

Naturally produced ethylene as a possibility to cause  
suicidal germination of hemi-parasitic *Striga*  
*hermonthica* seeds

*a case study with soil from Cameroon*

**Msc. Thesis Theoretical Production Ecology (F300-702)**

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### 3. organic matter

Evolution of  $C_2H_4$  from soils under anaerobic conditions was stimulated by amendment with cereal straw (Lynch and Harper, 1980; Goodlass and Smith, 1978). Peat and farmyard manure also resulted in enhanced  $C_2H_4$  evolution (Goodlass and Smith, 1978). Hay had no effect for ethylene production (Goodlass and Smith, 1978), though others reported enhanced ethylene production when soils were amended with hay (Babiker and Pepper, 1984). Humus does not provide a favourable substrate for microbial growth and consequently does not affect ethylene production (Lynch and Harper, 1980). Availability of the substrate is an important factor for ethylene production in soils. The nature and quantity of crop residues may significantly affect the levels of ethylene evolved under any given set of physical conditions (Smith and Dowdell, 1974). Lynch and Harper (1980) reported that anaerobic conditions were much more favourable for ethylene production when wheat and barley straw were incorporated in the soil than aerobic conditions.

Compounds that have been shown to stimulate ethylene production when added to soils include low molecular weight aliphatic organic acids, amino acids, phenolic acids, glucose and vitamins (Arshad and Frankenberger, 1990). Methionine, in combination with glucose as an energy source, greatly stimulated ethylene production (Lynch and Harper, 1980). The C/N ratio of organic material also influences the production of ethylene. More ethylene was produced per gram of added carbon with non-composted litter (C/N 12.6) than with composted litter (C/N 9.8) (Tang and Miller, 1993).

Ethylene production is enhanced by a high organic matter content under anaerobic conditions (Goodlass and Smith 1978) or under both anaerobic and aerobic conditions (Van Cleempot *et al.*, 1983; Tang and Miller, 1993). On the contrary Arshad and Frankenberger (1990) found no relationship between ethylene evolution and soil organic matter content.

### 4 temperature

Temperature is among the factors that determine concentration of ethylene in the soil, because of stimulation of growth conditions of the organisms producing ethylene (Smith and Dowdell, 1974).

### 5 pH

Acidic soils absorb less ethylene than basic soils (Witt and Weber, 1975). Goodlass and Smith (1978) have shown that the quantities of  $C_2H_4$  evolved under anaerobic conditions may be affected by pH.

### 6 redox potential

Smith and Restall (1971) found that all factors promoting reducing conditions, stimulate hydrocarbon production. Arshad and Frankenberger (1990) found that addition of oxidizers inhibited ethylene production. In the soil  $NO_3^-$  may be an important terminal electron acceptor for certain facultative anaerobic bacteria in the absence of  $O_2$  and can poised the redox potential sufficiently to prevent activity of strict anaerobes. Smith (1976) stated that there are other redox systems in soils, including the ferric–ferrous and manganic–manganous couples, which operate over the redox range. However, compared with  $NO_3^-$ , they will have little effect because they are insoluble in the oxidised form.

When soils were amended with  $NO_3^-$  at levels of 20–200  $\mu g\ g^{-1}$ ,  $C_2H_4$  was only produced after all  $NO_3^-$  was denitrified, while Van Cleempot *et al.* (1983) reported that the depressing effect on ethylene production was only pronounced at  $NO_3^-$  levels higher than 300  $\mu g\ g^{-1}$ . Other researchers reported that there was never a complete suppression of  $C_2H_4$  production even at levels up to 10,000  $\mu g\ NO_3^- g^{-1}$  soil (Goodlass and Smith, 1978; Smith and Restall, 1971).

## 7. Soil management

Bare, cultivated soil always produces less C<sub>2</sub>H<sub>4</sub> than if under crop, which, in turn, produces less than if the soil was uncultivated (Smith, 1976). Practices that increase moisture content or impede aeration as a result of smearing, can be expected to increase the incidence of anaerobic conditions and the levels of ethylene (Smith and Dowdell, 1974).

### 1.3.4 Transport and accumulation of ethylene in soils

Generally, accumulation of ethylene in soils is enhanced by:

- a physical barrier to diffusion presented by a high proportion of water-filled pores (Lynch and Harper, 1980; Smith and Dowdell, 1974; Glinski and Stepniewski, 1985);
- small aggregates that inhibit gaseous exchange with the atmosphere (Lynch and Harper, 1980);
- addition of organic matter to increase the amount of substrate for C<sub>2</sub>H<sub>4</sub> producers and low oxygen pressures (Hunt *et al.*, 1980).

The rate of diffusion of a gas through the soil is influenced by its molecular structure, soil temperature, presence of co-diffusing gases, continuity of soil pores, and the distribution of the gas between the air, water, and solid phases of the soil system. Ethylene rates of 0.42 kg/ha induced germination of witchweed seeds in sandy soils; heavy clay soils on the other hand require 1.1 kg/ha (Eplee, 1975).

The area on which this research is focussed is situated in the semi-arid tropics (SAT) of Africa. In the SAT soil tillage plays an important role in crop production (Hoogmoed, 1999). Tillage depth in lateritic soils is often shallow and 80-90% of the *Striga* seeds occur in the 0-5 cm top layer (Van Ast 1998). A large percentage of the soils found in these regions is light, contains non-swelling or shrinking clay minerals and has a low soil organic matter content. As a result, soils have a low structural stability and can be characterised as “sealing, crusting and hardsetting”. The formation of seals, crusts and hardset layers is aggravated by the aggressive and unpredictable nature of rainfall, which is typical for the SAT (Hoogmoed, 1999).

## 1.4 Problem definition

Since the discovery of ethylene as a germination stimulant for *Striga* seeds (Eplee, 1975), it was used to eradicate *Striga* infestations in the USA after sufficient conditioning (Eplee and Norris, 1995). In Kenya it has also been found that naturally produced ethylene caused suicidal germination under field conditions (Kuiper, 1997). Environmental and soil conditions as well as the economic standard of the people in the SAT are much different from the USA. The way of injecting ethylene in the soil is likely to be too expensive for most farmers.

With indications about effect of organic matter application on the ethylene production, this natural way of ethylene production is worth to be investigated. The main objective of this thesis work was to investigate whether naturally produced ethylene can be used to eradicate the *Striga* seed population.

From this main objective sub goals are defined that contains:

1. What are the main factors affecting production of ethylene for a SAT soil. What is the effect of organic matter on ethylene production. What is the effect of aerobic and anaerobic conditions and soil temperature.
2. What is the actual production of ethylene in a soil column after a simulated rainshower. What is the influence of smearing of the topsoil and amendment of the soil with organic matter.
3. What is the threshold concentration of  $C_2H_4$  to let the *Striga* seeds germinate.
4. Do technical solutions fit within the labour schedule of the African farmers.

