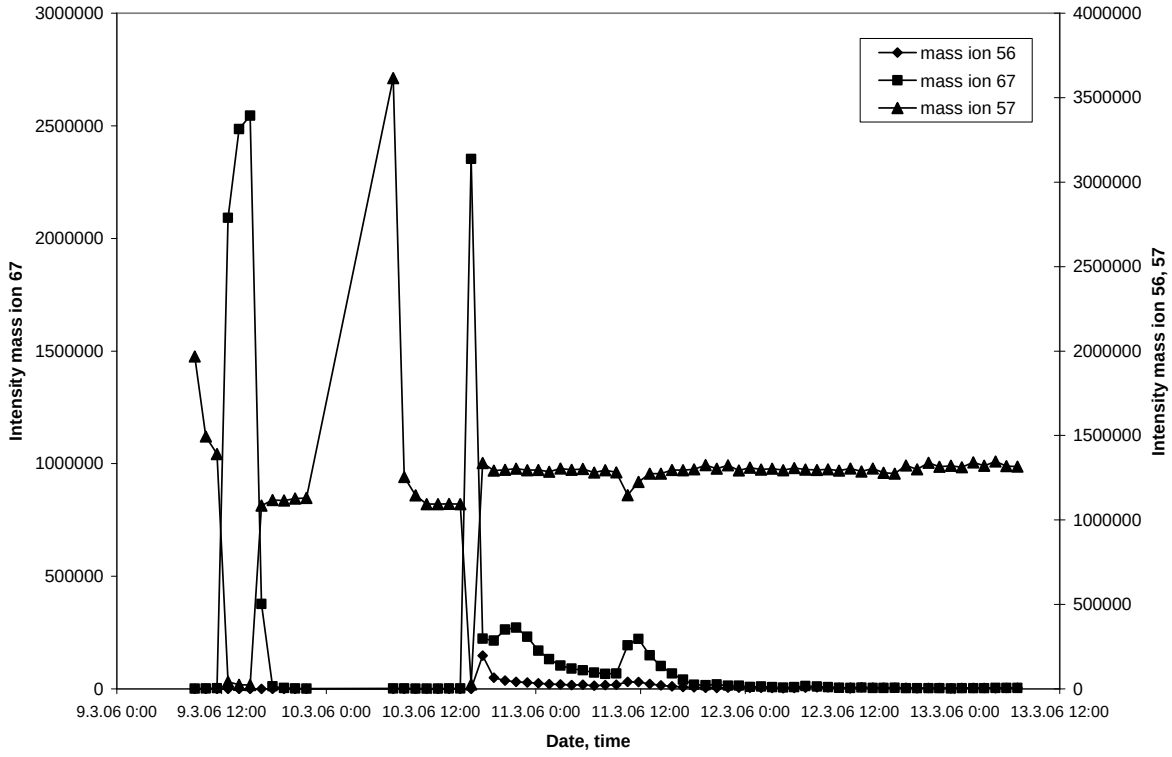

Figure 13: An example of LOX product emission after *B. cinerea* infection.

For principal component analysis (PCA), data from plants before and after inoculation with *B. cinerea* and control plants is used. The results of PCA for samples obtained for PCA are shown in Figure 14. The score plot shows clear distinction in the scores on PC 1 and PC 2 between samples obtained from the headspace of mild and severe *B. cinerea* infected tomato plants. The variation explained by the two PC's is over 50% (38.19 + 21.71). The samples in Figure 14 are numbered according to their class. Filenames belonging to these numbers are given in Appendix A; the corresponding chromatograms can be seen in Appendix C.

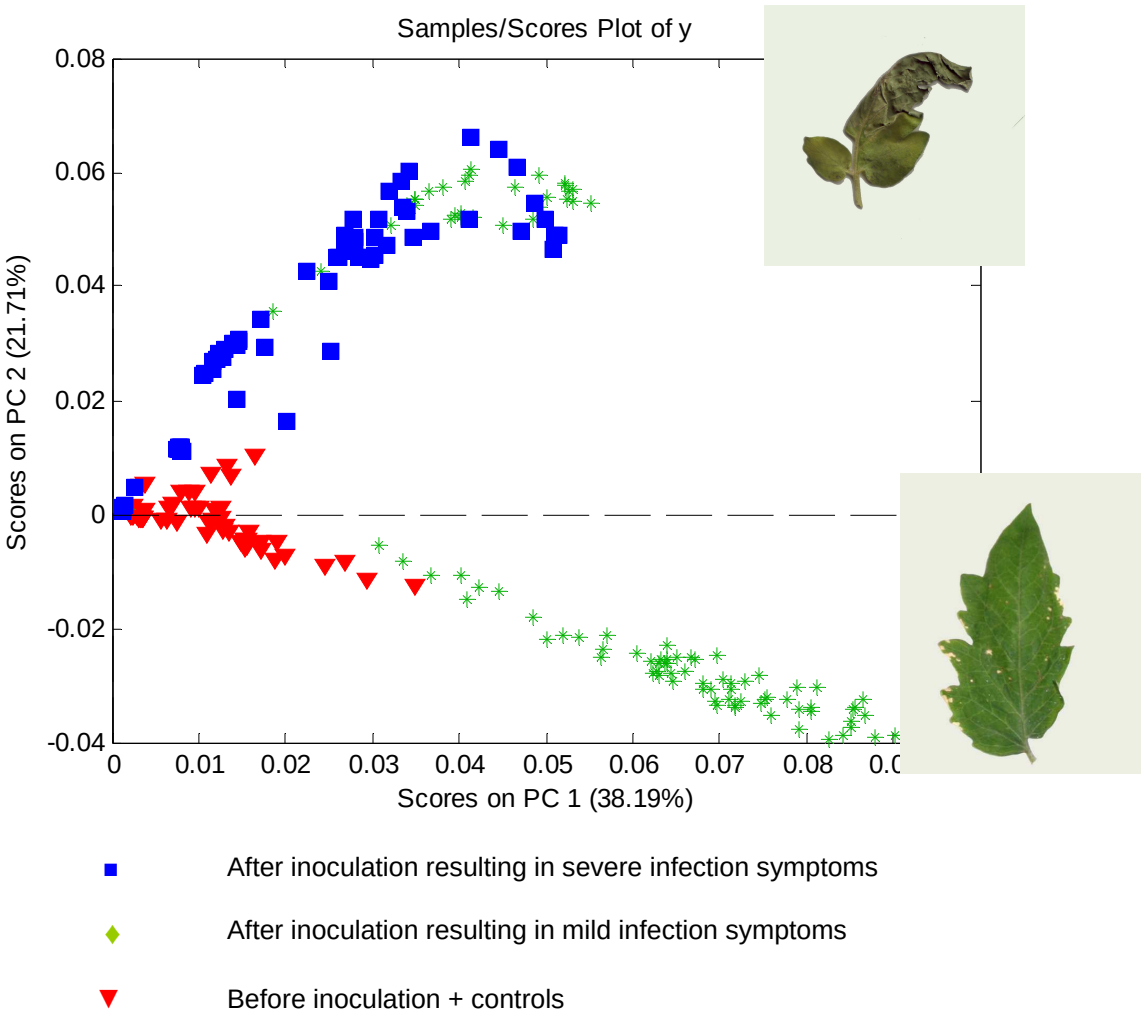

Figure 14: Score plot for samples obtained from control-plants and samples before and after inoculation with *B. cinerea*.

In the plot you can see separate clusters for 'same' treatment. The cloud of green points on the right-below corner consist of data from three replicates taken in the first visit to Juelich research centre. During this visit, the inoculation resulted in mild infection symptoms. The red dots consist of three replicates of data taken before plants were inoculated. The red cloud also includes data from a long term experiment with no intended stress application recorded in 2004. The blue en green dots in the left-above corner is from data taken from the two replicates of the 2nd visit to Juelich research centre resulting in sever infection symptoms.

3.3.3 Qualitative analysis

Statistical analysis was performed to identify compounds that significant differ between treatments. There were two experimental groups i.e. control plants and plants used for inoculation.

Comparisons were made between different phases during the infection process. These comparisons were all undertaken for samples obtained during the light phase (phase 3, phase 5 and phase 7) to minimise the effect of light intensity on emissions. Experiments 1-4 are described in the previous report (Jansen 2006). Experiments 5 and 6 are the new experiments resulting in severe infection symptoms.

Table 4: Selection of samples used for statistical comparison.

		Experiments					
Phase	**L[5]**	**1**	**2**	**3**	**4**	**5**	**6**
1		mv[1]	342T0001 06 Mar 06 5:24pm	343T0005 09 Mar 06 2:31pm	344T0107 20 Mar 06 5:46pm	mv	mv
2		339T0079[2] 28 Feb 06[3] 9:10pm[4]	342T0007 07 Mar 06 01:04am	343T0011 09 Mar 06 10:13pm	344T0112 21 Mar 06 12:09am	mv	mv
3		339T0085 01 Mar 06 11:53am	342T0015 07 Mar 06 10:01am	343T0014 10 Mar 06 11:43am	344T0121 21 Mar 06 11:41am	354T0251 22 Aug 06 11:15am	354T0311 25 Aug 06 1:00pm
Inoculation		01 Mar 06 4.15pm	07 Mar 06 5.12pm	10 Mar 06 3.59pm	21 Mar 06 4.12pm	22 Aug 06 4:12pm	25 Aug 06 3:05pm
4		mv	342T0025 08 Mar 06 12:06am	343T0024 10 Mar 06 11:31pm	344T0132 21 Mar 06 11:39pm	354T0281 23 Aug 06 10:50pm	357T008 25 Aug 06 11:11pm
5		341T0002 02 Mar 06 2:44pm	342T0035 08 Mar 06 11:36am	343T0033 11 Mar 06 11:01am	344T0141 22 Mar 06 11:06am	354T0291 24 Aug 06 11:135am	357T0018 26 Aug 06 11:53am
6		341T0009 02 mar 06 11:44pm	mv	343T0043 11 Mar 06 11:45pm	344T0151 22 Mar 06 11:51pm	354T0301 25 Aug 06 12:18am	357T0028 27 Aug 06 12:38am
7		341T0018 03 Mar 06 11:17am	343T0001 09 Mar 06 09:25am	343T0052 12 Mar 06 11:14am	344T0160 23 Mar 06 11:19am	354T0306 25 Aug 06 6:38am	357T0037 27 Aug 06 12:06pm
8		341T0027 03 Mar 06 10:46pm	mv	343T0061 12 Mar 06 10:44pm	mv	mv	357T0046 28 Aug 06 11:33am

[1]mv = missing value, [2]=filename, [3]=Date of analysis, [4]=Time of analysis.

No statistical differences between phase 3 and phase 5 could be found when binning the samples from experiments 1 to 6. Also no statistical difference could be found between phase 3 and phase 7 when binning the samples from experiment 1 to 6.

4 DISCUSSION

4.1 Disease progression

At 12 hours after inoculation, no visible symptoms were observed. Small lesions were first observed at 24 hours after inoculation. The lesion sizes increased progressively thereafter resulting in severe infection symptoms at 72 hours after inoculation when plants were removed from the chamber. Unfortunately it was no option to take pictures from the leaves during the experiment because the wall thickness of the glass chamber prevented sharp pictures.

4.2 Gas chromatography – mass spectrometry

Shortly after inoculation, a strong emission of volatile organic compounds from the octadecanoid pathway was observed. These compounds are commonly denoted as LOX products. The production of LOX products is stimulated under different stress conditions by plants such as wounding, pathogen attack and herbivore attack (Heiden *et al.* 2003). One of these acute responses is the induction of activity within the octadecanoid pathway and the subsequent emission of volatile LOX products (Figure 15).

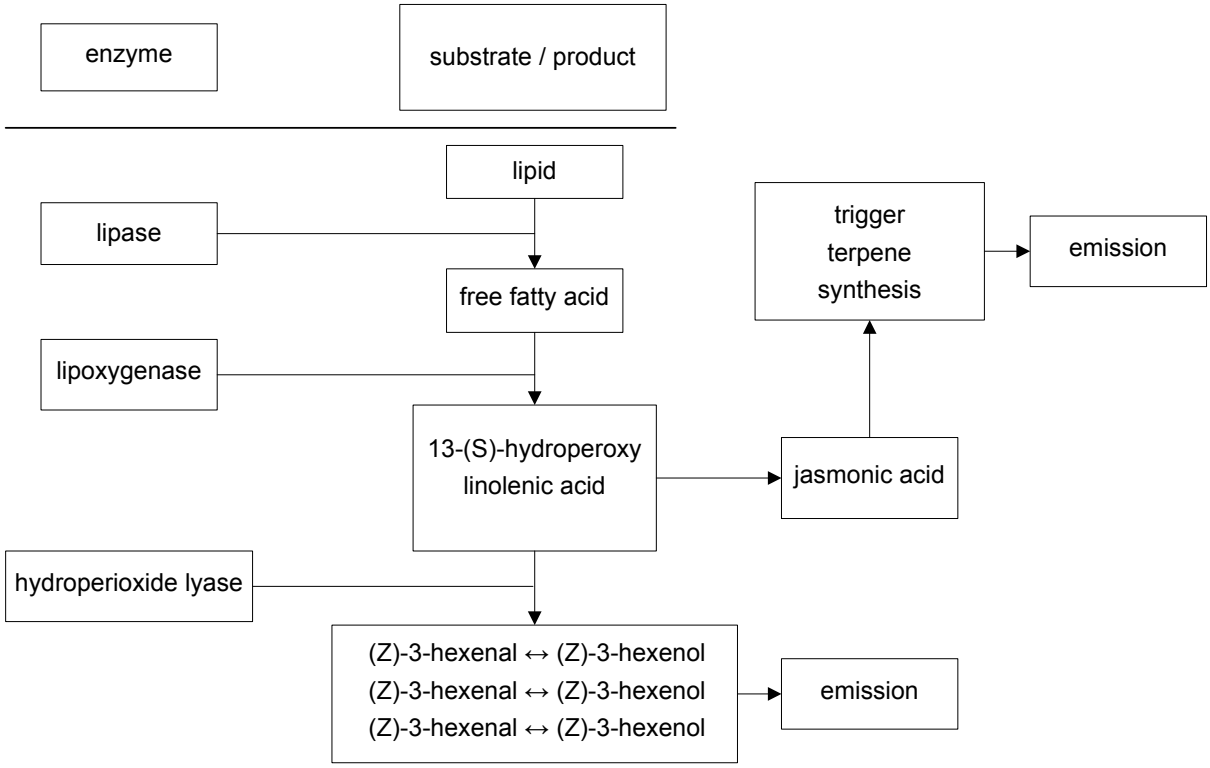

Figure 15: Biosynthesis of plant volatiles (Boland et al. 1998).

Mechanical damages on plant leaves are known for inducing the release of a succession of different LOX products. This induction is locally and starts immediately upon damage. Therefore it can be expected that the products are due to an autolytic oxidative breakdown of membrane lipids in response to plant cell damage.

Compared to previous measurements described in Jansen, 2006, we observed that the emission of LOX products was much more intense in the second series of headspace analysis described in this report. So this work strengthens the assumption that stressing with a pathogen gives no consistent stress signals. Reasons for these differences could be 1) vitality of plants and or spores; 2) the history of the plants and the inoculation procedure inside the chamber.

4.3 Pre-processing of GC-MS data

Normalization of the data was done by bringing the total area of each chromatogram to 1. This way of normalization seems accurate as the pattern of peaks remain intact. The normalization procedure is correcting in a way for differences in number of plants per experiment, the total leave area and variations in airflow encountered during the experiments. Another way to correct for differences in airflows is to make use of the internal standard. For doing this automatically, the peak corresponding to the internal standard should be perfectly aligned. The peak maximum amplitude of this internal standard can subsequently be determined. This peak maximum amplitude can then afterwards be used for normalization of the signal. It turned out to be difficult to assess the result of the alignment algorithm we used. Overlaying the chromatographic data is possible for limited number of chromatograms. For the number of chromatograms used in this study this is no option. In general, pre-processing is difficult to validate, and thus a correct strategy is by definition not possible.

4.4 Principal component analysis

Data reduction based on PCA turned out to be useful for the visual interpretation of the complex GC-MS data. The variation between mild and severe *B. cinerea* infected plants could be demonstrated.

5 CONCLUSION

The intensity of the stressor has a high impact on the emission of volatile compounds, both in quantitative as well in a qualitative way. This effect should be taken in account when evaluating and comparing volatile profiles among same, but also different stressors.

Acknowledgement

I would like to thank dr. Jürgen Wildt, dr. Einhart Kleist and Marco Miebach for their assistance during the experiments. I would also like to thank dr. Jan van Kan for providing the *Botrytis cinerea* strain. This research received grant-aided support from the European Science Foundation Scientific Programme “Volatile organic compounds in de Biosphere Atmosphere System (VOCBAS).

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Appendix A: Data structure

Class	Description
1	Before inoculation
2	After inoculation
3	Control data 2004
4	Control data 2006
5	Measurement error

Sample	Label	Include	Class	Description
1	243T0196.txt	1	5	Control experiment 2004
2	243T0197.txt	1	5	
3	244T0001.txt	1	3	
4	244T0002.txt	1	3	
5	244T0003.txt	1	3	
6	244T0004.txt	1	3	
7	244T0005.txt	1	3	
8	244T0006.txt	1	3	
9	244T0007.txt	1	3	
10	244T0008.txt	1	3	
11	244T0009.txt	1	3	
12	244T0010.txt	1	3	
13	244T0011.txt	1	3	
14	244T0012.txt	1	3	
15	244T0013.txt	1	3	
16	244T0014.txt	1	3	
17	244T0015.txt	1	3	
18	244T0016.txt	1	3	
19	244T0017.txt	1	3	
20	244T0018.txt	1	3	
21	244T0020.txt	1	3	
22	244T0021.txt	1	3	
23	244T0022.txt	1	3	
24	244T0023.txt	1	3	
25	244T0024.txt	1	3	
26	244T0025.txt	1	3	
27	244T0026.txt	1	3	
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29	244T0028.txt	1	3	
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42	244T0041.txt	1	3	

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44	244T0043.txt	1	3	
45	244T0044.txt	1	3	
46	244T0045.txt	1	3	
47	244T0046.txt	1	3	
48	244T0047.txt	1	3	
49	244T0048.txt	1	3	
50	244T0049.txt	1	3	
51	244T0050.txt	1	3	
52	244T0051.txt	1	3	
53	244T0052.txt	1	3	
54	244T0053.txt	1	3	
55	244T0054.txt	1	3	
56	244T0055.txt	1	3	
57	244T0056.txt	1	3	
58	244T0058.txt	1	3	
59	354T0250.txt	1	1	Botrytis experiment 1 -> outlier
60	354T0251.txt	1	1	
61	354T0252.txt	1	1	
62	354T0253.txt	1	1	
63	354T0254.txt	1	1	
64	354T0255.txt	1	1	
65	354T0256.txt	1	1	
66	354T0257.txt	1	2	Inoculation
67	354T0258.txt	1	2	
68	354T0259.txt	1	2	
69	354T0260.txt	1	2	
70	354T0261.txt	1	2	
71	354T0262.txt	1	2	
72	354T0263.txt	1	2	
73	354T0264.txt	1	2	
74	354T0265.txt	1	2	
75	354T0266.txt	1	2	
76	354T0267.txt	1	2	
77	354T0268.txt	1	2	
78	354T0269.txt	1	2	
79	354T0270.txt	1	2	
80	354T0271.txt	1	2	
81	354T0272.txt	1	2	
82	354T0273.txt	1	2	
83	354T0274.txt	1	2	
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94	354T0286.txt	1	2	
95	354T0287.txt	1	2	
96	354T0288.txt	1	2	

97	354T0289.txt	1	2	
98	354T0290.txt	1	2	
99	354T0291.txt	1	2	
100	354T0292.txt	1	2	
101	354T0293.txt	1	2	
102	354T0294.txt	1	2	
103	354T0295.txt	1	2	
104	354T0296.txt	1	2	
105	354T0297.txt	1	2	
106	354T0298.txt	1	2	
107	354T0299.txt	1	2	
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109	354T0301.txt	1	2	
110	354T0302.txt	1	2	
111	354T0303.txt	1	2	
112	354T0304.txt	1	2	
113	354T0305.txt	1	2	
114	354T0306.txt	1	2	
115	354T0310.txt	1	1	New Botrytis experiment -> Outlier
116	354T0311.txt	1	1	
117	357T0001.txt	1	1	
118	357T0002.txt	1	2	Inoculation
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134	357T0018.txt	1	2	
135	357T0019.txt	1	2	
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146	357T0030.txt	1	2	
147	357T0031.txt	1	2	
148	357T0032.txt	1	2	
149	357T0033.txt	1	2	
150	357T0034.txt	1	2	

151	357T0035.txt	1	2	
152	357T0036.txt	1	2	
153	357T0037.txt	1	2	
154	357T0038.txt	1	2	
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158	357T0042.txt	1	2	
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163	357T0047.txt	1	2	
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165	357T0049.txt	1	2	
166	357T0050.txt	1	2	
167	357T0051.txt	1	2	
168	357T0052.txt	1	2	
169	357T0053.txt	1	2	
170	357T0054.txt	1	2	
171	357T0055.txt	1	2	
172	357T0056.txt	1	2	
173	357T0057.txt	1	2	
174	357T0058.txt	1	2	
175	357T0059.txt	1	2	
176	357T0060.txt	1	2	
177	357T0061.txt	1	2	
178	357T0062.txt	1	2	
179	357T0063.txt	1	2	
180	357T0064.txt	1	2	
181	357T0065.txt	1	2	
182	357T0066.txt	1	2	
183	357T0067.txt	1	2	
184	357T0068.txt	1	2	
185	357T0069.txt	1	2	
186	357T0070.txt	1	2	
187	357T0071.txt	1	2	
188	357T0075.txt	1	2	
189	357T0076.txt	1	2	
190	357T0077.txt	1	5	Not enough data -> outlier -> cannot be read!
191	357T0078.txt	1	2	
192	357T0079.txt	1	2	
193	357T0080.txt	1	2	
194	357T0081.txt	1	2	
195	357T0097.txt	1	4	New control experiment
196	357T0098.txt	1	4	
197	357T0099.txt	1	4	
198	357T0100.txt	1	4	
199	357T0101.txt	1	4	
200	357T0102.txt	1	4	
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203	357T0107.txt	1	4	
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206	357T0110.txt	1	4
207	357T0111.txt	1	4
208	357T0112.txt	1	4
209	357T0113.txt	1	4
210	357T0114.txt	1	4
211	357T0115.txt	1	4
212	357T0116.txt	1	4
213	357T0117.txt	1	4
214	357T0118.txt	1	4
215	357T0119.txt	1	4
216	357T0120.txt	1	4
217	357T0121.txt	1	4
218	357T0122.txt	1	4
219	357T0123.txt	1	4
220	357T0124.txt	1	4
221	357T0125.txt	1	4
222	357T0126.txt	1	4
223	357T0127.txt	1	4
224	357T0128.txt	1	4
225	357T0129.txt	1	4
226	357T0130.txt	1	4
227	357T0131.txt	1	4
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229	357T0133.txt	1	4
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234	357T0138.txt	1	4
235	357T0139.txt	1	4
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242	357T0146.txt	1	4
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244	357T0148.txt	1	4
245	357T0149.txt	1	4
246	357T0150.txt	1	4
247	357T0151.txt	1	4
248	357T0152.txt	1	4
249	357T0153.txt	1	4
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251	357T0155.txt	1	4
252	357T0156.txt	1	4
253	357T0157.txt	1	4
254	357T0158.txt	1	4
255	357T0159.txt	1	4
256	357T0160.txt	1	4
257	357T0161.txt	1	4
258	357T0162.txt	1	4

259	357T0163.txt	1	4
260	357T0164.txt	1	4
261	357T0165.txt	1	4
262	357T0166.txt	1	4
263	357T0167.txt	1	4
264	357T0168.txt	1	4
265	357T0169.txt	1	4
266	357T0170.txt	1	4
267	357T0171.txt	1	4
268	357T0172.txt	1	4
269	357T0173.txt	1	4
270	357T0174.txt	1	4
271	357T0175.txt	1	4
272	357T0176.txt	1	4
273	357T0177.txt	1	4
274	357T0178.txt	1	4
275	357T0179.txt	1	4
276	357T0180.txt	1	4
277	357T0181.txt	1	4

Appendix B: Laboratory book

21.08.2006 (Monday)

12:22 Uhr Gerstel des Terpen-Gc gestoppt und mit 50min Anreicherungszeit wieder gestartet (354T0236 wird angereichert). Gerstel hat bisher nur 0.5 L angereichert

14:00 Uhr Eiche aus K3 ausgebaut, 3 Moneymaker eingebaut, zwei Temperaturfühler mit eingebaut (USB5 und USB6) USB6 wird als USB2 aufgezeichnet,

17:30 Uhr TP A K3 = 19.5℃ => Fluß in K3 erhöht: 40 -> 50L/min

22.08.2006 (Tuesday)

7:50 Uhr Terpen Gc hängt ab jetzt auch an K3, Lauf 354t0250 bei 32 min

13:20 Uhr Moneymaker aus GK2 ausgebaut und letzte Eiche aus dem Keller eingebaut, Oxy Gc Lauf 354s0223 bei 34 min an GK2 gehängt, neue Versuchsnummer 356

13:47 Uhr zusätzliche Verdünnungsluft via 20L/Min-FC (angesteuert über FC-Steuerung der Quellen K16) an den Ausgang K3 gehängt => Luft aus K3 soll verdünnt werden da der Taupunkt für die Infiltration hoch gesetzt werden muss, Kammer 3 auf 15℃ runtergeregelt
ACHTUNG: Zusätzlich zur Luft vom FC Luft K3 (WMR20 K11) gehen noch Soll=6 L/min über den FC Luft KK2 (WMR20 K20) und den linken Ozongenerator in die K3 !!
Taupunkt K3 Eingang jetzt auf 15℃ eingeregelt

16:12 Uhr Infiltrate Tomato plants with botrytis cinerea, lights switched of, Terpen GC Run 354t0257 at 5 min

18:17 Uhr dew point inlet chamber 3 now 14.6 ℃, no water in the tubings, only little emissions of TMTT, nothing more

23.08.2006 (Wednesday)

7:40 Uhr Lichtrechner K1 und K2 waren wohl in der Nacht ausgefallen. Zeitabgleich für beide war bei 60 min während PC-Zeit bei etwa 500 war.
⇨ Zeitabgleich durchgeführt, ab jetzt wieder Licht in K2

8:00 Uhr Lichtrechner K3 wieder auf Automatik

9:03 Uhr Lichtprogramm K2 geht von 5:00 – 20:00 Uhr, K3 von 5:00 – 18:00 Uhr

10:42 Uhr Keine Emissionen aus K3 = zu nass ???, Luft durch Befeuchter reduziert,
Eingangsluft hat jetzt etwa 7℃ Taupunkt

11:20 Uhr T K3 von 15 auf 20℃

13:00 Uhr Terpen GC wieder ohne Verdünnung an K3, Lauf 354t0273 bei 30 min

14:35 Uhr LOX ist da, Lauf 354t0274

24.08.2006 (Thursday)

10:20 Uhr Zusatzluft K2 gemessen = 20 L/min, Befeuchter K2 aufgefüllt, Zuluft geht jetzt über den Befeuchter in GK2, WMR 20 Kanal 6 von 30 auf 20, Kanal 18 auf 5,
Taupunkt Kammerausgang ca. 18℃, Eingang ca. 14℃

12:16 Uhr WMR 20 Kanal 18 auf 15 und Kanal 6 auf 15
12:34 Uhr WMR 20 Kanal 18 auf 20 und Kanal 6 auf 10,

ANMERKUNG:
Dies konnte nur bedingt einen Einfluß auf GK2 haben, da die Leitung vom FC WMR20 K18 in die Halle geht !!
Es wurde der FC QK16 (ein Reserve-FC 20L/min angeschlossen !!!),
d.h. es wurde nur der Fluss über FC GK2 (WMR K6) reduziert

Anzeige TP GK2 murx, da Magnetventile auf Automatik und Steuerpgm alter Kammerrechner K1 nicht aktiv !!
Ab. 16:10 Messung am Ausgang GK2

12:42 Uhr Taupunkt Eingang GK2 jetzt 15,5 ℃
13:00 Uhr Taupunkt am Ausgang über 21℃! WMR 20 Kan al 18 auf 15 und Kanal 6 auf 15
14:07 Uhr Taupunkt am Ausgang über 21℃! WMR 20 Kan al 18 auf 10 und Kanal 6 auf 20
16:10 Uhr Messung mit TP und Binos am Ausgang GK2
Fluss GK2 ab jetzt 10 L (SOLL, 50%) / Min via Befeuchter + 20 L/min via
FC Luft GK2
Licht K2 auf Dauerlicht

25.8.2006 (Friday)

7:20 Uhr Befeuchter für GK2 überbrückt, Flüsse konstant gelassen
356S0077 wird gleich angereichert
10:50 Uhr Removed the infected plants from chamber nr. 3.
11:00 Uhr Put three new plants in chamber nr. 3.
11:43 Uhr New file is 354T0310. This is the first file for new plants
13:37 Uhr Temperatur K3 von 20 auf 15℃, Terpen GC an die Quellen gehängt, Neue Versuchsnummer 357, Befeuchter nachgefüllt
14:30 Uhr Befeuchter an GK2 angeschlossen, 10 min later lights off
15:05 Uhr Tomato plants infested with botrytis cinerea, lamps off
16:09 Uhr Luft durch GK2 von 20+10 L/min. auf 10+10 L/min gesetzt.
Taupunkt Eingang von 2,5 auf 9 ℃ herauf
Da scheint Ethanol im Wasser zu sein

26.08.2006 (Saturday)

11:33 Uhr Ventil für befeuchtete Luft an Schalttafel K3 aufgedreht und K3 Licht auf Automatik gesetzt (= alle Lampen an), K3 ist bis oben hin mit Wassertropfen beschlagen
14:10 Uhr Kammer 3 immer noch nass. Befeuchter K3 kurzgeschlossen TP Eingang von 9,8 auf -17 ℃. K3 auf 15 Grad gelassen und Terpen GC dran Es läuft 357T0020.
Kammer 2 Befeuchter kurzgeschlossen, es läuft 356S0111

27.08.2006 (Sunday)

9:40 Uhr K3 jetzt trocken – Tomaten sehen echt krank aus, wieder viele LOX Produkte, Pflanzen K3 gegossen

28.08.2006 (Monday)

8:45 Uhr Kammer 2 Lichtsteuerung auf Automatik, an. Lauf 356S0159 ist bei 18 Minuten
:

29.08.2006 (Tuesday)

8:25 Uhr Terpen GC Sequenz zu Ende. Letzter Lauf 357T0071 heute morgen 7:20 Uhr Neustart mit 357T0075
OXY-GC Sequenz auch zu Ende Letzter Lauf 356S0176
11:20 Uhr Kühlung an Wurzel Eiche GK2 angestellt, vorsichtig Temperaturfühler Nummer 9 in den Boden eingeführt
12:04 Uhr Kammer 2 auf Dauerlicht

13:40 Uhr Lauf 357t0080 komplett angereichert, Tomaten aus K3 ausgebaut und eingefroren
14:17 Uhr Zwei neue Moneymaker in K3 eingebaut, Lauf 357t0079 bei 27 min, Fluss K3 von 50 auf 40 L/min

17:03 Uhr Terpen GC Ofen war ausgeschaltet! Nach anschalten Ofenheizung weiter gelaufen
Ist der ab Mittag überhaupt gelaufen??

30.08.2006 (Wednesday)

9:00 Uhr Terpen GC steht, keine Fehlermeldung aber Ofen aus. Sequenz abgebrochen, Neustart. Uhr des TerpenGC Rechners geht etwa 10 min. vor

9:25 Uhr Tomaten in Kammer 3 gegossen

31.08.2006 (Thursday)

8:30 Uhr Terpen GC steht seit gestern Abend. Warning Oven shut off. Ofen angeschaltet Läuft weiter
OXY-GC steht auch aber schon seit gestern Nachmittag . wohl beim Verlängern der Sequenz abgestürzt
Ca. 11:30 Uhr In Kammer 2 2 Lampen aus, war Kontrolle für Blatttemperaturanzeige

12:30 Files 357T0103 (letzter gestern Abend, war überhaupt nichts drin und File 357T0104 (erster heute morgen MeSA und TMTT Doppelt) gelöscht, sind Unsinn

13:15 Uhr Kammer 2 noch 2 Lampen an
TPeingang und Ausgang K2 hängen wohl schon seit einiger Zeit zusammen

14:15 Uhr Licht in Kammer 2 ganz aus

17:30 Uhr Kammer 3 Kontrollpflanzen: das sieht ziemlich schlimm aus. Alle da außer LOX und jetz schon richtig deftig obwohl den Pflanzen selbst nichts anzusehen ist

1.09.2006 Friday

8:45 Uhr Licht wieder an in K2.

10:30 Uhr Ausbau der Eiche aus Kammer 2. Licht per Hand alles aus

11:57 Uhr OXY GC: Kalibrationen sind stabil, zusammen mit Terpen GC an Tomate. Aus Überlapp kann zur Not eine Kalibration gemacht wereden

2.09.2006 Saterday

14:16 Uhr Tomaten in K3 gegossen

3.09.2006 Sonday

11:45 Uhr MeJa in K3 gehängt, Start Lauf 357t0163 in ca. 5 min

4.09.2006 Monday

11:45 Uhr Da scheint sich ja nichts zu tun, Wenn überhaupt, nicht viel Ocimen, kein DMTT, Indol nur kurz in der Nacht. Entweder nicht genug MeJA oder zu kalt?
Temperatur K 3 von 15 auf 35 ℃ gesetzt Lauf 357T 0181 fertig chromatogr,. Lauf357T0182 hat noch 2 min. zum anreichern

14:50 Uhr Baumwolle in GK2 eingebaut, gegossen, 20 l/min, 25 C eingestellt. UR_2006_4_9
Gerade eben muss Terpen GC an Baumwolle gehangen worden sein.

15:36 Uhr Kein messbarer Druck am Ende der Teflonleitung A GK2 => Baumwolle neu eingebaut und besser am Stamm abgedichtet
Statt Leitung Q-FC16 (max 10L/min, auf 50%) jetzt wieder Zusatzluft GK2 (WMR20 K18, Soll: 10L/min) dazu
Druck GK2 statt 200 Pa jetzt 500 Pa und am Ende der Teflonleitung A GK2
120 Pa

Appendix C: Raw data

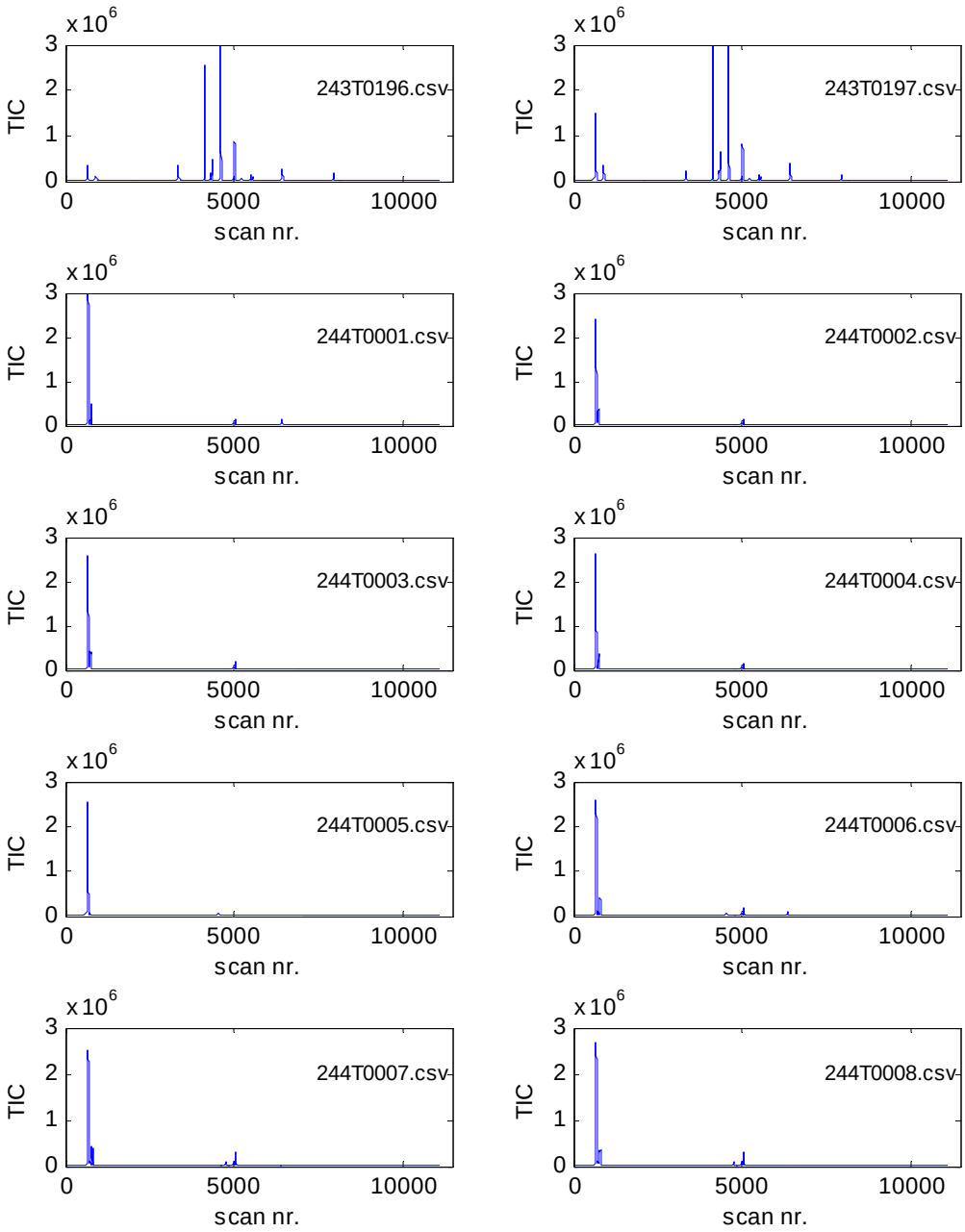

Figure 16: Control data (2004)