If the actual production in the tested SAT soil meets the threshold concentration of ethylene to let the *Striga* seeds germinate, field experiments may be designed to test new management in practice.

## 2.1 CHARACTERISATION OF MAIN ETHYLENE PRODUCTION AFFECTING FACTORS IN A SOIL FROM CAMEROON

### 2.1.1 Introduction

This experiment was designed to gain understanding about the major factors that influence ethylene production in a re-wetted soil from Cameroon. Temperature was chosen realistically compared with Cameroonese conditions. Organic matter amendments and moisture content were established at favourable, but less realistic values to test their potential influence. Types of organic matter used were available sources in Cameroon and application rates. With the results of this experiment, treatments for the following soil column experiment could be designed. This experiment was executed at the Department of Microbiology.

### 2.1.2 Materials and methods

#### 2.1.2.1 Soil and organic matter characteristics

The soil used to determine the potential and actual production of ethylene was from Garoua province in northern Cameroon. All soil used in the experiments passed a 4-mm sieve. Table 1 presents some chemical properties of the soil, analysed according to the procedures described in *Soil Analysis Procedures, Extraction with 0.01 M CaCl<sub>2</sub>* (Houba *et al.*, 1999) and C-Kurmies as described in *Other Procedures* (Houba *et al.* 1997). Table 2 presents the C/N and C/P ratio's of the types of organic matter used in this experiment and the experiment in which the actual C<sub>2</sub>H<sub>4</sub> production was determined (chapter 2.2). Total C, N, and P were determined according to the procedure described by *Other Procedures* of (Houba *et al.* 1997). The texture of the soil was a

All determinations were executed by the Department of Soil Science and Plant Nutrition.

Table 1 *Chemical properties of the soil from Garoua, Cameroon*

| pH  | N-NO <sub>3</sub><br>[mg/kg] | N-NH <sub>4</sub><br>[mg/kg] | N-total<br>[mg/kg] | P<br>[mg/kg] | Na<br>[mg/kg] | K<br>[mg/kg] | C-Kurmies<br>[g/kg] |
|-----|------------------------------|------------------------------|--------------------|--------------|---------------|--------------|---------------------|
| 6.4 | 2                            | 4                            | 12                 | 1.7          | 0             | 83           | 6.2                 |

Table 2 *C/N and C/P ratios of brewery sludge, cotton milling waste and cotton seed cake*

| type of organic matter | brewery sludge | cotton milling waste | cotton seed cake |
|------------------------|----------------|----------------------|------------------|
| C/N ratio              | 14.4           | 42.0                 | 7.2              |
| C/P ratio              | 127.9          | 611.5                | 38.1             |

### 2.1.2.2 Experimental set-up

The potential  $C_2H_4$  production of the soil was determined by incubating soil slurries in 120-ml serum bottles that were made airtight with a rubber septum. Gas samples were taken with injection needles. In order to get used to taking gas samples and to be sure the gas chromatograph could detect the  $C_2H_4$  concentrations, a preliminary experiment was carried out. In this experiment the effect of the  $O_2$  pressure and amendments with two different organic matter sources at two application rates were tested, see Table 3. The organic matter applications as well as the  $O_2$  pressure had no clear effect on the  $C_2H_4$  production. More details about experimental set-up and results of the preliminary experiment can be found in Appendix I.

Table 3 *Outline of the treatments of the preliminary experiment*

| environment | organic matter |                              |
|-------------|----------------|------------------------------|
|             | source         | application rate<br>[ton/ha] |
| anaerobe    | -              | -                            |
| anaerobe    | CMW            | 1                            |
| anaerobe    | CSC            | 1                            |
| aerobe      | CMW            | 1                            |
| aerobe      | CSC            | 1                            |
| anaerobe    | CMW            | 5                            |
| anaerobe    | CSC            | 5                            |
| aerobe      | CMW            | 5                            |
| aerobe      | CSC            | 5                            |

CMW = cotton milling waste; CSC = cotton seed cake

In the main experiment available organic matter from Cameroon was tested again. The organic matter used was cotton milling waste (CMW), cotton seed cake (CSC), and brewery sludge (BS). C/N ratios of these sources are, according to Table 2, for CMW 42.0, for CSC 7.2 and for BS 14.4 respectively. The chosen application rates were fixed at 1 and 5 ton/ha. The dry bulk density  $^b\rho_d$  of loose dumped soil from Cameroon was determined beforehand as  $1.66\text{ g/cm}^3$ . The density of the solid part of the soil  $-\rho_s$  -was assumed to equal  $2.65\text{ g/cm}^3$ , so the porosity than was calculated as 37.4% of the soil volume. Per bottle of 120 ml, a slurry of 91.9 g was applied. This slurry consisted of 75.0 g of dry soil and 16.9 g of water to reach saturation. The mean headspace minus the septum was equal to  $71.7\text{ cm}^3$ . The water was mixed through the dry soil in the form of ice scales to create better mixing conditions compared with mixing liquid water with dry soil. For more details about the experimental set-up see Appendix II. Two series with a different temperature regime were used: one series with a constant temperature of  $35^\circ\text{C}$  and the other series was kept 12 hours at  $25^\circ\text{C}$  and 12 hours at  $45^\circ\text{C}$  to approach field circumstances in Cameroon. The non-microbiological production of  $C_2H_4$  was determined by autoclaving the soil during 20 minutes at  $120^\circ\text{C}$  and 2 bar pressure to prevent boiling of the water. Anoxic conditions at both temperature regimes were tested for comparison with the initial aerobic condition of the other treatments. All treatments had four replications. An outline of the treatments is presented in Table 4.

Table 4 Outline of the treatments in which the effect of temperature regime, organic matter application,  $O_2$  condition, and autoclaving was determined for ethylene production

| initial conditions | temperature [°C] | organic matter |                           |
|--------------------|------------------|----------------|---------------------------|
|                    |                  | type           | application rate [ton/ha] |
| aerobe             | 35               | -              | -                         |
| aerobe             | 35               | BS             | 1                         |
| aerobe             | 35               | CMW            | 1                         |
| aerobe             | 35               | CSC            | 1                         |
| aerobe             | 35               | BS             | 5                         |
| aerobe             | 35               | CMW            | 5                         |
| aerobe             | 35               | CSC            | 5                         |
| anaerobe           | 35               | CMW            | 5                         |
| aerobe             | 25/45            | -              | -                         |
| aerobe             | 25/45            | BS             | 1                         |
| aerobe             | 25/45            | CMW            | 1                         |
| aerobe             | 25/45            | CSC            | 1                         |
| aerobe             | 25/45            | BS             | 5                         |
| aerobe             | 25/45            | CMW            | 5                         |
| aerobe             | 25/45            | CSC            | 5                         |
| anaerobe           | 25/45            | CMW            | 5                         |
| autoclaved         | 35               | -              | -                         |

Gas samples were taken 1, 6, 8, 12, 19, and 26 days after the start of the incubation. Some septa of the serum bottles were leaking after 19 days as was found by injecting He gas into the bottles and analysing at the next sampling date.

Statistical analyses were carried out by using the general linear models procedure (GLM). With GLM factor analysis was done for temperature, types of organic matter, and initial condition.

### 2.1.2.3 Gas chromatography

The used gas chromatograph had a Fused Silica column with a CP-PoraPLOT Q coating with a film thickness of 10  $\mu$ m. The length of the column was 27.5 m and the inside diameter was 0.32 mm. The used detector was a flame-ionisation detector. The operational temperature was kept at 60°C and the carrier gas was N<sub>2</sub> under a pressure of 100 kPa relative to the atmospheric pressure. The split fraction of the injected sample was 0.0934 so that about 10% of the injected sample was led through the column whereas the major part was emitted. The split flow through the column was 40 ml/min.

The sample size during all experiments was 0.2 ml. Picture 1 shows the used injection needle for sampling and Picture 2 shows the used gas chromatograph. Erlenmeyers containing 1000 and 10 ppm of C<sub>2</sub>H<sub>4</sub> were prepared by dilution of pure C<sub>2</sub>H<sub>4</sub> and used as calibration samples. The peak areas were integrated by means of a computer program. Retention times of the samples were compared with the calibration samples. Time and temperature were determined at the moment of sampling.



Picture 1 A 0.2 ml gas sample is injected through the septum of the gas chromatograph

### 2.1.3 Conversion of concentration to $C_2H_4$ production

$C_2H_4$  accumulated both in the headspace with a certain volume  $V_{hs}$  and was dissolved in the water phase  $\theta$  of the incubated soil. A calculation example how to proceed from peak area of  $C_2H_4$  at the gas chromatograph to total production of  $C_2H_4$  in [mg/kg dry soil] and [nmol/kg dry soil] is given below:

- (1) date: June 23 (145 hours incubation)
- (2) incubation temperature: 308 K
- (3) peak area  $C_2H_4$  by GC: 1664

(4) average peak area standard sample 10 ppm: 3339

(5) concentration  $C_2H_4$  in head space  $[C_2H_4]_{hs} = \{(3)/(4)\} 10 = 4.9835$  ppm

(6) aimed mixture of soil and water: 16.9 g water + 75.0 g dry soil; total weight of added slurry 91.9 g

(7) volume water in sample,  $V_w$ :  $16.06 \cdot 10^{-6} \text{ m}^3$ . Determined by drying the soil for 48 hours at 110°C. The amount of water [g] equals 16.06 g

(8) volume headspace ( $V_{hs}$ ), determined after  $C_2H_4$  determinations by filling the headspace with water:  $71.60 \cdot 10^{-6} \text{ m}^3$

(9) real added amount of dry soil  $S_w$ , calculated as the total amount of slurry – the amount of water in the sample = (6) – (7) = 75.84 g

(10)  $1 \text{ ppmV} = \frac{P}{RT} \cdot \frac{Mw}{10^6} \cdot 10^3 = 1.1256 \text{ mg/m}^3$ ,  
 $P = 1 \text{ atm} = 101325 \text{ Pa}$ , sampling temperature  $T = 303.15 \text{ K}$ ,  $R = 8.3142 \text{ J kg}^{-1} \text{ K}^{-1}$ ,  $Mw$  (molecular weight) = 28 g/mol

(11) total amount of gas in the headspace [mg],  $TAOG_{hs} = [C_2H_4]_{hs} \cdot 1.1256 \cdot V_{hs} = (5) (10) (8) = 4.016 \cdot 10^{-4} \text{ mg}$

(12) distribution-constant for water and air ( $K_{w,a}$ ) at  $T = 30^\circ\text{C}$ : 0.098 (Seidell, 1941). For more details, see Appendix III

(13) total amount of gas in the water phase [mg],  $TAOG_w = K_{w,a} \cdot [C_2H_4]_{hs} \cdot 1.1256 \cdot V_w = (12) (10) (7) (5) = 8.180 \cdot 10^{-6} \text{ mg}$

(14)  $TAOG = TAOG_{hs} + TAOG_w = (11) + (13) = 4.098 \cdot 10^{-4} \text{ mg}$

(15)  $TAOG \cdot 10^3 / S_w = C_2H_4 \text{ [mg/kg dry soil]} = \{(14) / (9)\} \cdot 10^3 = 5.403 \cdot 10^{-3} \text{ mg/kg dry soil}$

(16)  $TAOG \cdot 10^9 / (Mw \text{ dry weight soil}) = C_2H_4 \text{ [nmol/kg dry soil]} = 192.98 \text{ nmol/kg dry soil}$

This way of calculation involves the following assumptions:

- soil was perfectly mixed with water;
- the gas phase of the soil  $\mathcal{O}_g$  has been added to the volume of the head space  $V_{hs}$
- a correction for withdrawal of volumina by previous sampling was negligible

### 2.1.4 Results

The production of ethylene was highest directly after the start of incubation of the wetted soil, see Figure 2. Under initial aerobic conditions 45% of the total  $C_2H_4$  production took place at the first day of incubation compared with 32% for strict anoxic conditions. The total production of ethylene per kg dry soil was significant higher ( $\alpha = 0.05$ ) for initial aerobic compared with strict anaerobic conditions during the first 12 days of incubation. Figure 1 shows the accumulated  $C_2H_4$  production for the two conditions together with the standard deviation  $\sigma$  per time per treatment, represented by bars. The data points for initial aerobic and strict anaerobic conditions are based on all treatments which had initial aerobic conditions and the two treatments that had strict anaerobic conditions respectively. One exception was made for the values of accumulated ethylene after 290 hours incubation at 35°C. These values were omitted due to differing values compared with the 25°/45°C regime at the same incubation time. However, this assumption is contestable.

To get the best fitted line the assumption was made that  $C_2H_4$  production started after 5 hours. This was done to avoid the natural logarithm of zero and to let  $R^2$  increase to maximum. The data points gave rise to fitting with a natural logarithm (Ln) function.

The equation for the total  $C_2H_4$  production  $y$  [nmol/kg dry soil] for initial aerobic conditions is, when incubation time  $t$  is in hours:  $y = 58.276\ln(t) - 85.156$ ;  $R^2 = 0.993$ . For strict anaerobic conditions the equation is:  $y = 52.195\ln(t) - 88.603$ ;  $R^2 = 0.9929$ . The production rate  $y$  [nmol/kg dry soil \* hour] is the slope of the equation for the total production and equals for initial aerobic conditions:  $y = 58.276 / t$ ; the slope can be calculated by taking the  $f'$  of the functions for total accumulated  $C_2H_4$  production. For strict anaerobic condition the production rate was equal to:  $y = 52.195 / t$ . The production rates are presented in Figure 2.