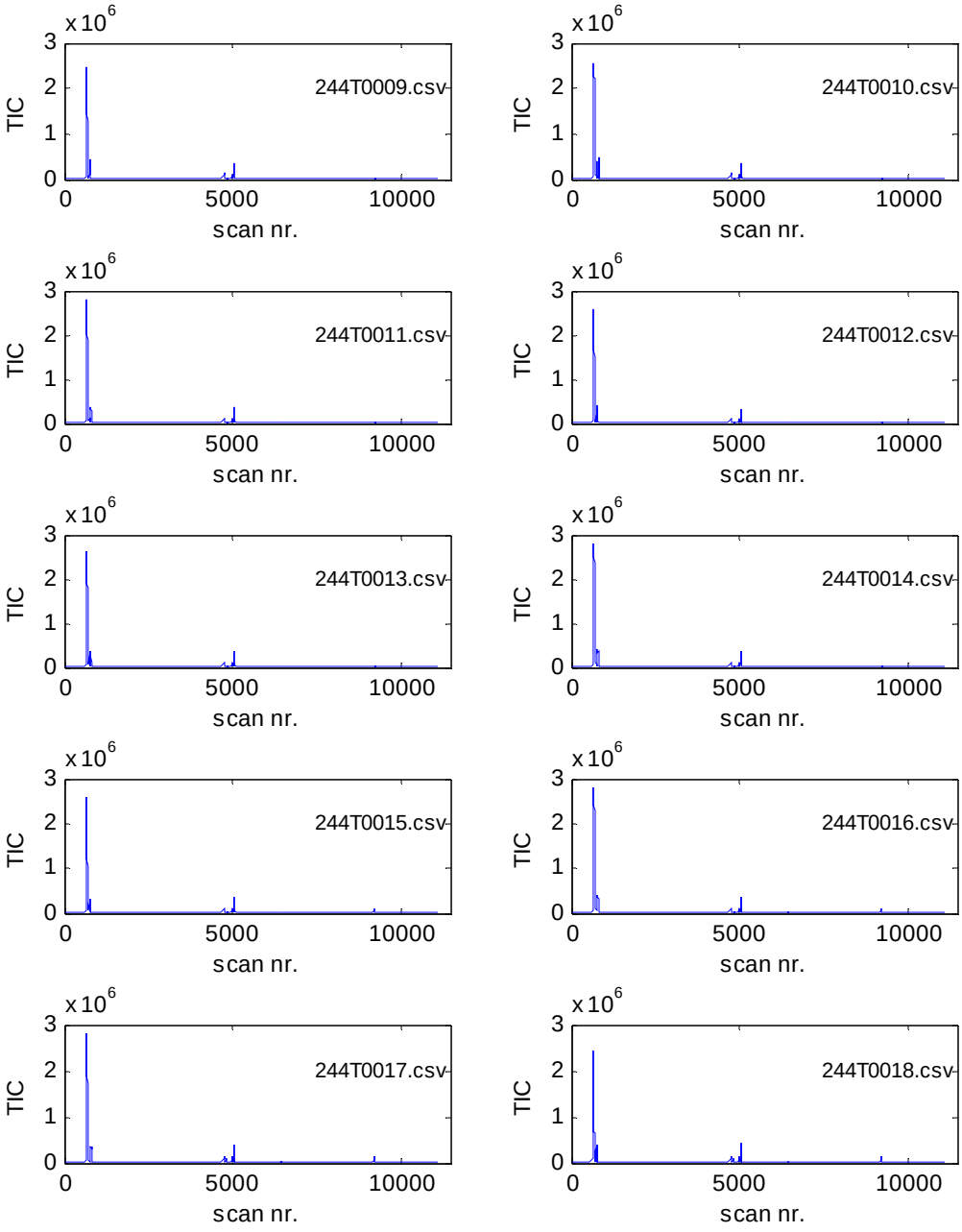

Figure 17: Control data (2004)

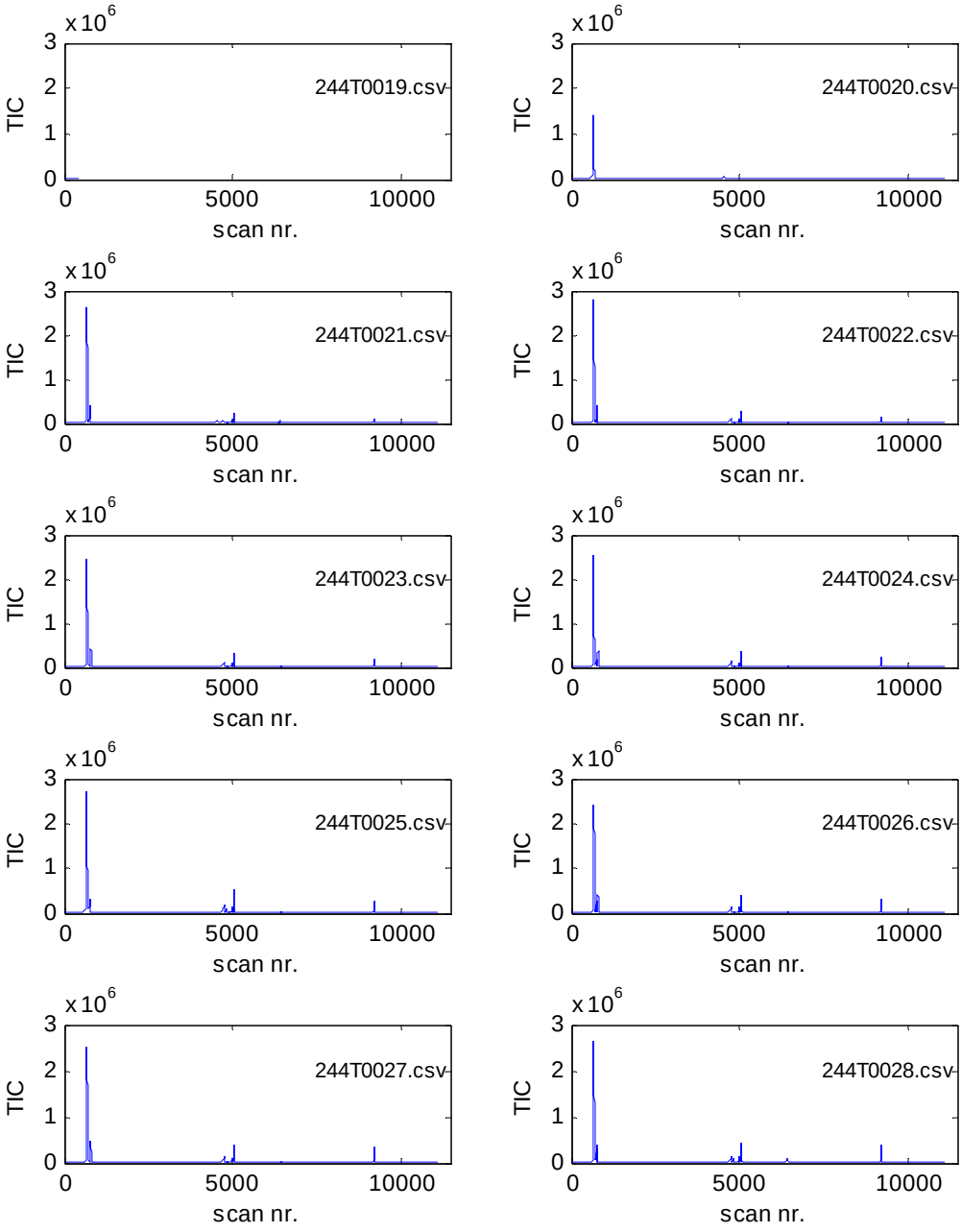

Figure 18: Control data (2004)

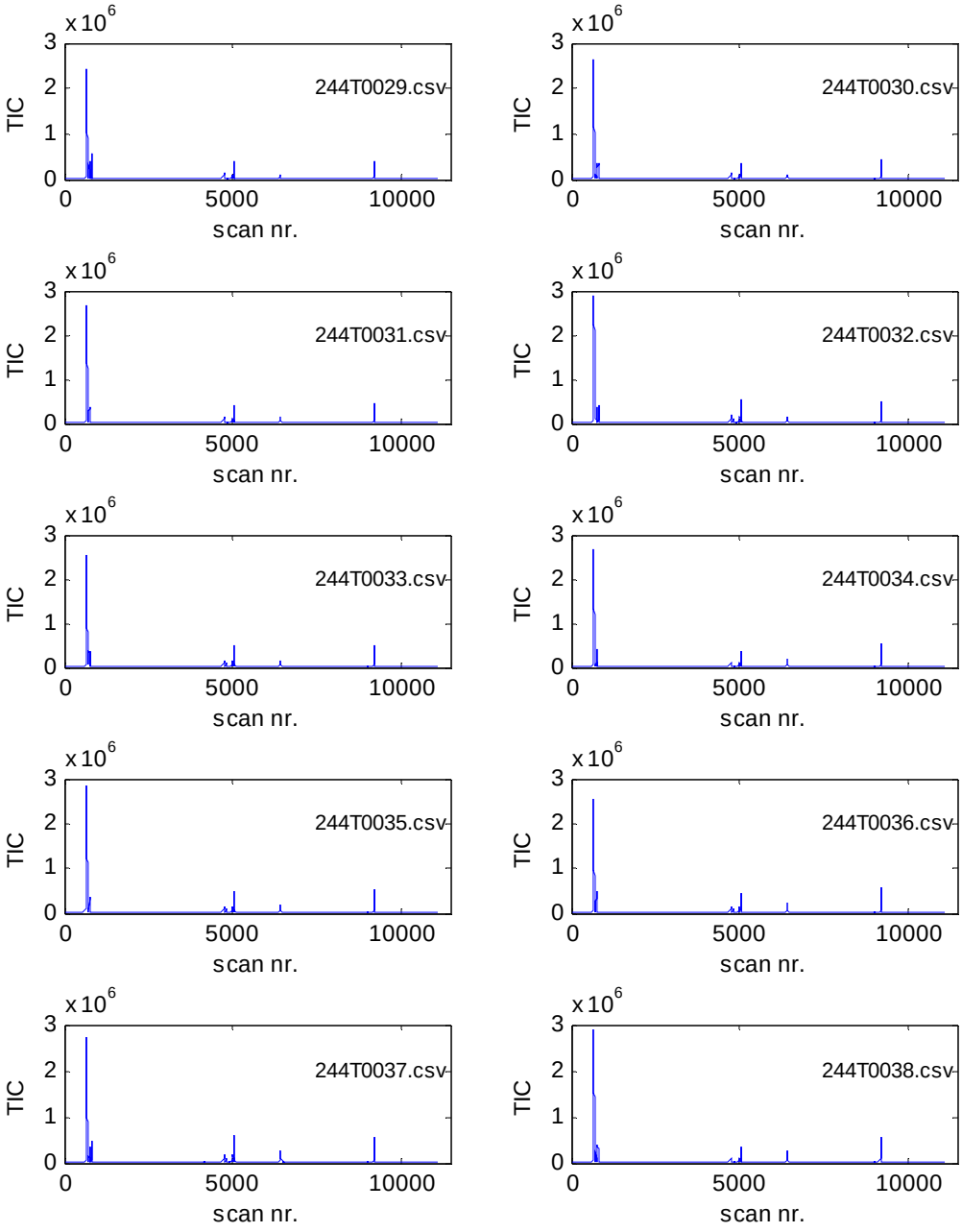

Figure 19: Control data (2004)

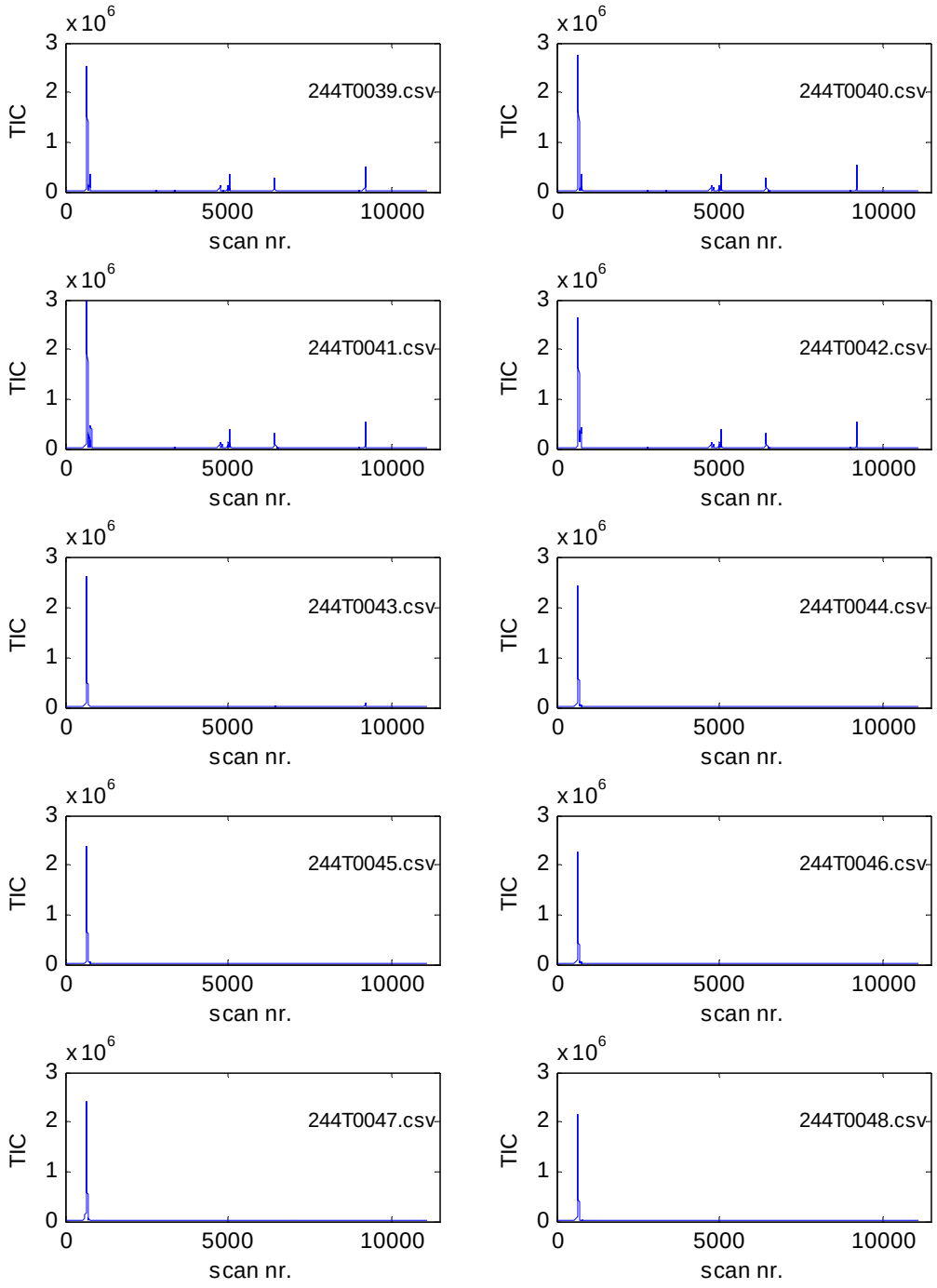

Figure 20: Control data (2004)

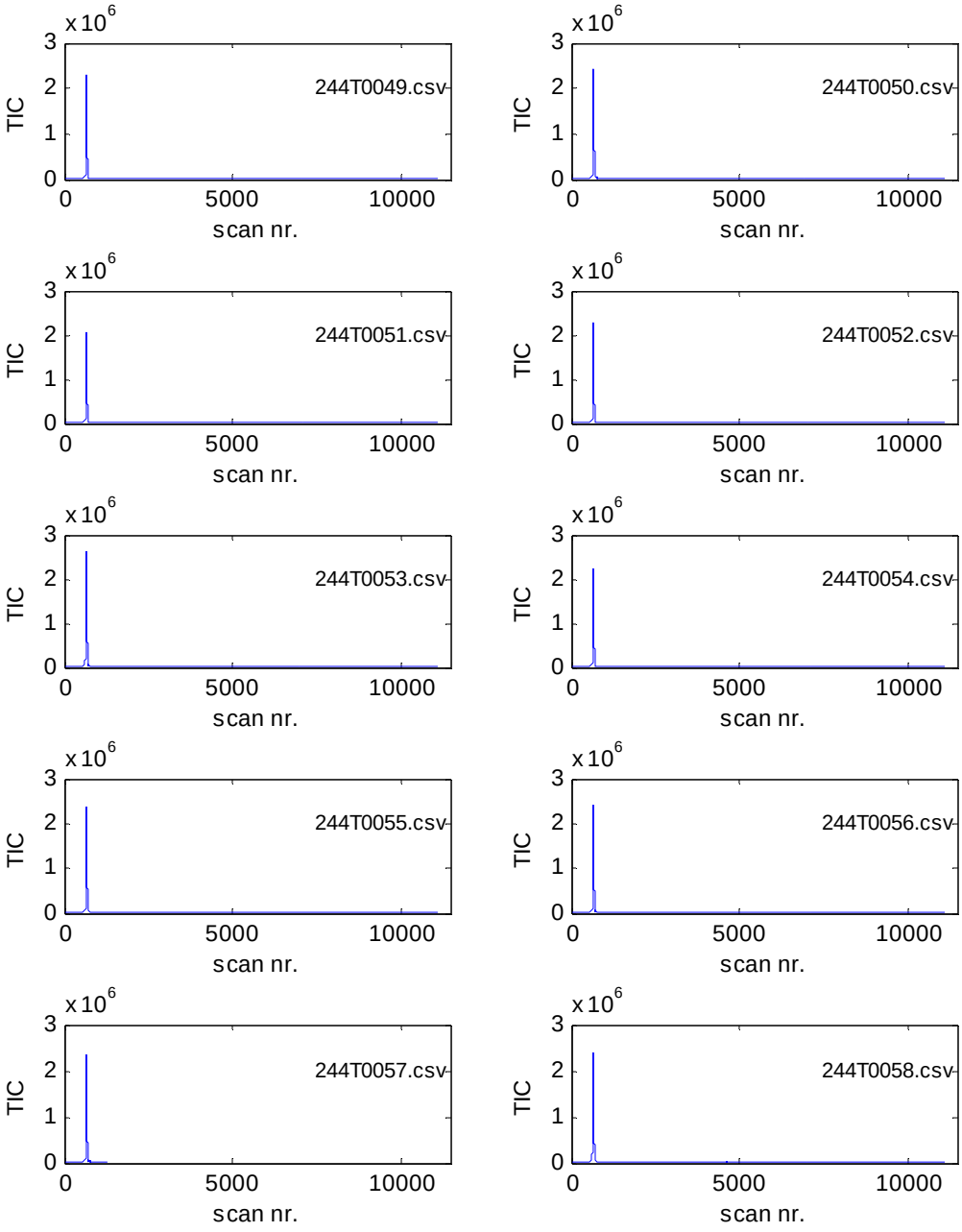

Figure 21: Control data (2004)