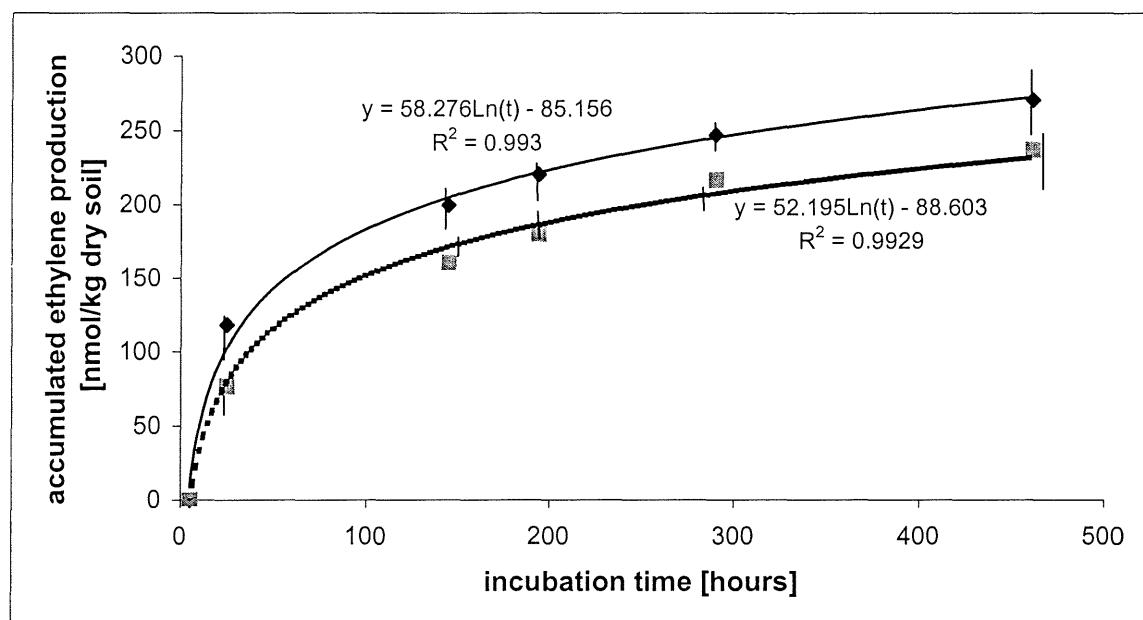


Figure 1 *Accumulated ethylene production under initial aerobic conditions as a mean of all initial aerobic treatments (continuous line) and strict anaerobic (dashed line) conditions as a mean of the two anaerobic treatments together with standard deviations*

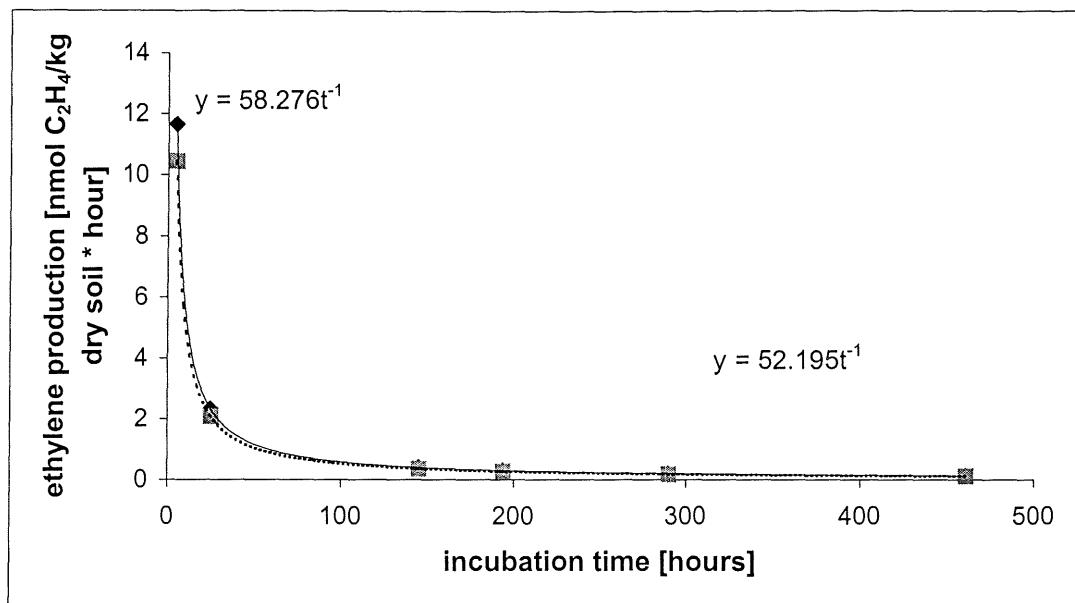


Figure 2 *Production rate of ethylene under initial aerobic (continuous line) and anaerobic conditions (dashed line)*

In general, during the whole incubation time the 25°/45°C temperature regime did not significantly differ in C<sub>2</sub>H<sub>4</sub> production with the 35°C regime. Only after 8 days (192 hours) the 25°/45°C regime had a significant higher production than the 35°C regime. Results of the accumulated production of C<sub>2</sub>H<sub>4</sub> for all different treatments are presented in Table 5. From 6 to 12 days after the start of incubation, the application of cotton seed cake hampered ethylene production significantly. In general the different types of organic matter and the application rates did not affect C<sub>2</sub>H<sub>4</sub> production. Ethylene was of microbiological origin for the autoclaved bottles did not produce any ethylene during the first 3 days and the amounts after 3 weeks were still negligible compared with the non-autoclaved bottles, see Tables 5 and 6.

## 2.2 ACTUAL ETHYLENE PRODUCTION AND DIFFUSION IN A SOIL FROM CAMEROON

### 2.2.1 Introduction

The experiment, in which the potential ethylene production was determined, showed that production of ethylene started almost immediately after the soil was wetted, see Figures 1 and 2. This experiment is focussing on imitating the field conditions. Soil will be moistened and drained, simulating rain. Concentration of ethylene at various depths in time is important to predict its role as germination stimulant. The objectives of this experiment are:

- to determine the  $C_2H_4$  concentrations at 2.5, 7.5 and 12.5 cm depth in a soil profile after wetting the soil;
- Are wet conditions and/or a low  $O_2$  pressure favourable for  $C_2H_4$  production?
- How takes  $C_2H_4$  diffusion place in the upper 15 cm of a soil profile.

### 2.2.2 Materials and Methods

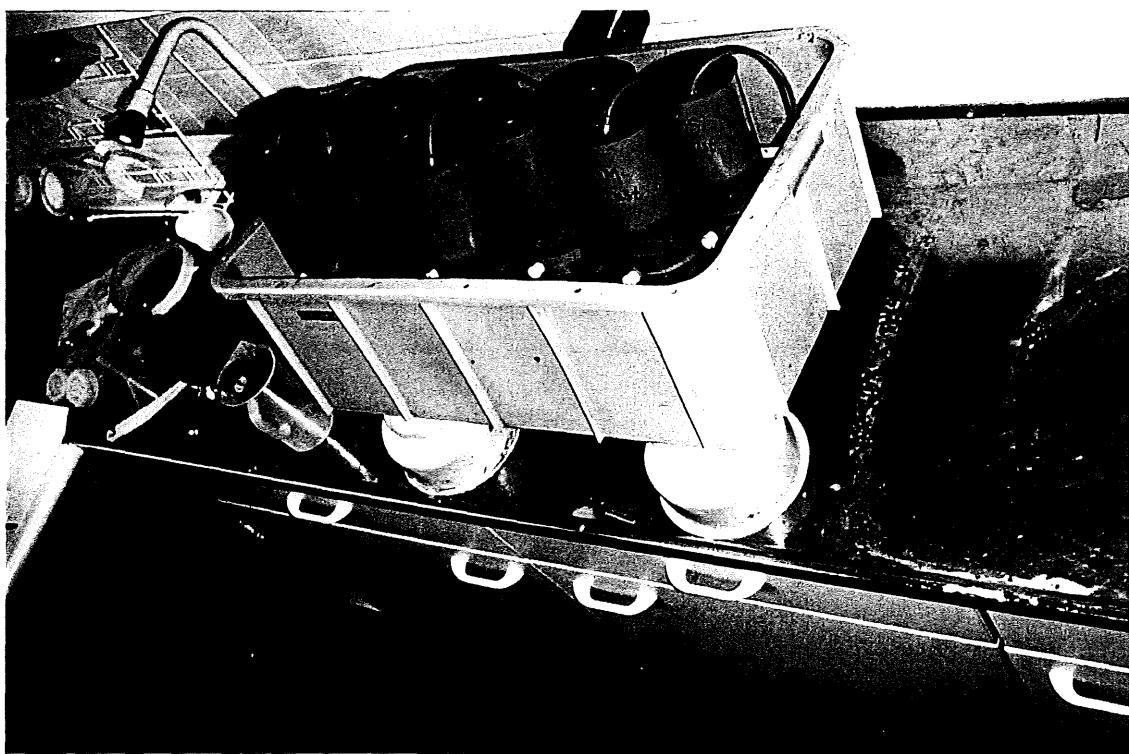
The soil used for this experiment was from Garoua, Cameroon from which the characteristics are enumerated in Table 1. The soil was packed in a column made of PVC with a height  $h$  of 17.5 cm and a radius  $r$  of 3.84 cm. The bottom was sealed with a piece of linen. The column was sawn in parts in order to determine the moisture content afterwards. The height of the three parts was from top to bottom 5.0, 5.0, and 7.5 cm. On top of the soil column a headspace tube with a height of 12.5 cm with the same  $r$  was put to determine the escaped ethylene from the soil. The headspace was closed with a cap with a height of 2.5 cm so that the headspace volume became  $463\text{ cm}^3$ . This volume was supposed to be large enough to be suitable for sampling while the diffusion rate back into the soil was assumed to be negligible. The dry bulk density  $^b\rho_d$  was strived to be equal to  $1.80\text{ g/cm}^3$ . To achieve the fixed  $^b\rho_d$  the soil was tamped down every few centimetres. Gas samples from the soil were taken from gas diffusion chambers. These chambers were made of cut syringes with an estimated volume of  $0.5\text{ cm}^3$ . The chambers were connected with a septum. In the wall of the PVC tube, holes with a radius of 2.9 mm were made at 2.5, 7.5, and 12.5 cm from the top of the soil. The septa just fitted in this hole and grease was used to ensure air tightness. Pictures 2-5 give an impression about the design.

The soil was moistened from below, to prevent air inclusion, at an angle of  $11^\circ$  with the septa at the higher side so that when the column was saturated, water could not enter the gas chambers. The height of the water level outside the columns was gradually increased from below to the top of the soil column. The soil was saturated in a time period of one hour. To equalise the outside air pressure with inside air pressure an injection needle with a small tube was pinned through the cap. Picture 5 shows the set-up at the time of wetting the soil from below.

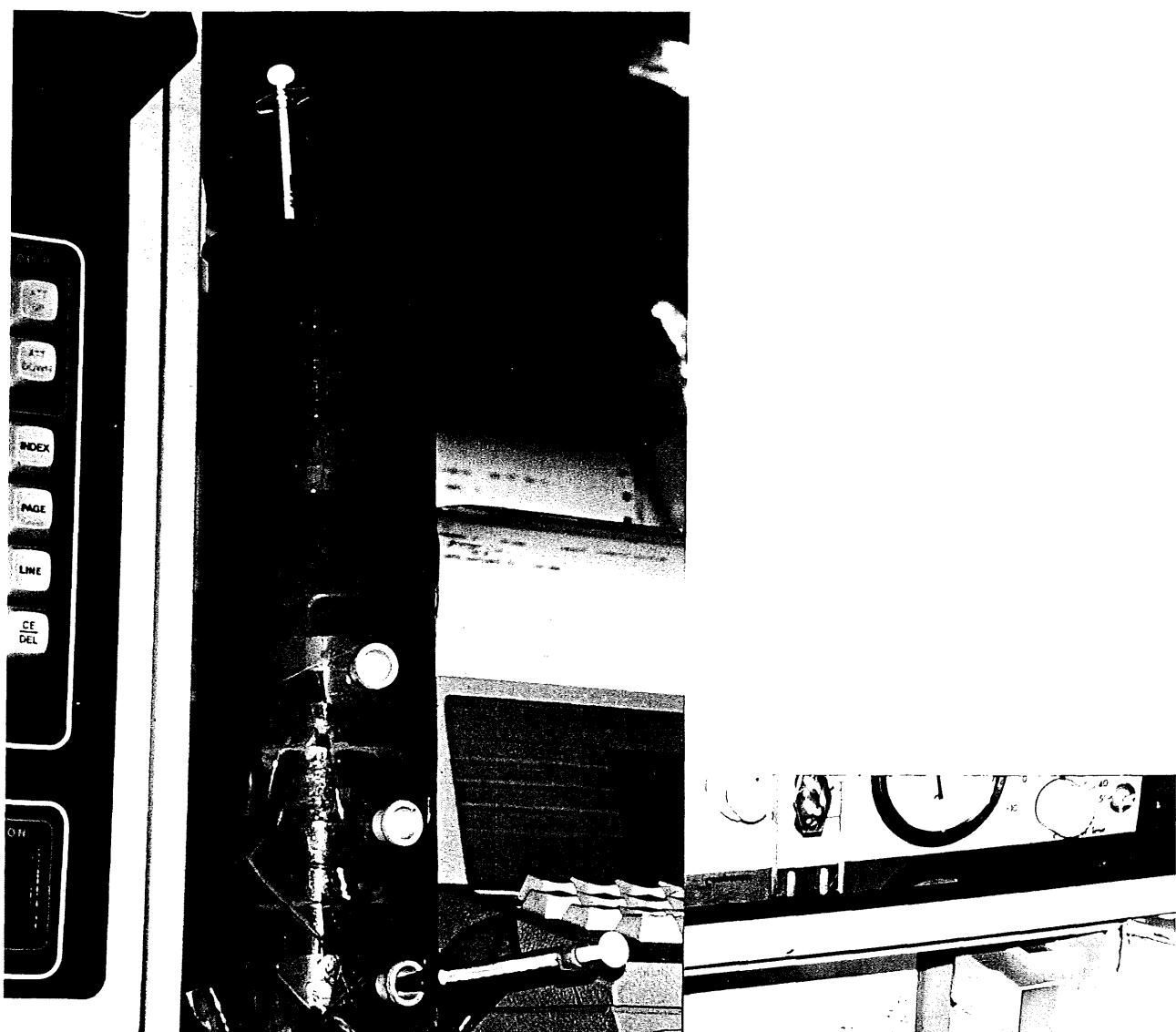
The soil columns were drained for 35 hours in order to create conditions that may approach those in a soil after the onset of the rainy season. A preliminary experiment was executed in order to establish the drainage rate of the column. This experiment showed a decrease of the volumetric water content  $\theta$  from 0.32 to 0.26 after 35 hours draining, see Figure 1 in Appendix V. The bottomside of the columns was sealed with plastic. The gas chambers were injected with air to remove all possibly entered water. More details about built-up of the columns are shown in Appendix V.