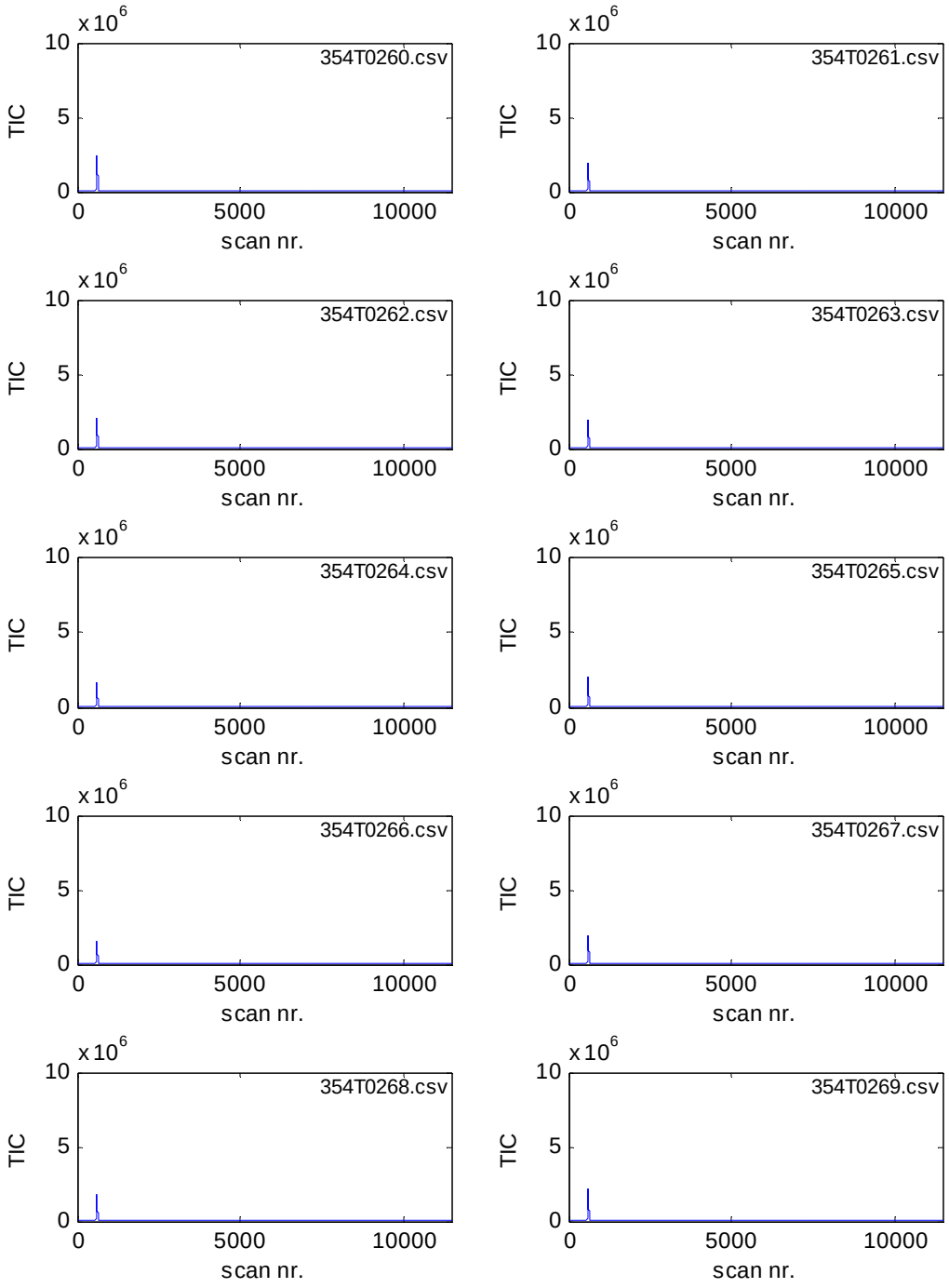

Figure 22: Botrytis experiment

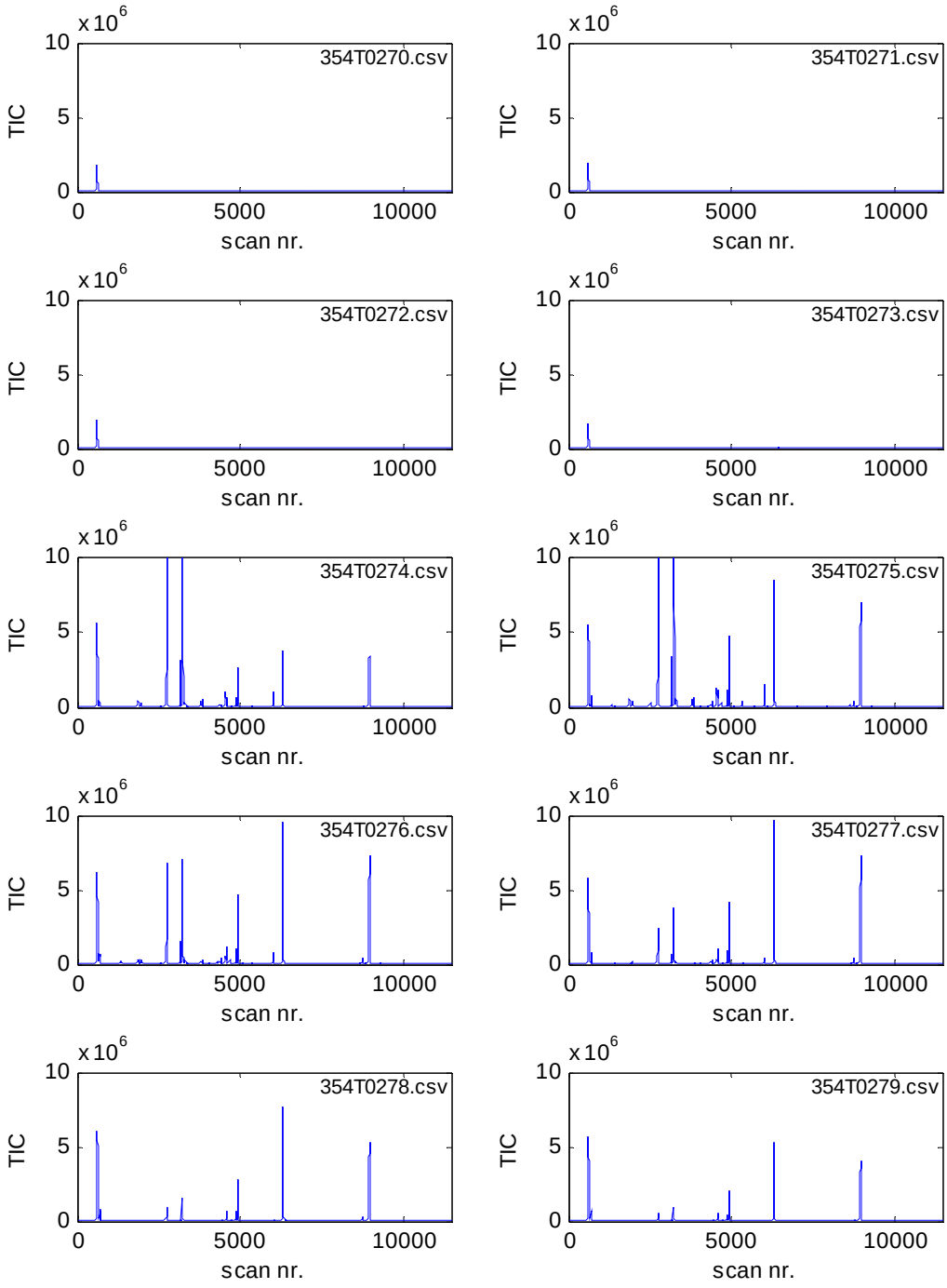

Figure 23: Botrytis experiment

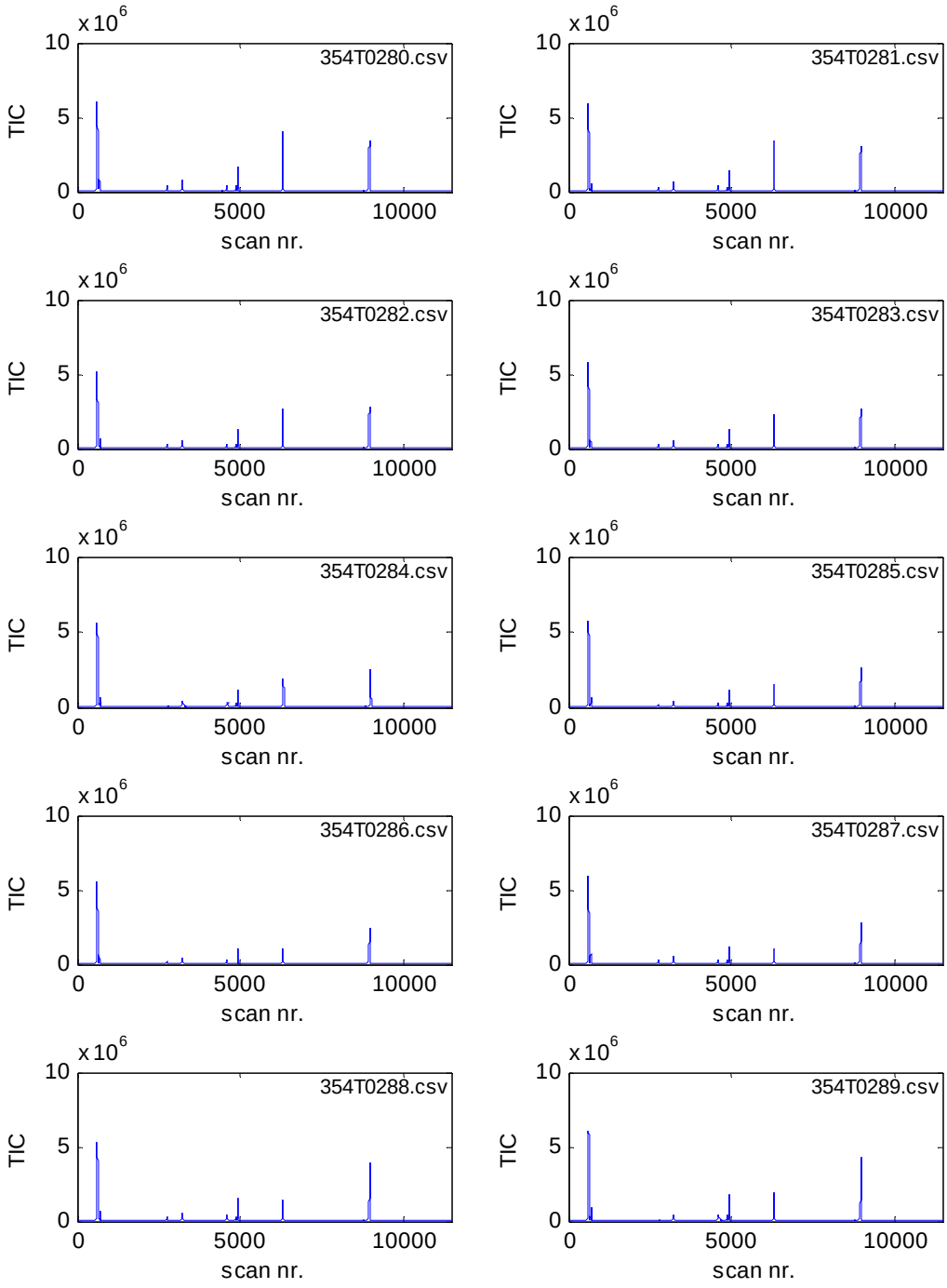

Figure 24: Botrytis experiment

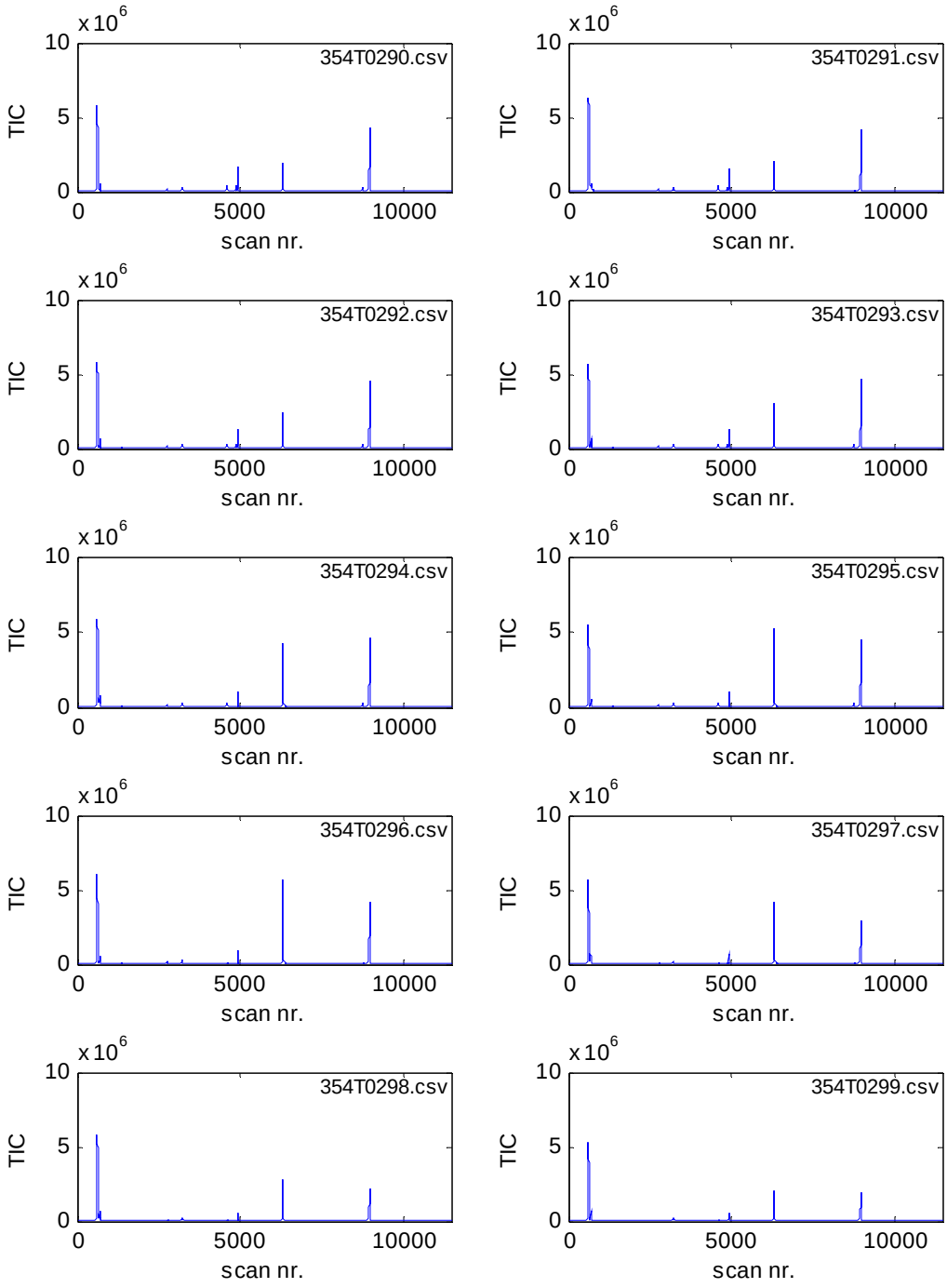

Figure 25: Botrytis experiment

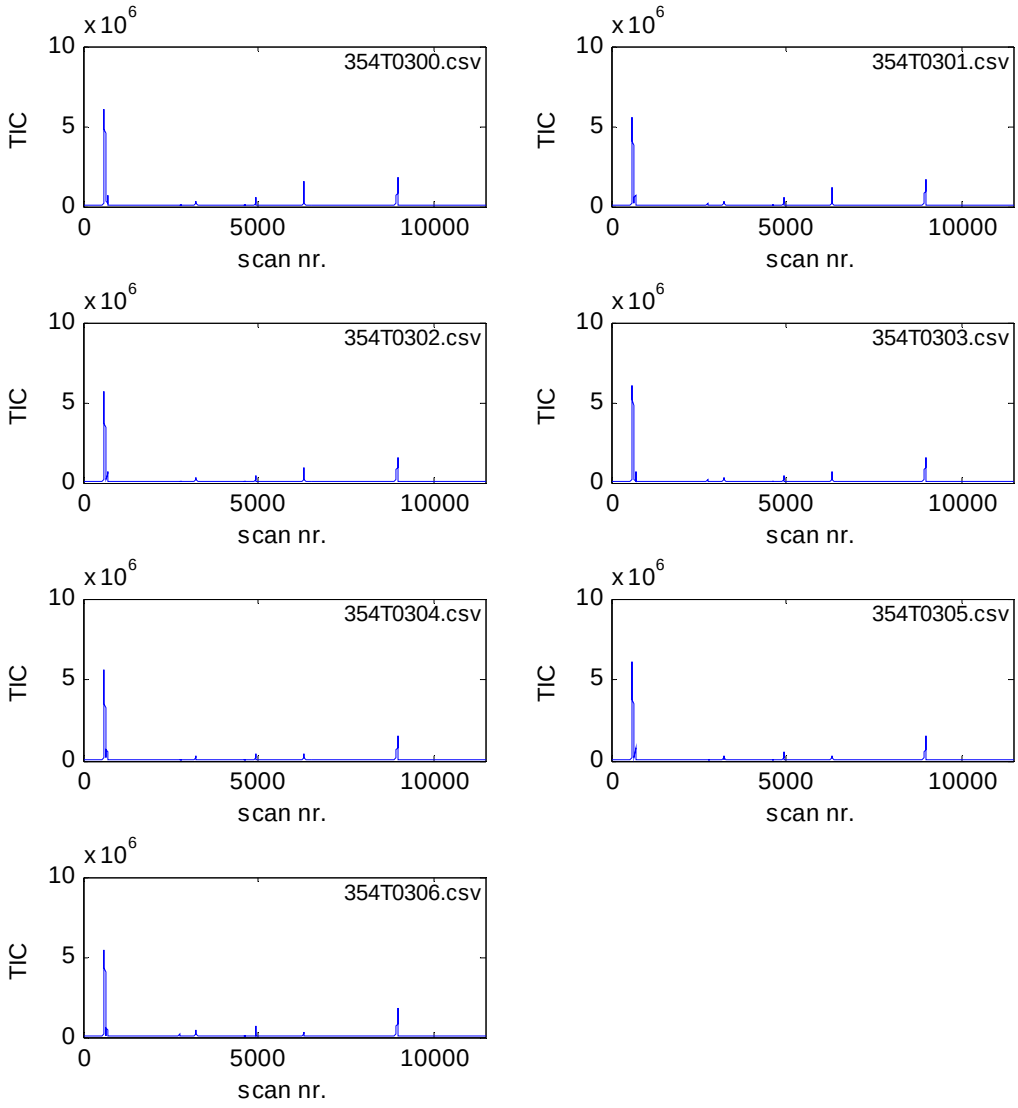

Figure 26: Botrytis experiment