*Picture 2 Location of gas chamber at  $z=7.5$  cm depth in the column*

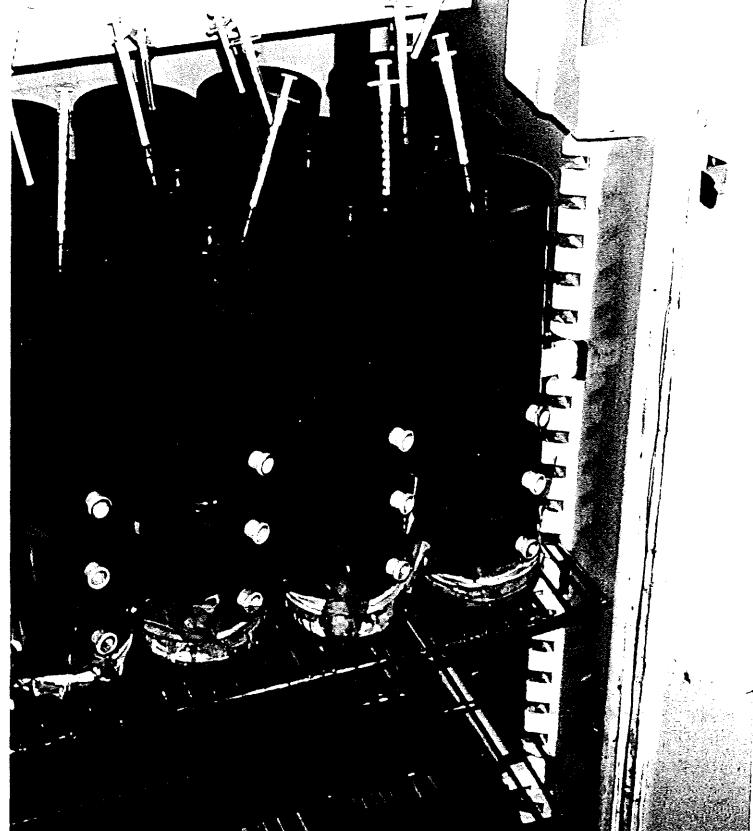


*Picture 3 The columns are moistened from below under an angle of  $11^\circ$*



*Picture 4 Column 1 is sampled  
at depth  $z=12.5$*

*Picture 5 The soil columns in the  
temperature regulator* →



Ethylene concentration in a soil depends on the production and the diffusion rate of ethylene. The latter is affected by physical barriers like moisture content, crust formation, and solubility of the gas in water. Crust formation was simulated in this experiment by smearing the top layer of the soil with a teaspoon. Organic matter amendments were tested again, to determine their role in the actual production of ethylene of a wetted soil. The source of organic matter used was cotton milling waste (CMW) and the application rate was fixed at 5 ton/ha only applied in the top 5 cm. For characteristics of CMW see Table 2. The soil columns were incubated at a temperature regime of 12 hours at 25°C and 12 hours at 45°C. An outline of the treatments is presented in Table 7.

Table 7 *Outline of the treatments determining the actual ethylene concentration in the soil profile*

| treatment # | cotton milling waste | crust formation |
|-------------|----------------------|-----------------|
| 1           | -                    | -               |
| 2           | +                    | -               |
| 3           | -                    | +               |
| 4           | +                    | +               |

Treatments were replicated 3 times. Sampling for C<sub>2</sub>H<sub>4</sub> was first executed 5 hours after 35 hours of draining the soil. Experiments were executed at the Department of Microbiology using the same gas chromatograph as in the experiment investigating the potential production, see chapter 2.1.2.3. Statistical analyses were carried out by using the General Linear Models (GLM) procedure. The probability level  $\alpha$  used, was 0.05 for all analyses. The concentrations of ethylene at the four sampling depths were analysed by application of contrasts to the GLM procedure. A contrast exists of comparing two individual treatments.

### 2.2.3 Diffusion theory

With the obtained C<sub>2</sub>H<sub>4</sub> concentrations at depths z=2.5, 7.5 and 12.5 production rates and diffusion rates can be calculated. The results of calculating the production rates can be compared with the experiment in which the potential ethylene production was determined and literature values. Diffusion is considered the major mechanism by which gases move in soils and is described by Fick's equation:

$$q = -D_g \frac{dc}{dz} \quad (1)$$

where

q = flux of gas [kg m<sup>-2</sup> s<sup>-1</sup>]

D<sub>g</sub> = diffusion coefficient gas in soil [m<sup>2</sup>/s]

c = concentration of gas in the total gas phase [kg/m<sup>3</sup>]

z = depth [m]

Fick's equation can be applied because concentration and depth are known, and the diffusion coefficient for C<sub>2</sub>H<sub>4</sub> in soil can be calculated (De Jong and Schappert, 1972).

The diffusion coefficient in soil  $D_g$  is related to the diffusion coefficient in air  $D_0$  with the following equation:

$$D_g = \tau \phi_g D_0 \quad (2)$$

where

$D_g$  = diffusion coefficient in soil [ $\text{m}^2/\text{s}$ ]

$\tau$  = tortuosity factor [ $\text{m}/\text{m}$ ] or unitless

$\phi_g$  = fraction air filled pores, calculated as ( $\text{m}^3$  gas volume/ $\text{m}^3$  soil volume) and assuming isotropic soil is used as surface reduction factor through which gas diffuses

$D_0$  = diffusion coefficient in free air [ $\text{m}^2/\text{s}$ ]

(Koorevaar *et al.*, 1983)

Equation (3) is valid for a quasi steady-state condition. In this experiment this state was reached after about one day.

The tortuosity  $\tau$  depends on  $\phi_g$  as is presented in Table 8. The used tortuosity was calculated by linear interpolation of the values of Table 8. The values per layer per column for  $\phi_g$ ,  $\theta$ , and  $D_g$  are in Appendix VI.

Table 8 Tortuosity at various air-filled porosities according to Leistra *et al.*, cf Leffelaar (1987)

| $\phi_g$ | 0.0 | 0.035             | 0.07 | 0.10 | 0.15 | 0.20 | 0.30 |
|----------|-----|-------------------|------|------|------|------|------|
| $\tau$   | 0.0 | $2 \cdot 10^{-4}$ | 0.01 | 0.03 | 0.06 | 0.10 | 0.20 |

In a steady state process the production of  $\text{C}_2\text{H}_4$ ,  $\alpha_{\text{C}_2\text{H}_4}$ , can be calculated with the equation:

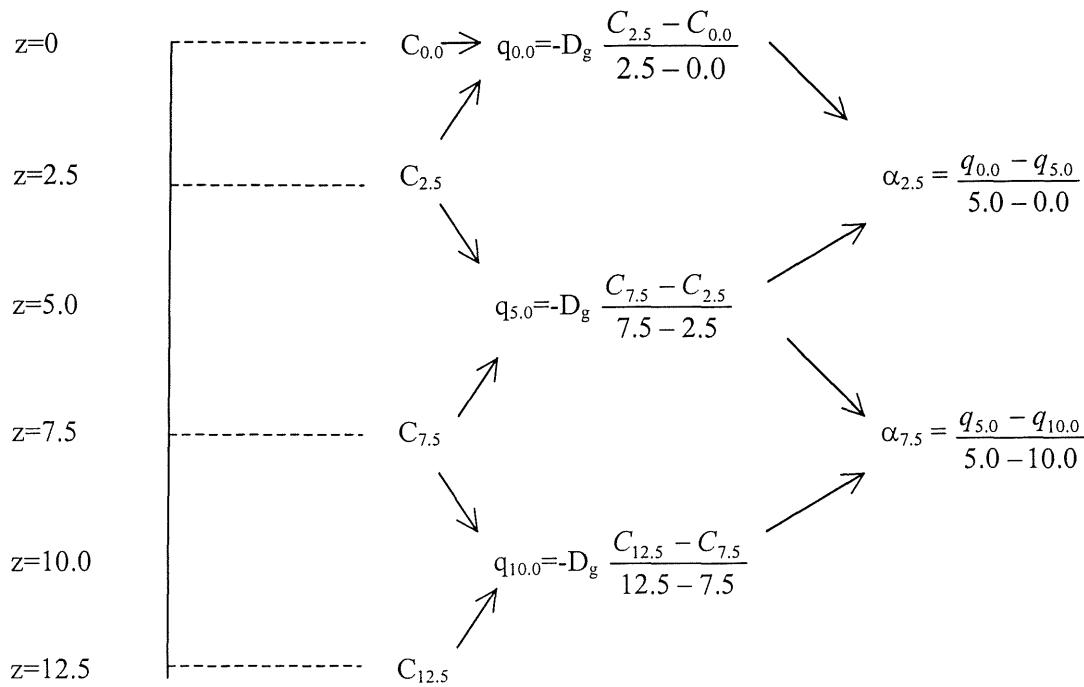
$$\alpha = \frac{dq}{dz} \quad \frac{\text{kg}}{\text{m}^2 \text{s}} \quad \frac{1}{\text{m}} \quad (3)$$

where

$\alpha$  = the consumption or respiration of a gas [ $\text{kg s}^{-1} \text{m}^{-3}$ ]

(De Jong and Schappert, 1972)

Figure 3 shows how from the measured concentrations at depth  $z=2.5$ , 7.5 and 12.5 cm and the negligible  $\text{C}_2\text{H}_4$  concentration in the headspace the flux density  $q$  and the production rate  $\alpha$  at various depths was calculated.



the value for  $q_{0.0}$  was based on the ethylene concentration at  $z=2.5$  cm depth and the assumed negligible concentration in the headspace

Figure 3 *Calculation scheme for flux density at  $z=0.0, 5.0$  and  $10.0$  cm depth and production rate at  $z=2.5$  and  $7.5$  cm depth*

The production or respiration is positive when the gas is evolved and negative when the gas is consumed. For the  $D_g$  values at depth  $z=0$  the  $D_g$  for the top 5-cm was taken. For  $z=5.0$  the mean  $D_g$  of the layers  $z=0$  to  $z=5.0$  and  $z=5.0$  to  $z=10.0$  cm was taken. Similarly for  $z=10.0$  the mean  $D_g$  of the layers  $z=5.0$  to  $z=10.0$  and  $z=10.0$  to  $z=12.5$  cm was taken. A calculation example is shown in Appendix 7.

#### calculation of diffusion coefficient for $C_2H_4$ in a $N_2$ dominant atmosphere

No experimental values could be found for the diffusion coefficient of  $C_2H_4$  in air. The following expression is used to calculate the diffusion coefficient for gas in air:

$$D_{ij} = \frac{3}{8} \sqrt{\frac{(k^3 N)}{\pi}} \frac{\sqrt{T^3 (M_i + M_j) / (2 M_i M_j)}}{p \sigma_{ij}^2 \Omega_{ij}} \quad (4)$$

Two parameters or force constants -  $\sigma_{ij}$  and  $\varepsilon_{ii}$  - are characteristics of the colliding molecules with dimension length. This length is the value of the intermolecular separation distance where the potential energy is zero and may be considered as the effective molecular size.  $\varepsilon_{ii}$  is the maximum energy of attraction occurring between molecules.

$$\sigma_{ij} = (\sigma_{ii} + \sigma_{jj}) / 2$$

$$\varepsilon_{ij}/k = [(\varepsilon_{ii}/k) (\varepsilon_{jj}/k)]^{1/2}$$

$\Omega_{ij}$  comes from (Hirschfelder *et al.*, 1964)

The symbols represent:

$D_{ij}$  : binary diffusion coefficient [ $\text{m}^2/\text{s}$ ]