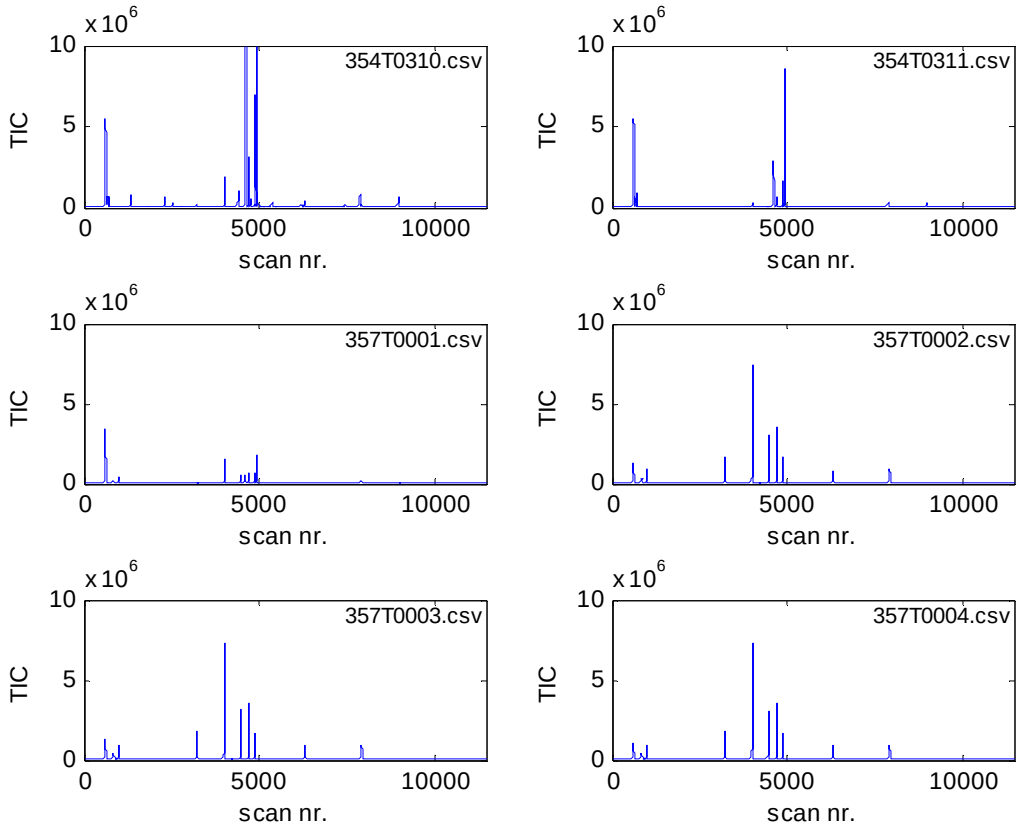

Figure 27: Botrytis experiment

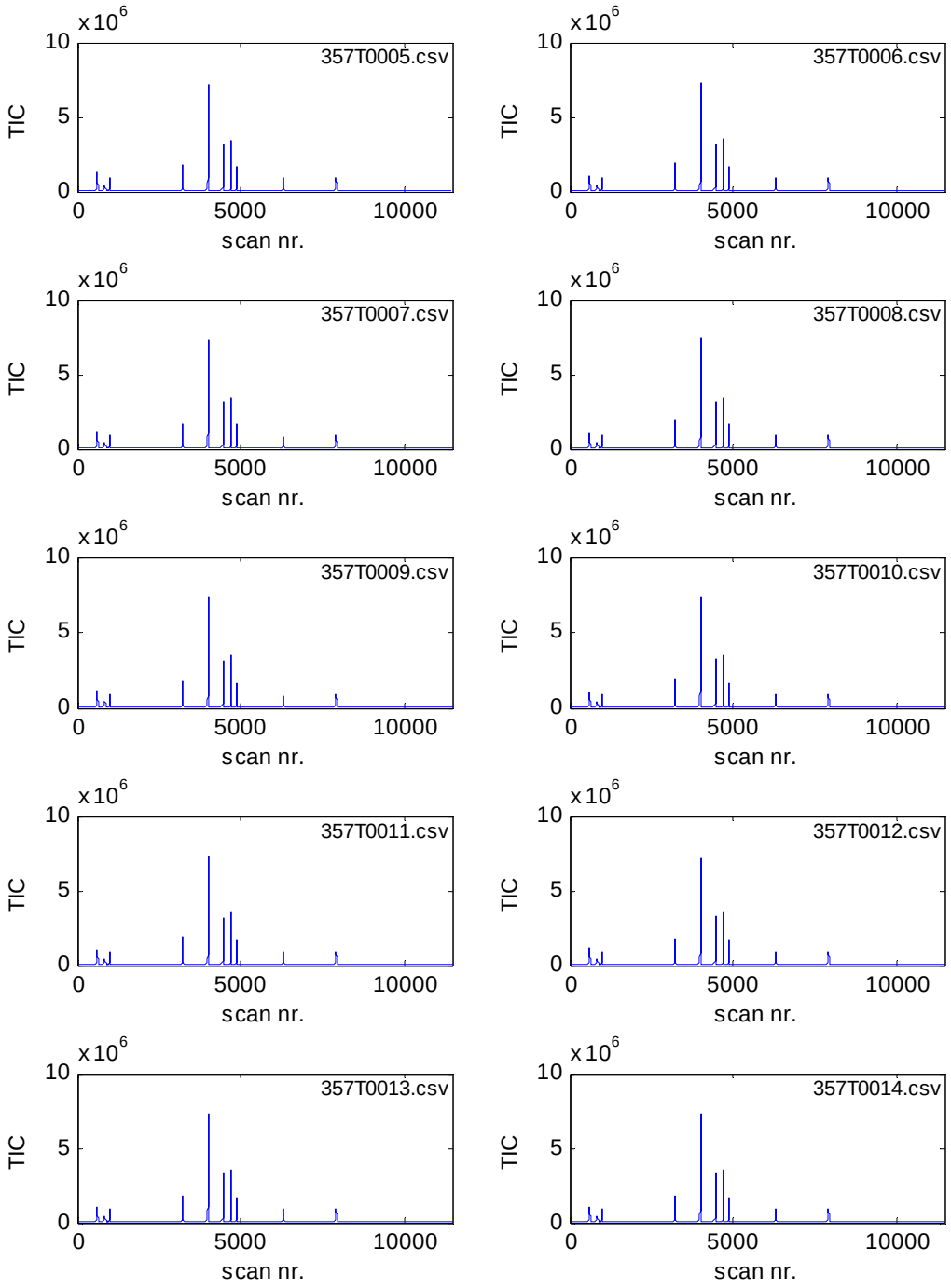

Figure 28: Botrytis experiment

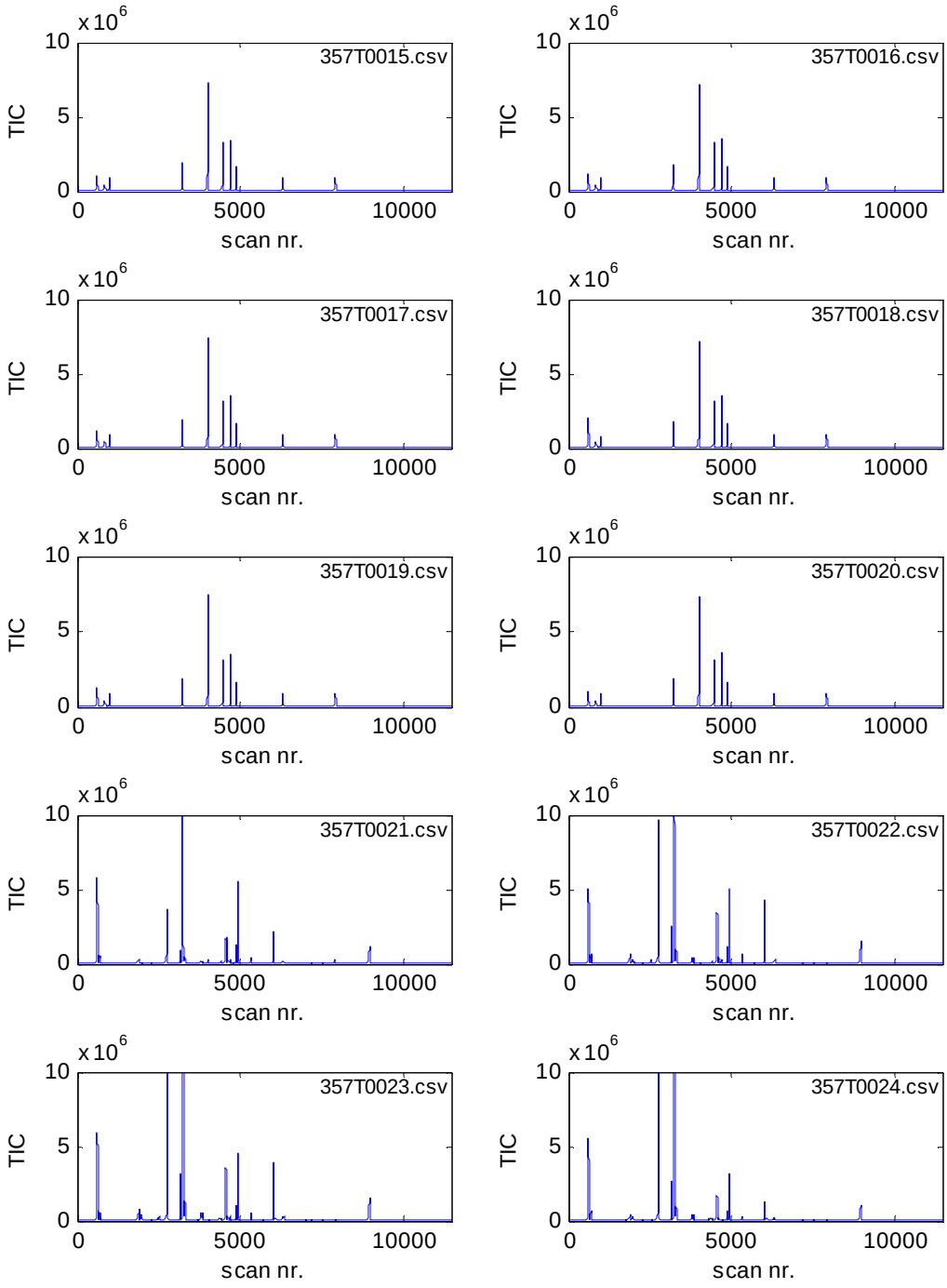

Figure 29: Botrytis experiment

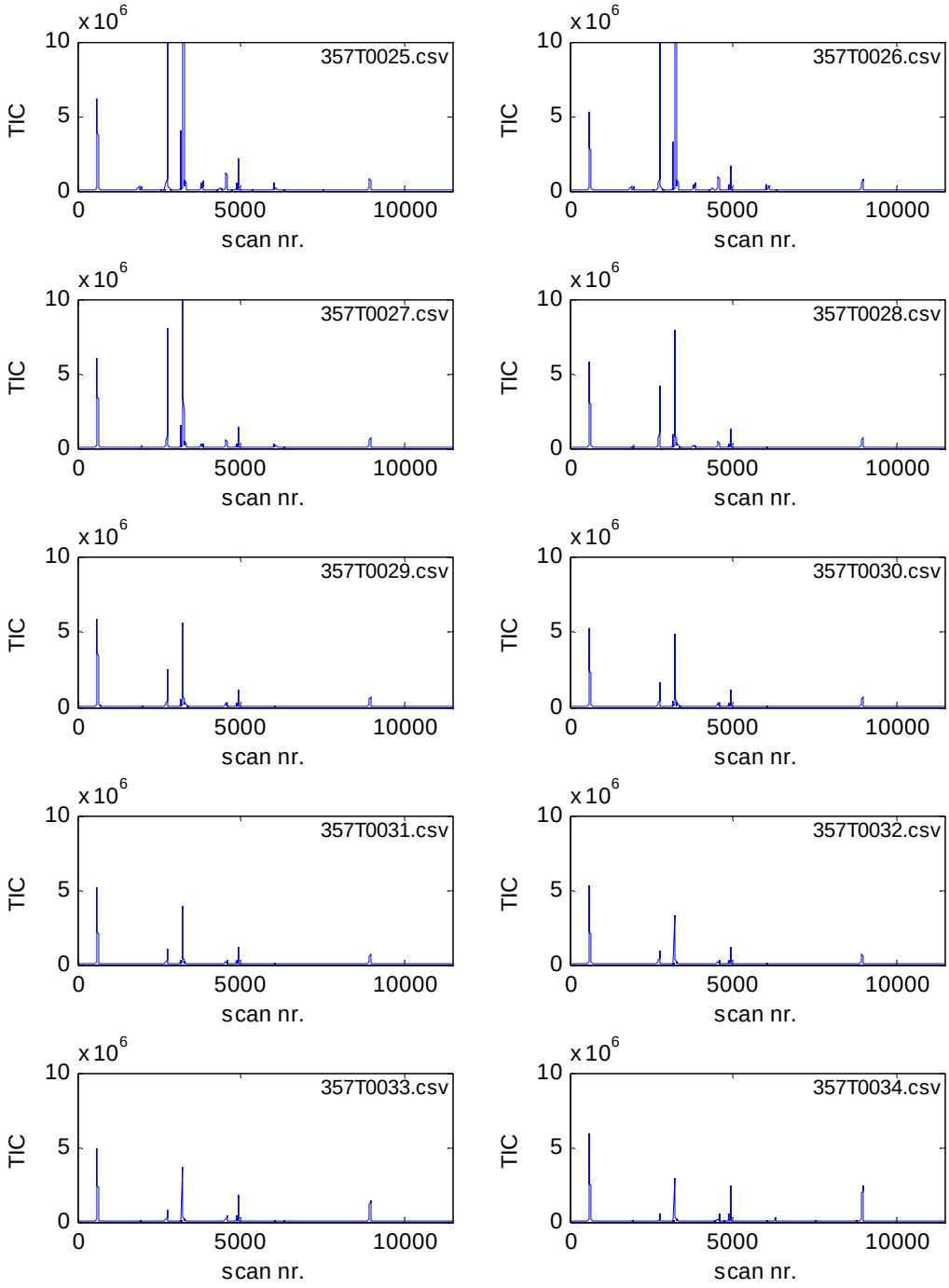

Figure 30: Botrytis experiment

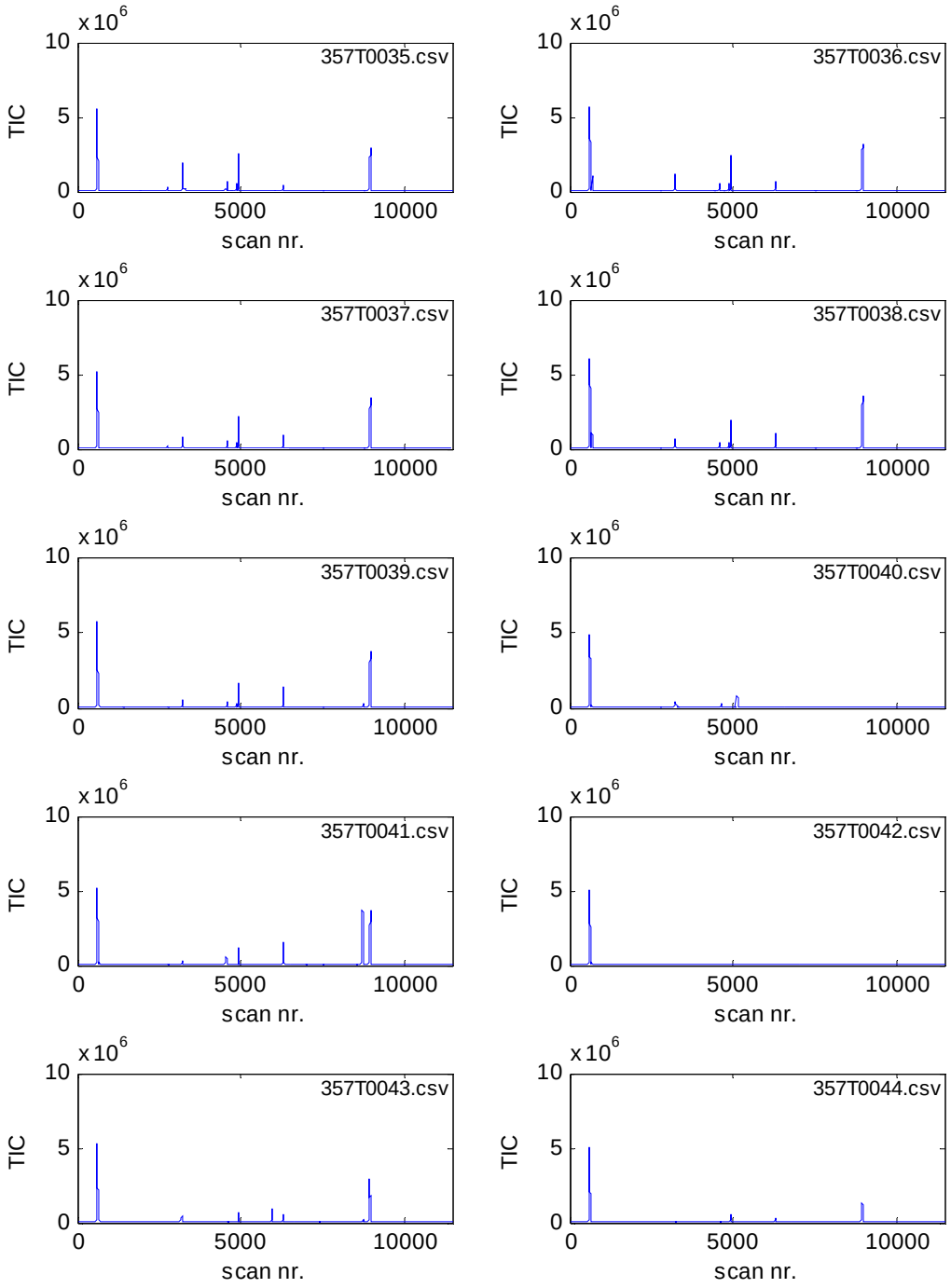

Figure 31: Botrytis experiment

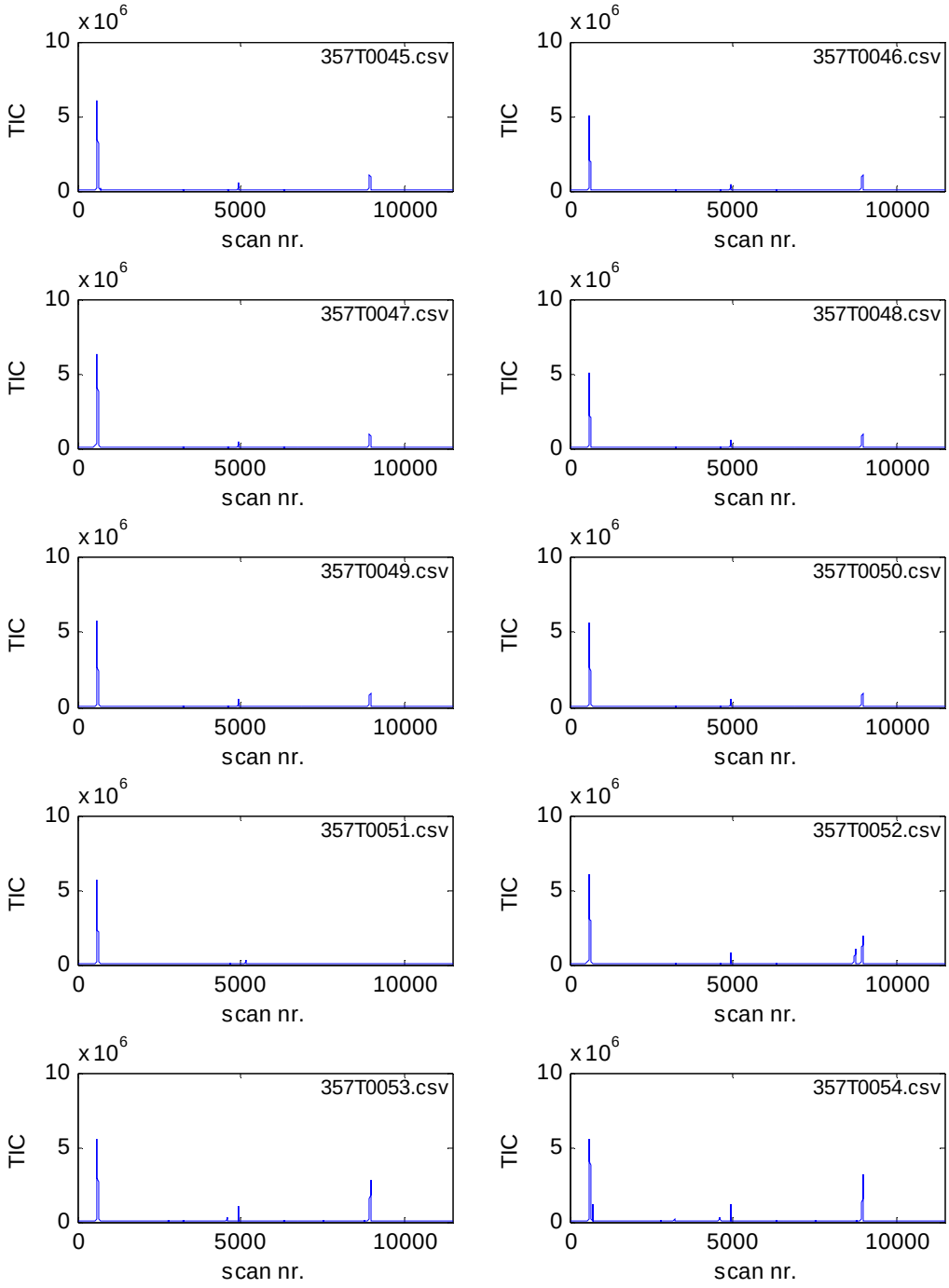

Figure 32: Botrytis experiment

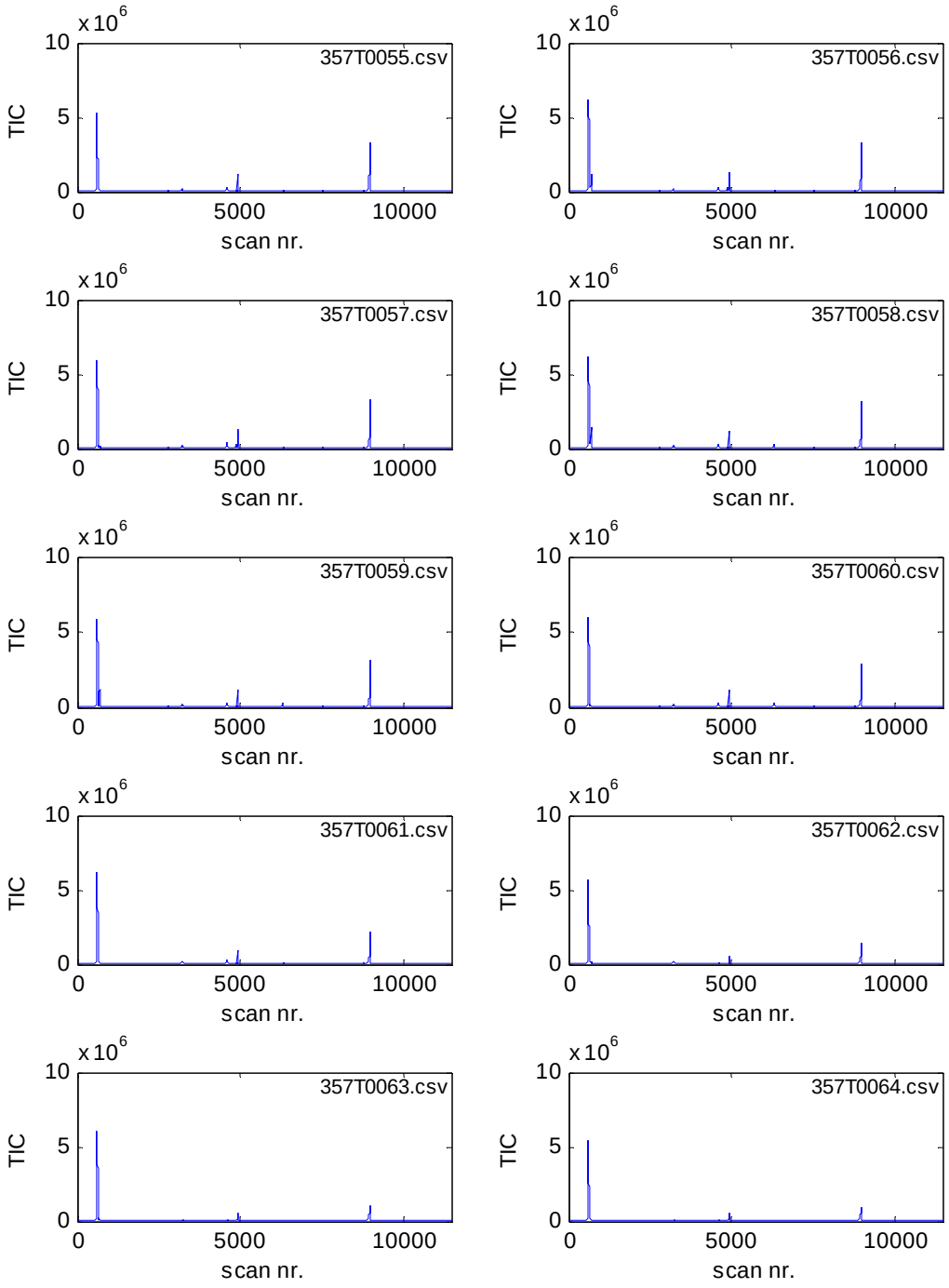

Figure 33: Botrytis experiment

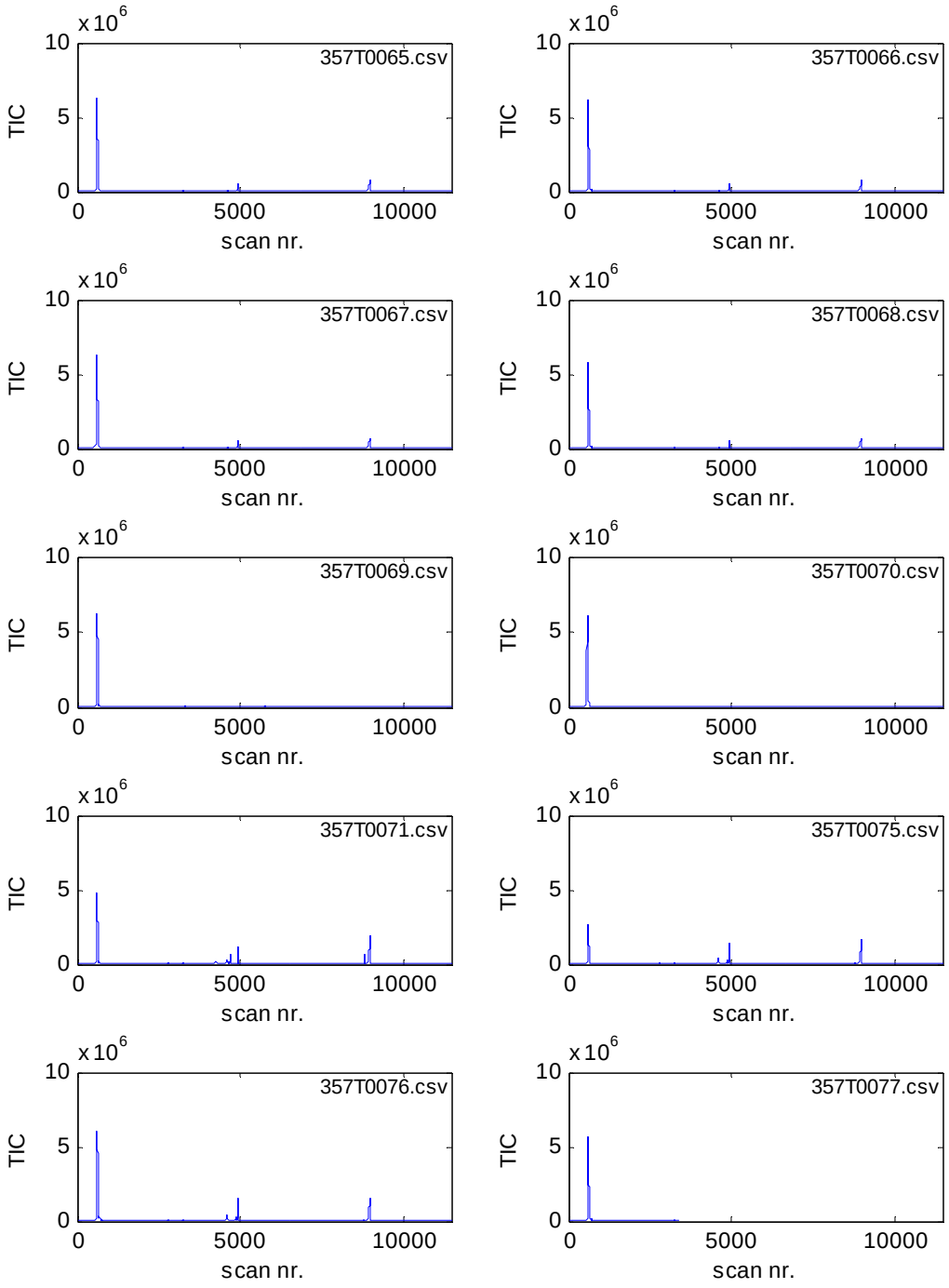

Figure 34: Botrytis inoculated (22-8-2006)

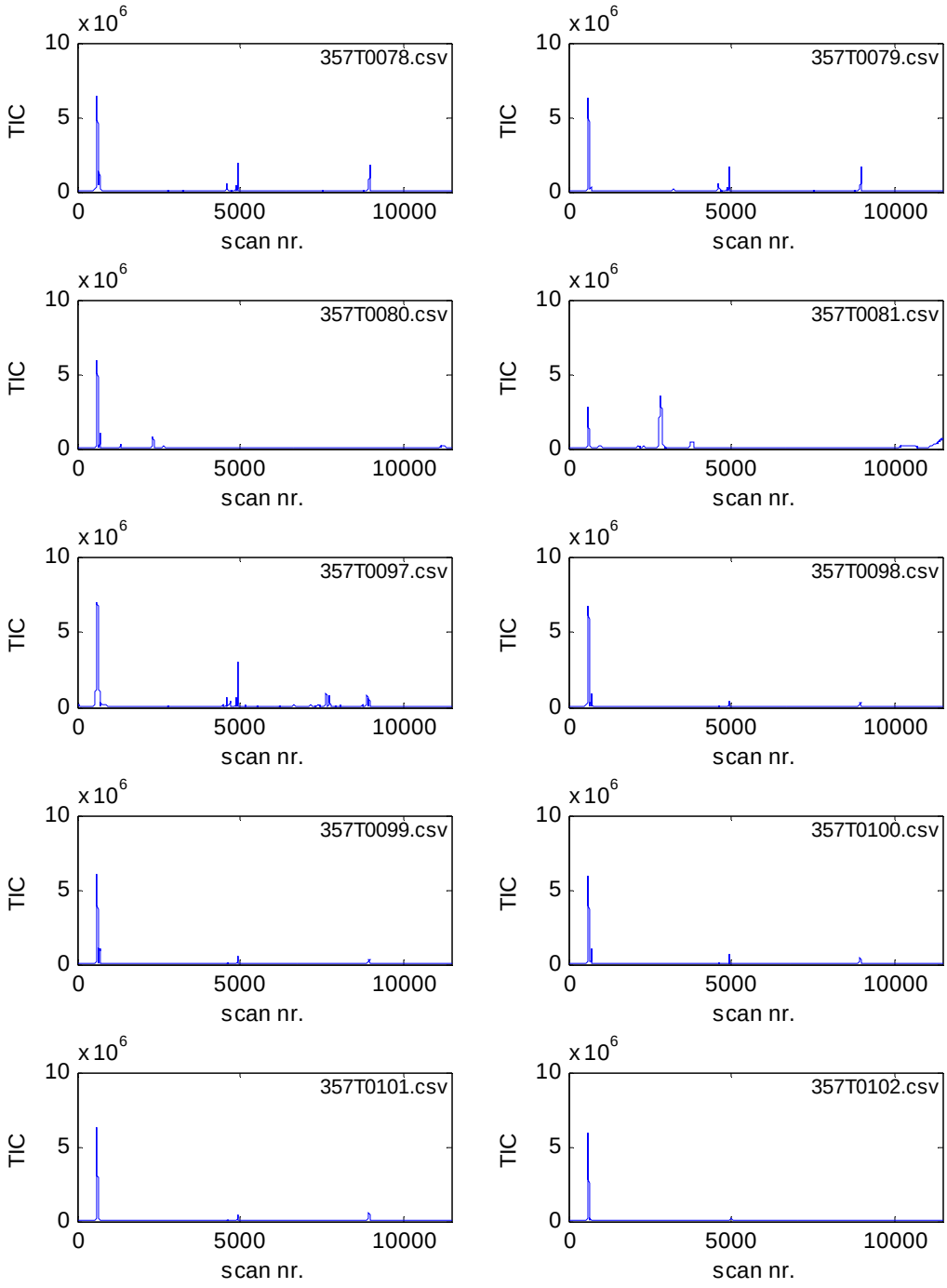

Figure 35: Control experiment (from 357T0097)

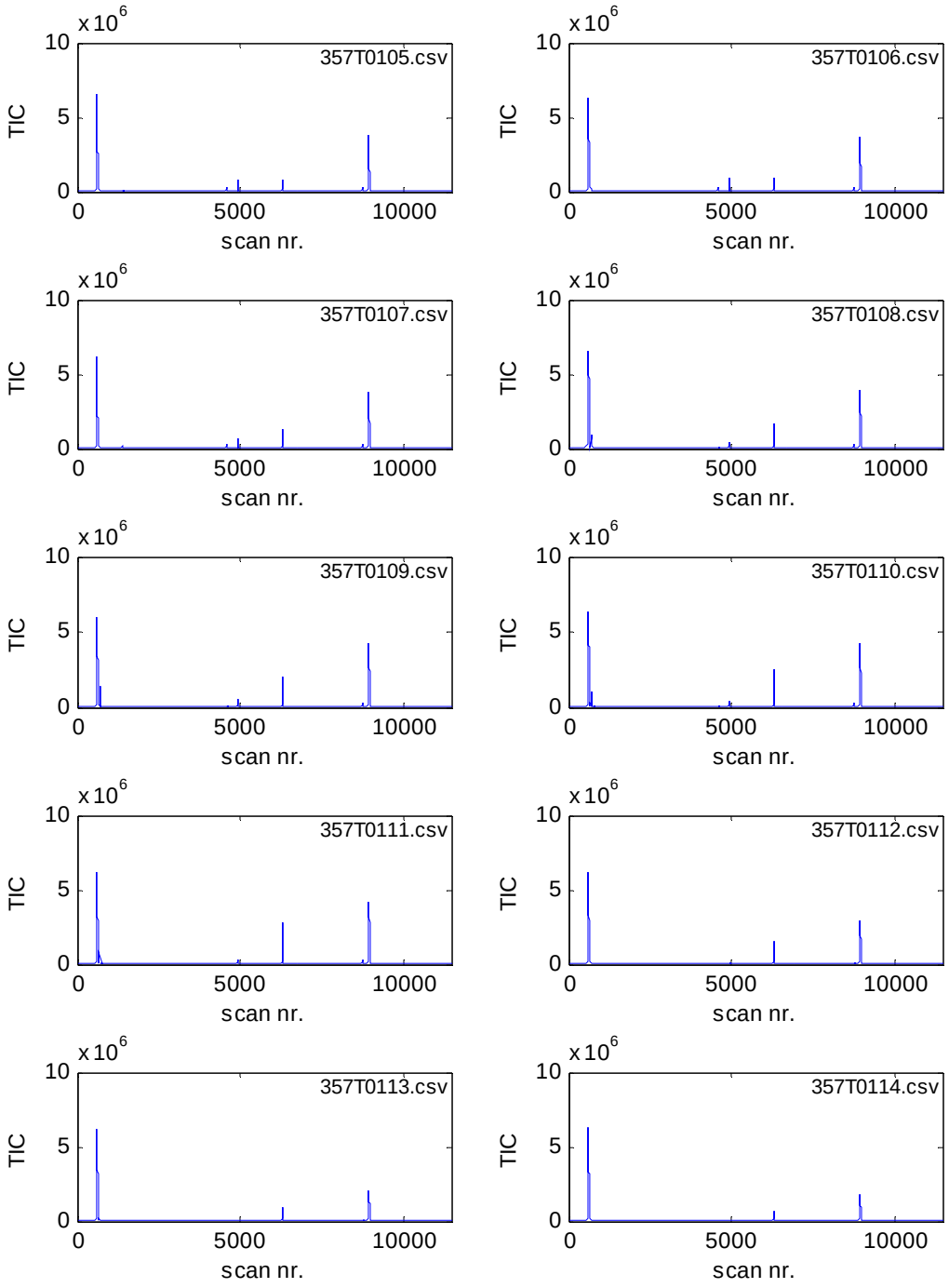

Figure 36: Control experiment

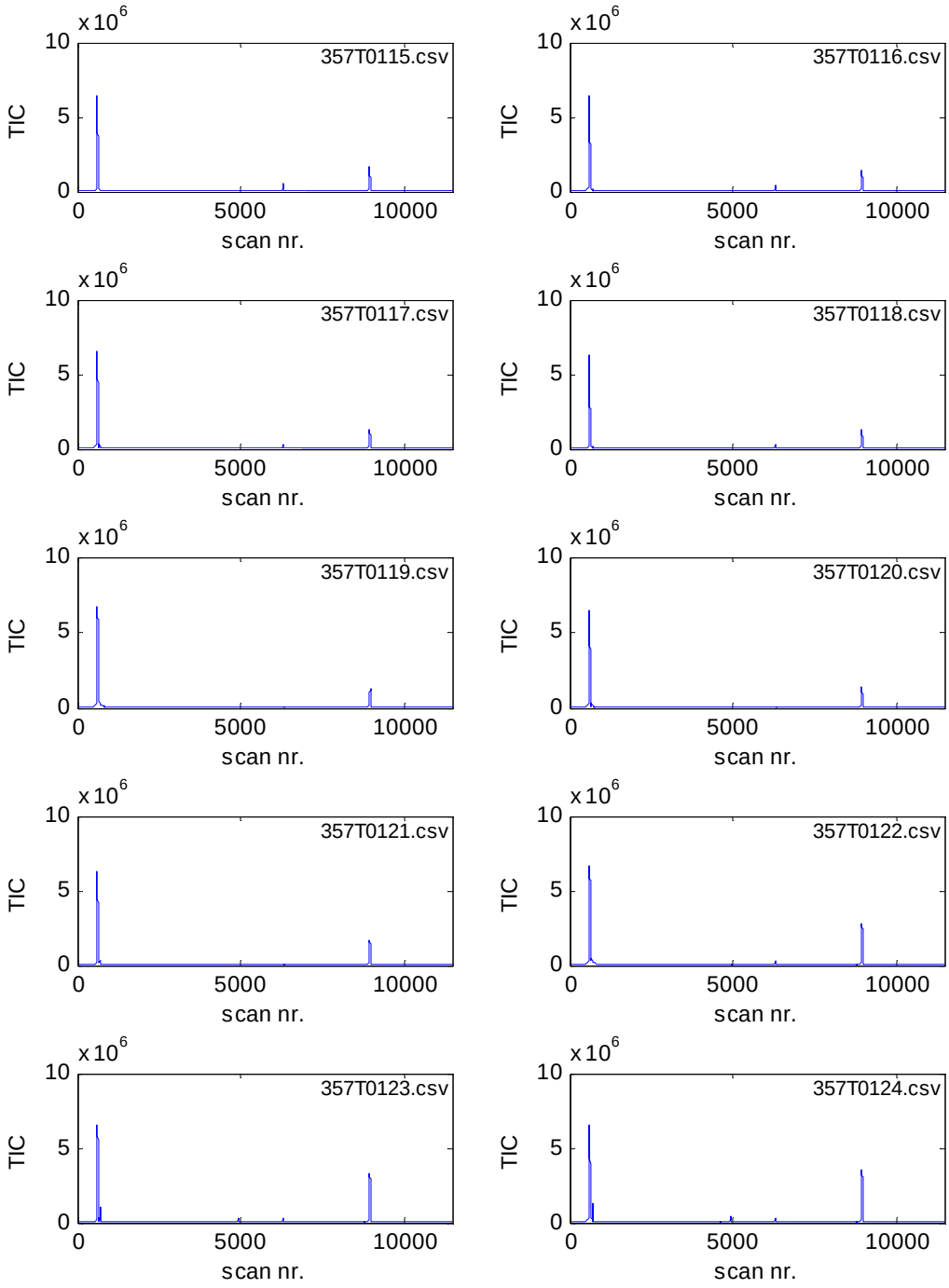

Figure 37: Control experiment 2

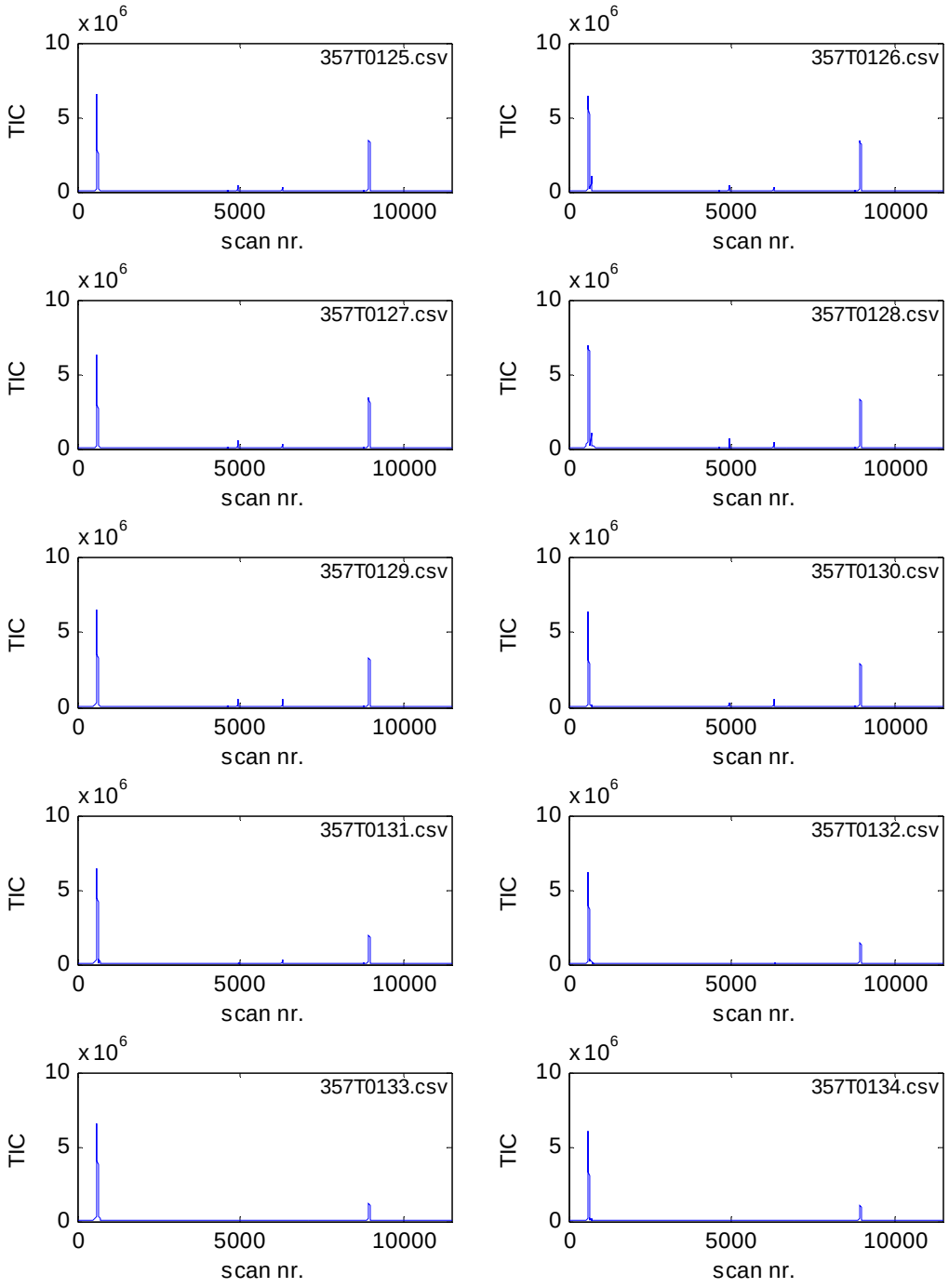

Figure 38: Control experiment

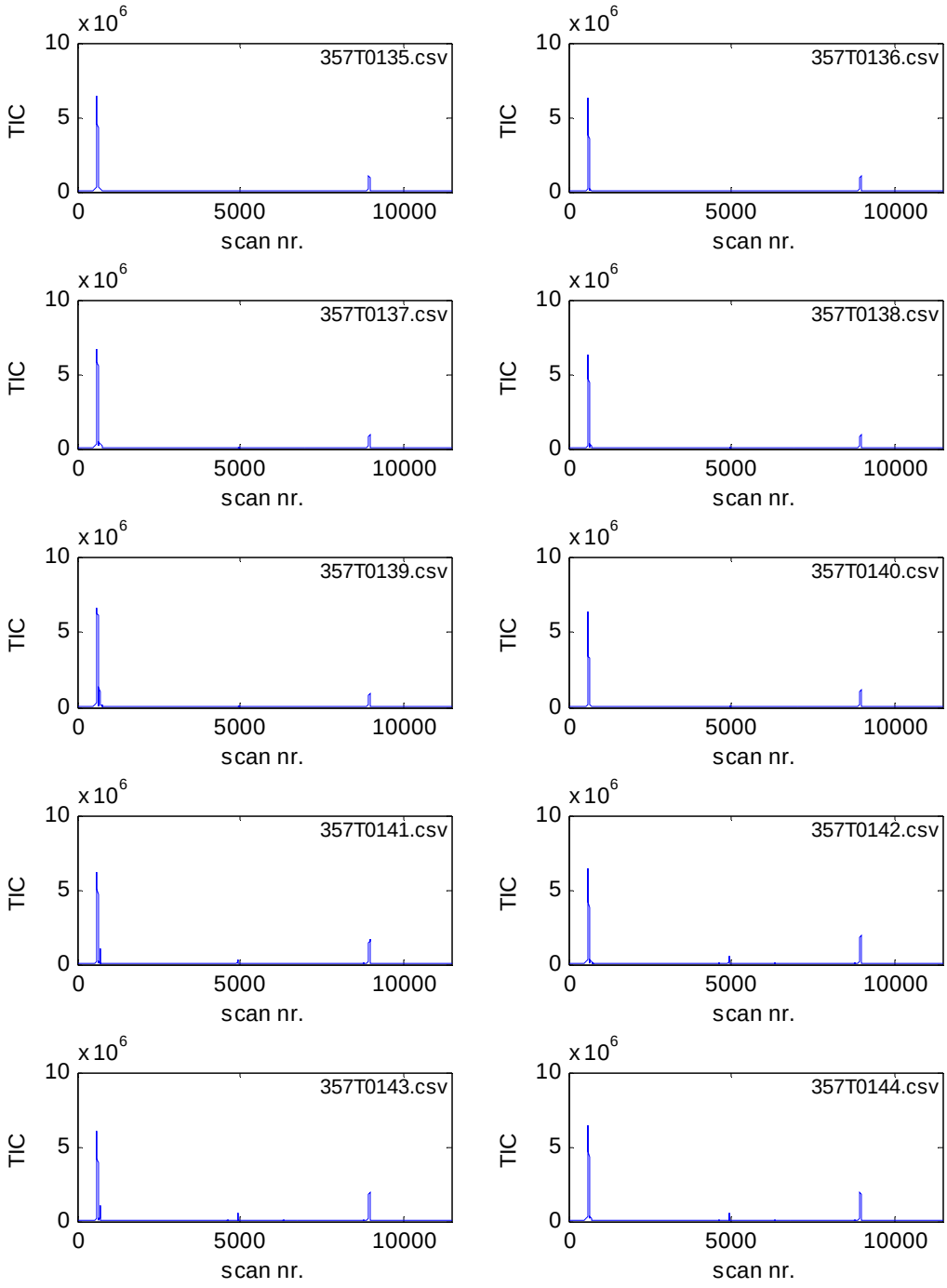

Figure 39: Control experiment

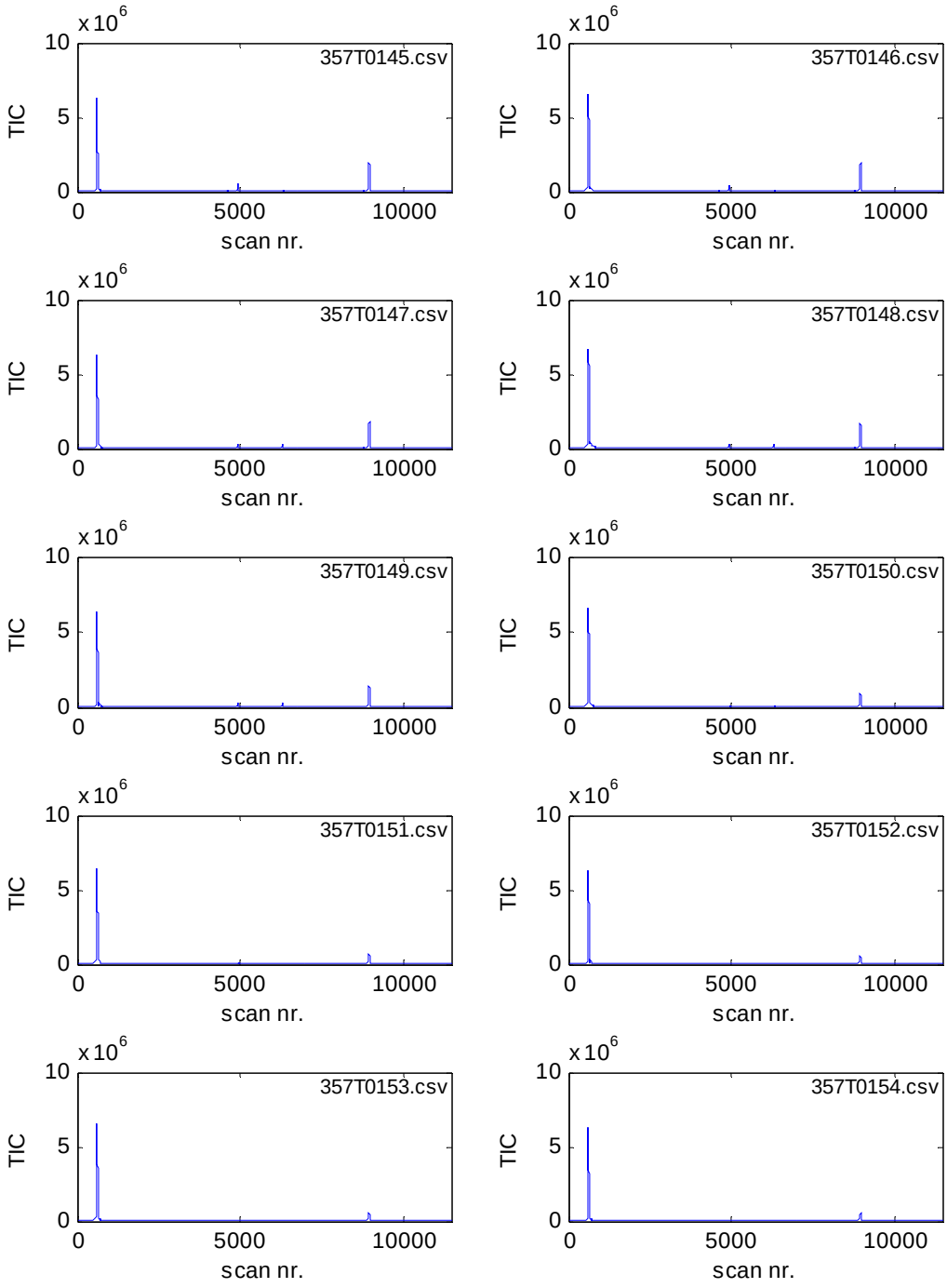

Figure 40: Control experiment

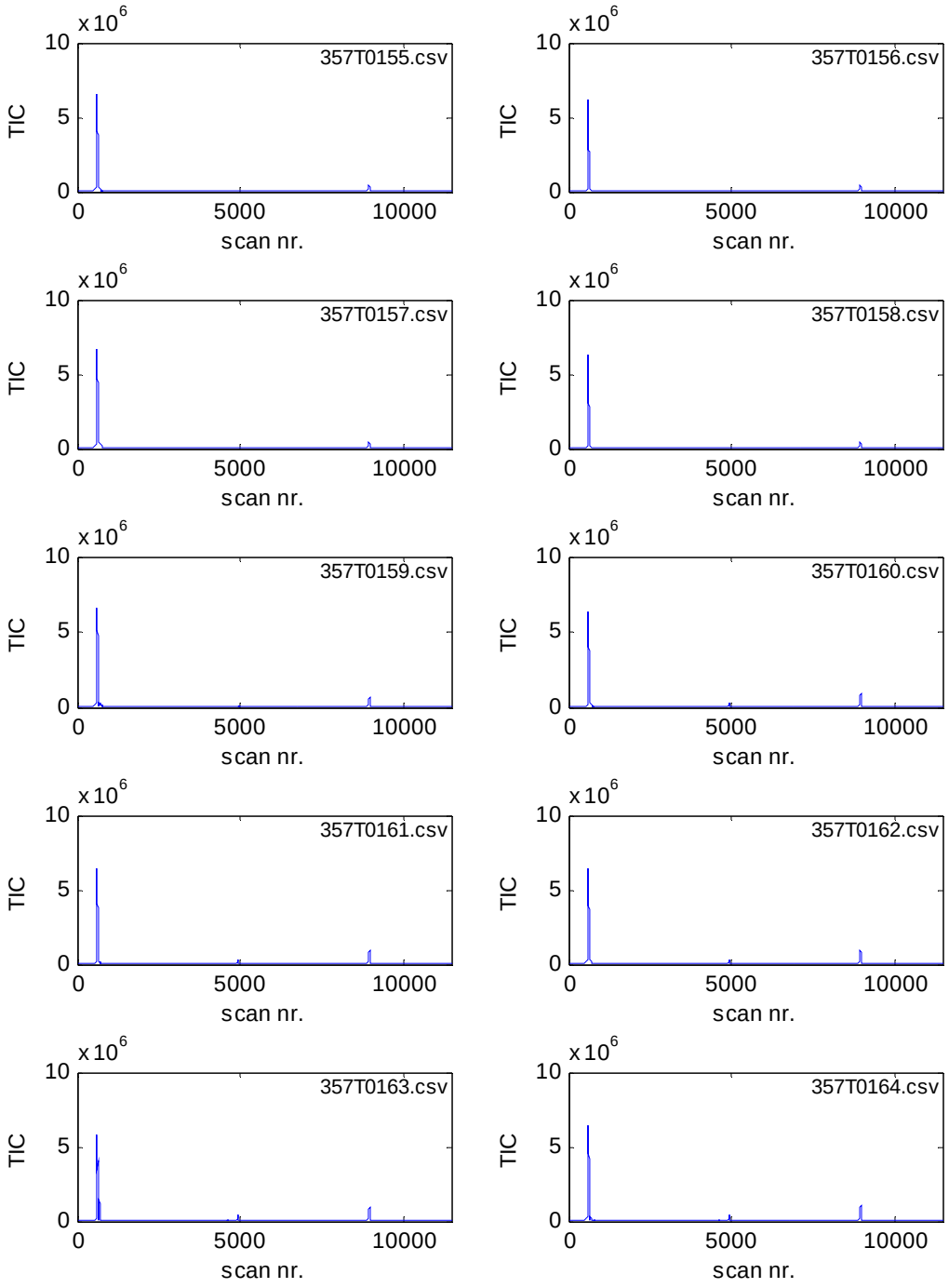

Figure 41: Control experiment

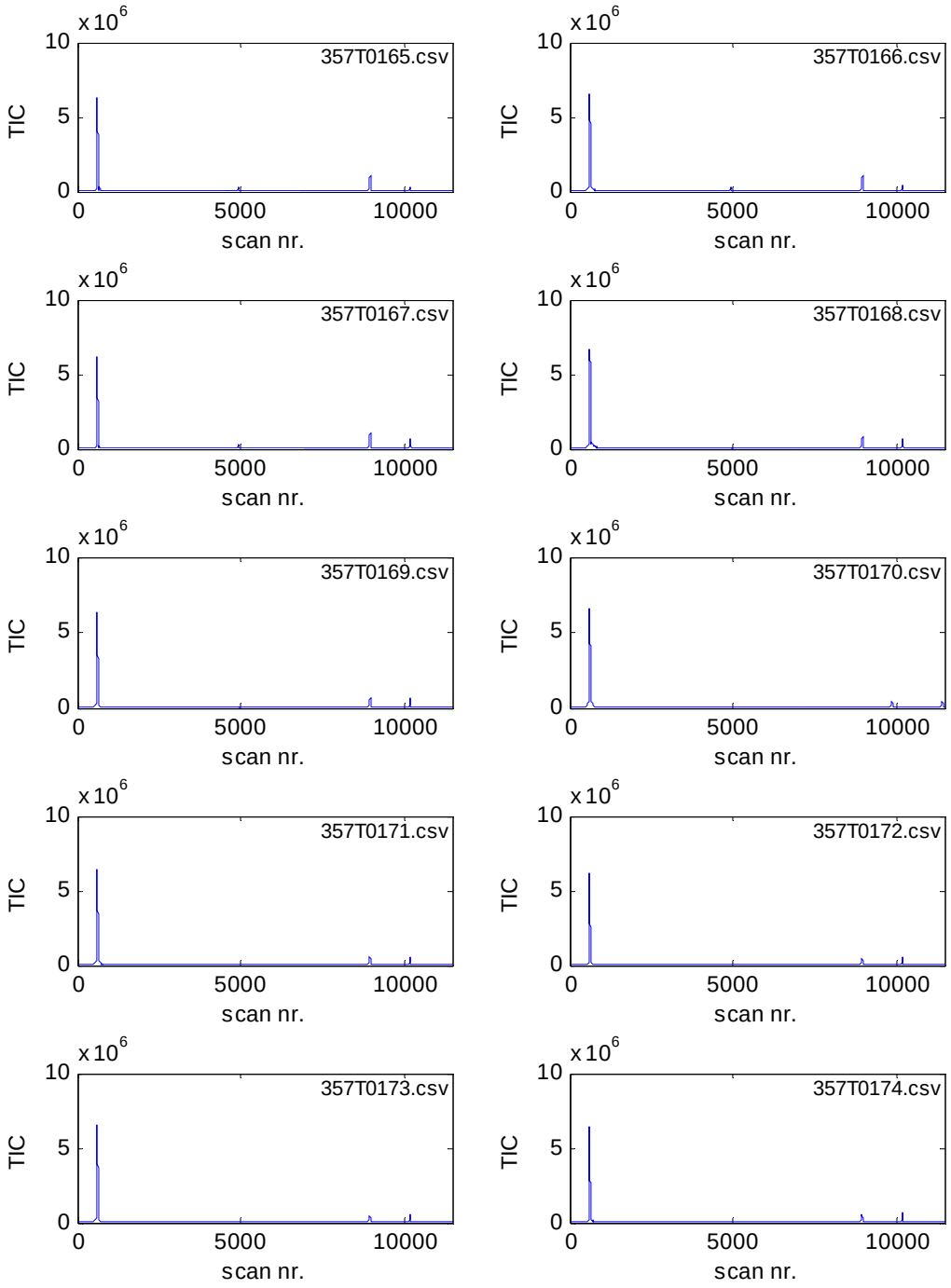

Figure 42: Control experiment

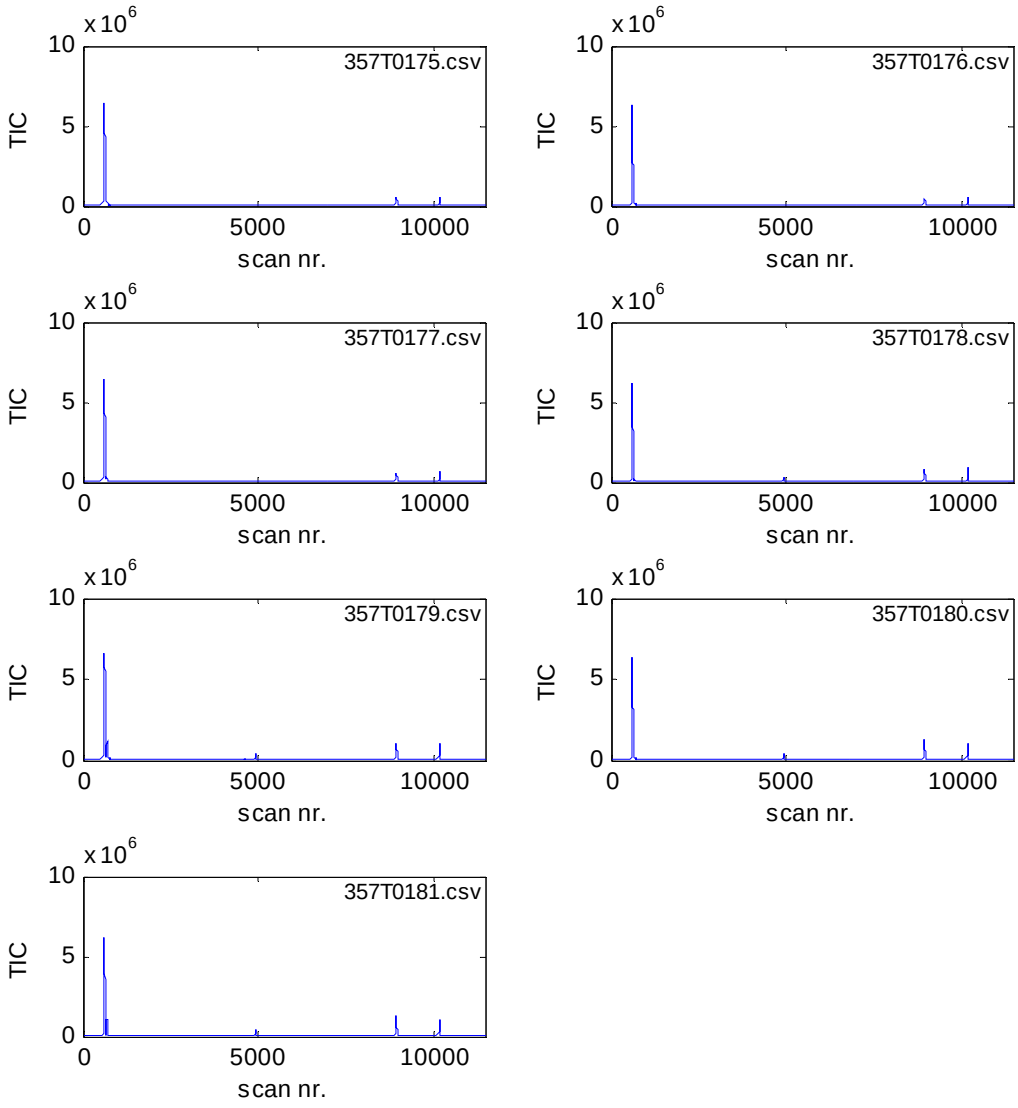

Figure 43: Control experiment

Appendix D: Climatic data

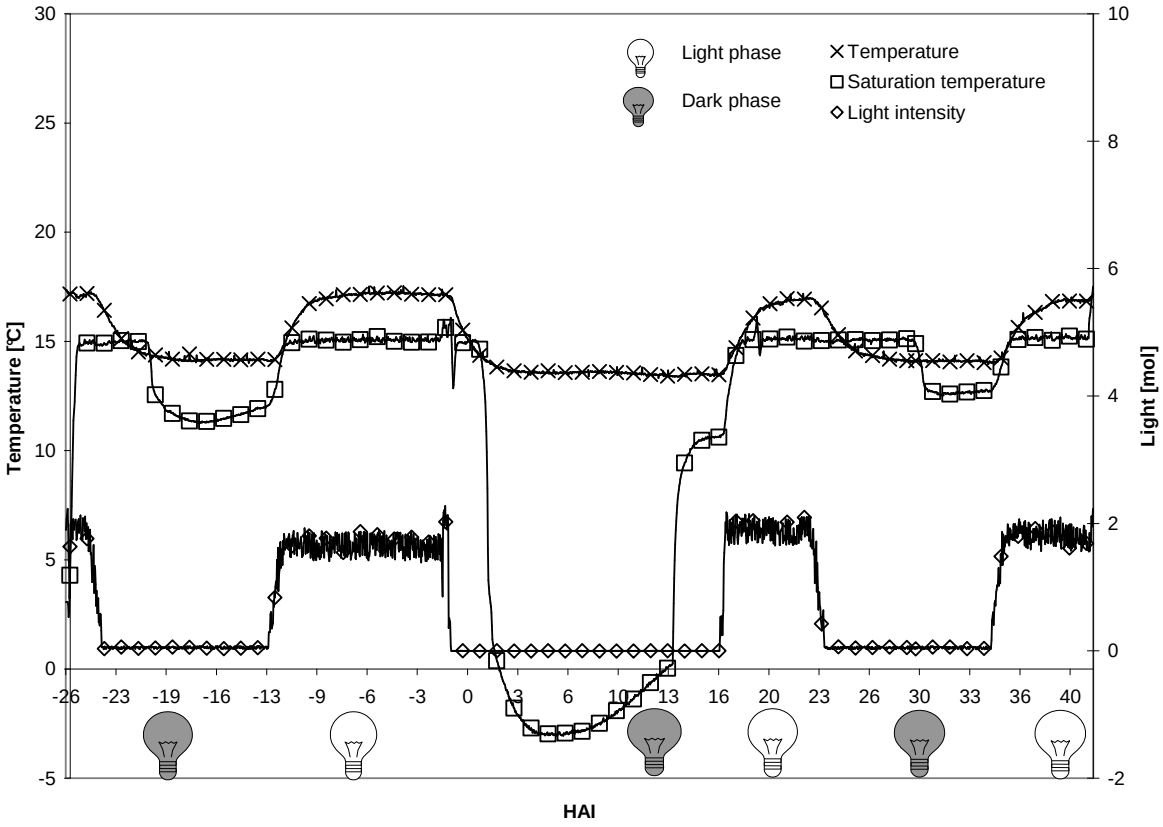

Figure 44: Period: 6.3.06 15:47 till 09.03.2006 11:47. -26 HAI: Plants put in. 0 HAI: Plants removed, inoculated with botrytis and introduced into the chamber again. 41 HAI: plants touched at the hairs of the stem. 41.5 HAI Plants removed.

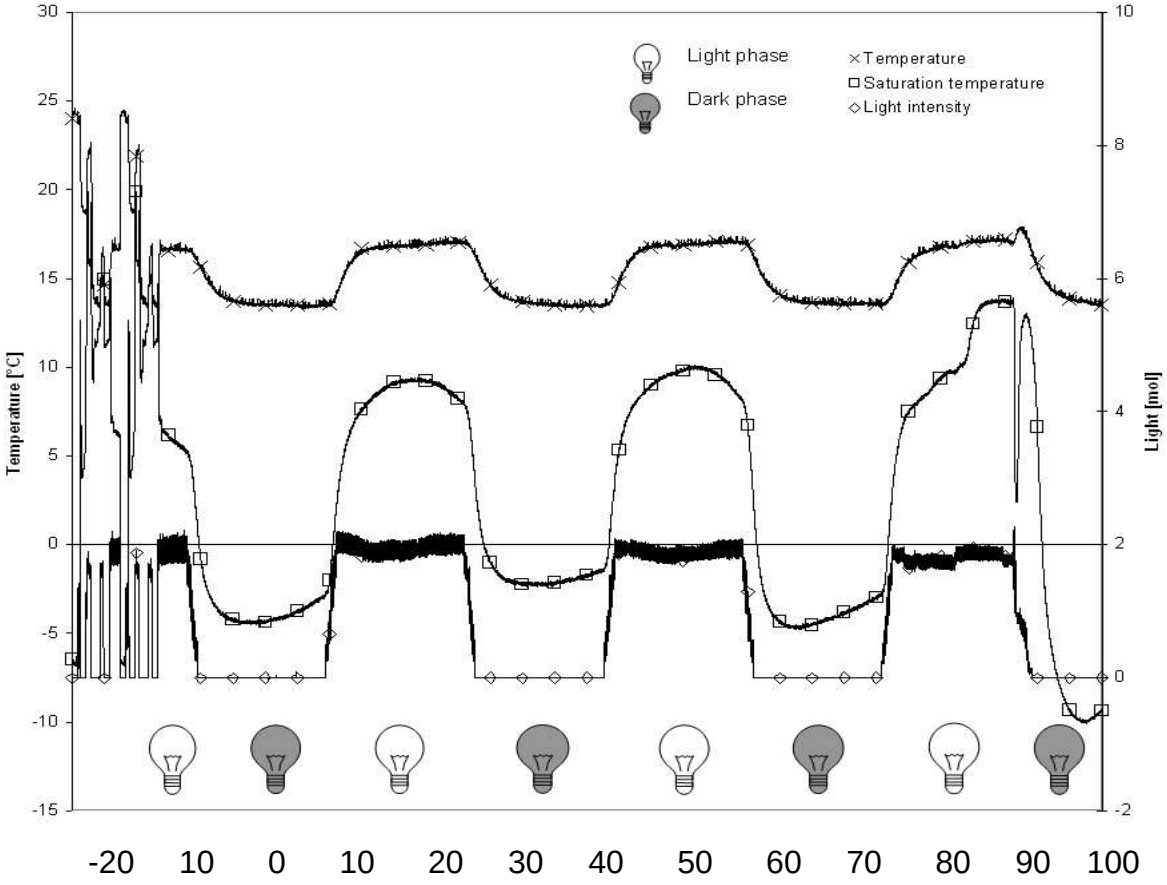

Figure 45: Period: 14-03-2006 16.00 till 20-03-2006 Waterspraying and ozone experiment. -21 HAO: Spraying with water 0 HAO: Ozone on, 1 HAO: Ozone of, 100 HAO: plants removed

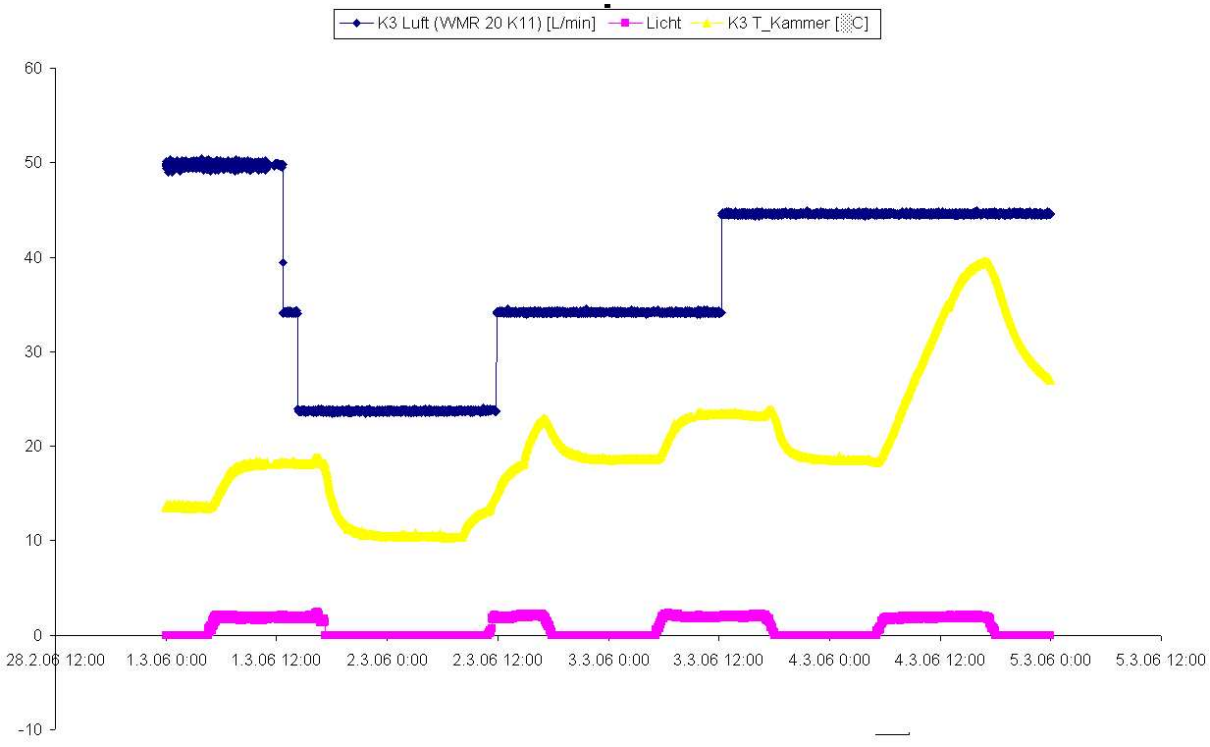

Figure 46: 1st experiment (28-2-06 till 04-3-06) (05-03-06 -> temperature too high!)

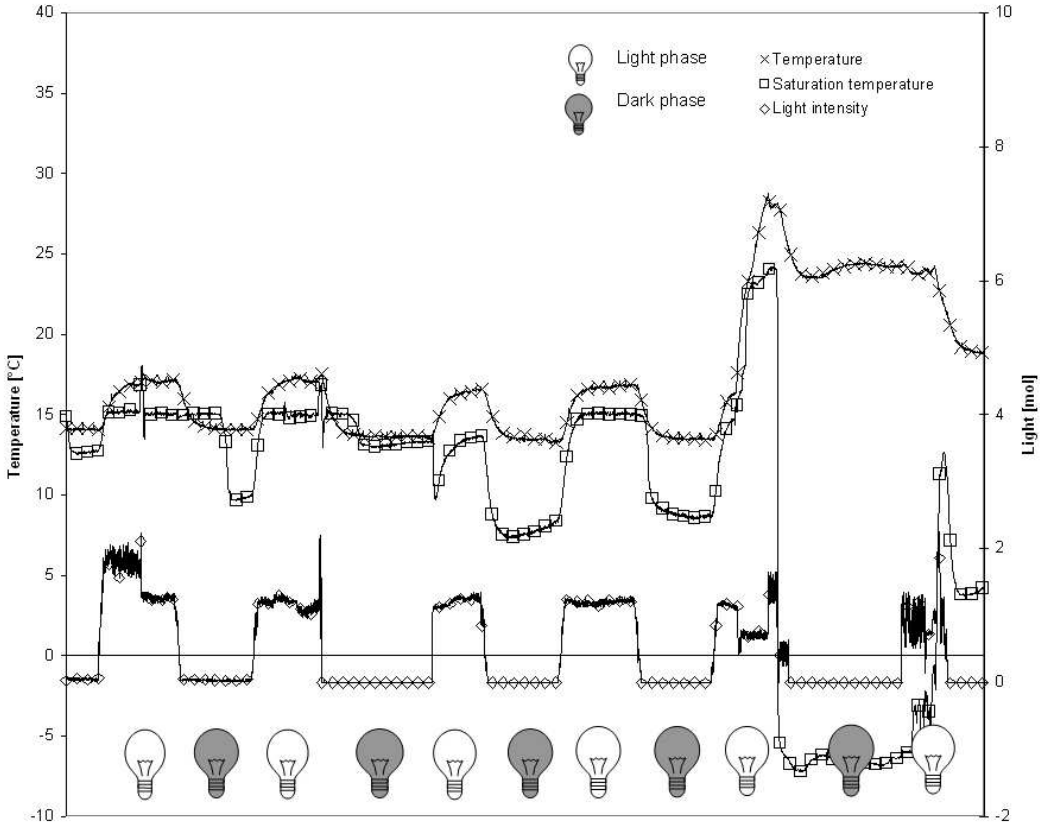

Figure 47: 2nd experiment Period: 09.03.2006 00:01 till 14.03.2006 23:59.

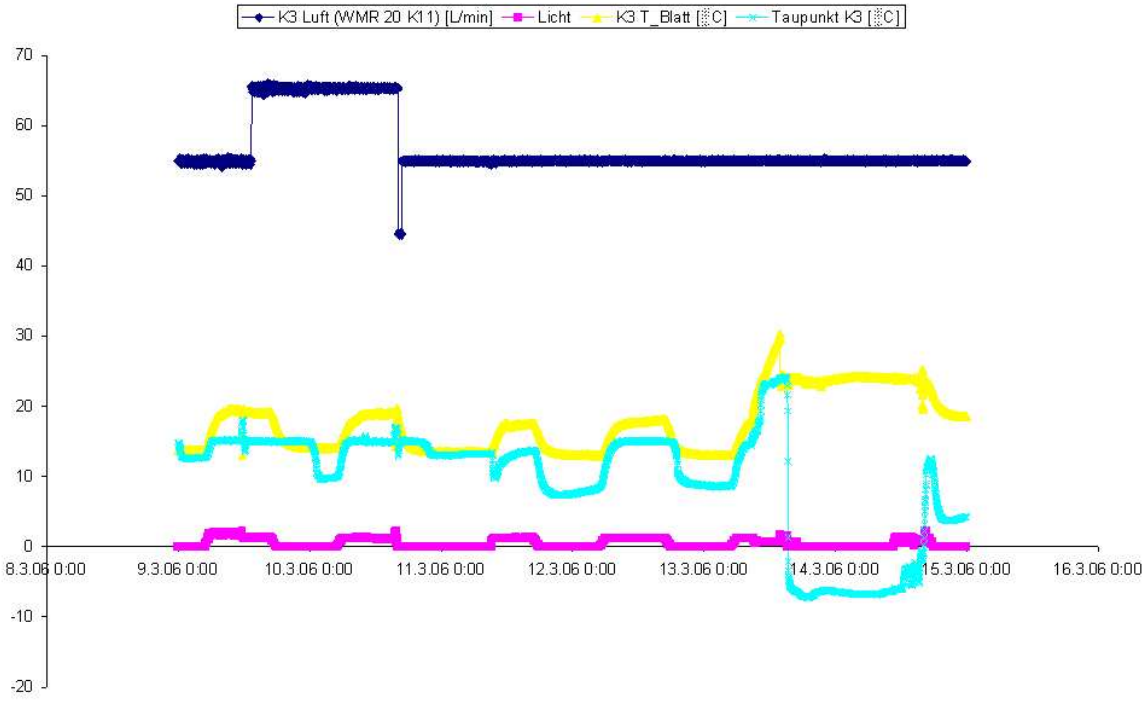

Figure 48: 2nd experiment Period: 09.03.2006 00:01 till 14.03.2006 23:59

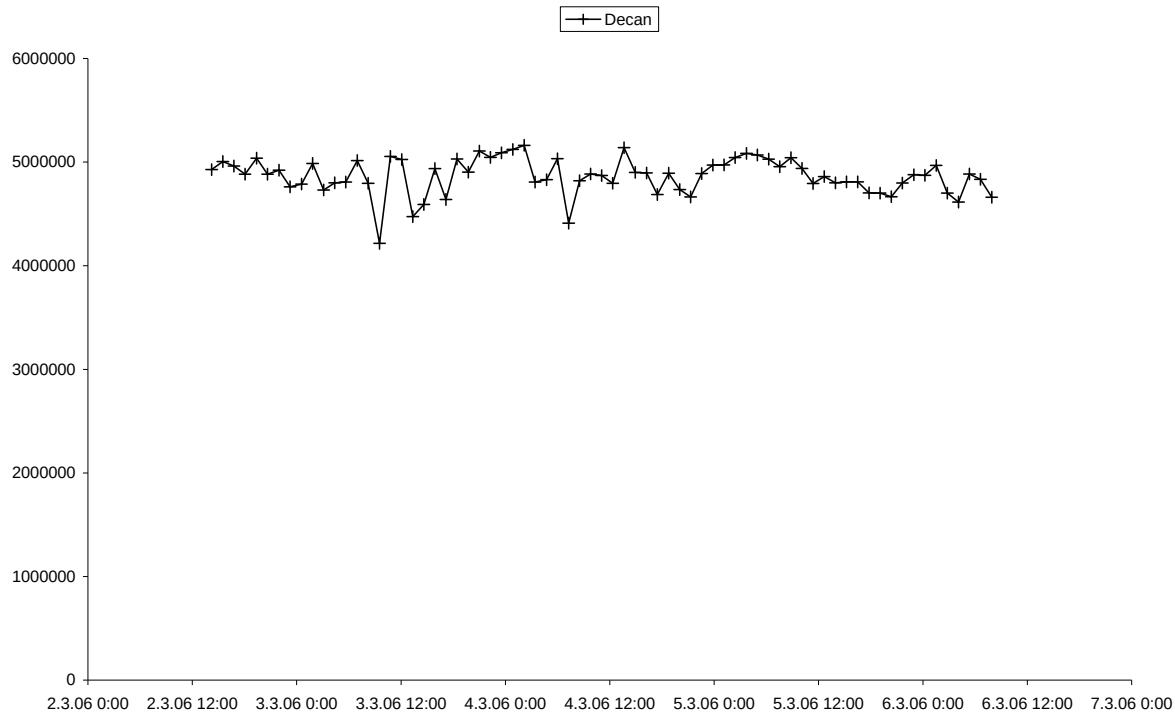

Figure 49: Stability of the system -> Decane in an internal standard