$k$  : Boltzmann constant;  $k=R/N=1.3805 \cdot 10^{-23}$  [J/K]

$N$  : Avogadro's number,  $N=6.0225 \cdot 10^{23}$

$T$  : absolute temperature [K]

$M_i$  : molecular weight of component  $i$  [kg/mol]

$p$  : pressure [Pa]

$\varepsilon_{ii}$  : Lennard-Jones potential parameter; maximum energy of attraction between like molecules of component  $i$  [J]

$\sigma_{ii}$  : Lennard-Jones potential parameter; collision diameter for like molecules of component  $i$  [m]

$\Omega_{ij}$  : dimensionless collision integral based on the Lennard-Jones intermolecular potential field

The supposed gas next to  $\text{C}_2\text{H}_4$  in the soil was  $\text{N}_2$  accounting for 80% of the atmospheric pressure.

$$M_{\text{C}_2\text{H}_4} = 28 \cdot 10^{-3} \text{ kg/mol} \quad M_{\text{N}_2} = 28 \cdot 10^{-3} \text{ kg/mol}$$

$$\varepsilon_{\text{C}_2\text{H}_4}/k = 205 \text{ K} \quad \varepsilon_{\text{N}_2}/k = 91.5 \text{ K}$$

$$\sigma_{\text{C}_2\text{H}_4} = 4.232 \cdot 10^{-10} \text{ m} \quad \sigma_{\text{N}_2} = 3.681 \cdot 10^{-10} \text{ m}$$

$$\Omega_{\text{C}_2\text{H}_4/\text{N}_2} = 1.0449$$

(Leffelaar, 1987 and Hirschfelder *et al.*, 1964)

For  $p = 101325 \text{ Pa}$ ;  $T = 298 \text{ K}$  the diffusion coefficient for  $\text{C}_2\text{H}_4$   $D_{\text{C}_2\text{H}_4}$  in  $\text{N}_2$  dominant air equals  $1.56 \cdot 10^{-5} \text{ m}^2/\text{s}$ .

The value for the term  $\frac{3}{8} \sqrt{(k^3 N) / \pi}$  in equation (4) equals  $8.42 \cdot 10^{-24} \text{ J}^3 \text{ K}^{-3} \text{ mol}^{-1}$ .

## 2.2.4 Results

The treatment effect for ethylene concentrations at all depths was not significant during the measuring period. The maximum  $\text{C}_2\text{H}_4$  concentrations at  $z=2.5$  and 7.5 cm depth occurred at 25 hours of incubation and were 7.1 and 21.9 ppm respectively. At  $z=12.5$  cm depth the maximum measured concentration was found later and was equal to 47.3 ppm. Between 104 and 194 hours of incubation the  $\text{C}_2\text{H}_4$  concentration at  $z=2.5$  and 7.5 cm dropped below 1 ppm, while after more than two weeks at  $z=12.5$  the  $\text{C}_2\text{H}_4$  concentration was still higher than 1 ppm (361 hours after stopping drainage). There was no significant effect of the applied cotton milling waste and the simulated crust on the  $\text{C}_2\text{H}_4$  concentration. Table 9 shows ethylene concentrations of the 4 different treatments at  $z=2.5$ , 7.5 and 12.5 cm calculated as averages of the replicates. In Table 10 the average ethylene concentrations per depth of the four treatments are presented together with significant differences that are expressed by different letters. All measured ethylene concentrations are in Appendix VIII and results of statistical analyses are in Appendix IX.

Table 9 Ethylene concentrations in the soil at  $z=2.5$ ,  $7.5$  and  $12.5$  cm depth per treatment during the incubation time

| treatment                                   |       | time after stopping drainage [hours] |      |      |      |      |     |
|---|-------|--------------------------------------|------|------|------|------|-----|
| organic matter<br>(only in top 5 cm)        | crust | 5                                    | 25   | 54   | 104  | 194  | 361 |
| $C_2H_4$ concentration [ppm] at $z=2.5$ cm  |       |                                      |      |      |      |      |     |
| -   | -     | 1.0                                  | 2.1  | 3.0  | 0.8  | 0.6  | 0.3 |
| +   | -     | 3.5                                  | 4.0  | 3.4  | 2.4  | 0.6  | 0.0 |
| -   | +     | 1.2                                  | 2.7  | 3.8  | 2.4  | 0.3  | 0.0 |
| +   | +     | 5.9                                  | 5.4  | 3.6  | 2.0  | 0.3  | 0.5 |
| $C_2H_4$ concentration [ppm] at $z=7.5$ cm  |       |                                      |      |      |      |      |     |
| -   | -     | 12.1                                 | 27.7 | 13.8 | 6.7  | 0.7  | 0.0 |
| +   | -     | 6.6                                  | 25.0 | 3.7  | 1.5  | 0.0  | 0.0 |
| -   | +     | 5.2                                  | 21.8 | 9.4  | 2.2  | 0.0  | 0.0 |
| +   | +     | 2.7                                  | 13.0 | 5.8  | 1.8  | 0.0  | 0.0 |
| $C_2H_4$ concentration [ppm] at $z=12.5$ cm |       |                                      |      |      |      |      |     |
| -   | -     | 10.3                                 | 36.8 | 40.8 | 35.4 | 11.4 | 0.9 |
| +   | -     | 8.5                                  | 27.9 | 48.5 | 34.0 | 5.2  | 4.8 |
| -   | +     | 7.3                                  | 30.5 | 48.6 | 30.7 | 5.6  | 0.5 |
| +   | +     | 7.8                                  | 34.0 | 51.2 | 37.8 | 2.7  | 0.4 |

Table 10 Overall ethylene concentrations in the soil at  $z=2.5$ ,  $7.5$ , and  $12.5$  cm depth during the incubation time

| depth [cm] | $C_2H_4$ concentration [ppm]         |        |        |        |       |        |
|------------|--------------------------------------|--------|--------|--------|-------|--------|
|            | time after stopping drainage [hours] |        |        |        |       |        |
|            | 5                                    | 25     | 54     | 104    | 194   | 361    |
| 2.5        | 2.9 a*                               | 3.2 a  | 3.5 a  | 4.3 a  | 0.5 a | 0.2 ab |
| 7.5        | 6.7 b                                | 21.9 b | 8.2 ab | 3.1 a  | 0.2 a | 0.0 a  |
| 12.5       | 8.5 b                                | 32.3 c | 47.3 c | 34.5 b | 6.2 b | 1.7 b  |

\*Letters express significant differences.  $C_2H_4$  concentrations in the same column with only different letters are significant different by application of General Lineair Models Procedure

In Figure 4 the  $C_2H_4$  concentrations at 194 hours of incubation are plotted against the  $\%O_2$ . The figure shows that higher  $C_2H_4$  concentrations only occur at low  $\%O_2$ .

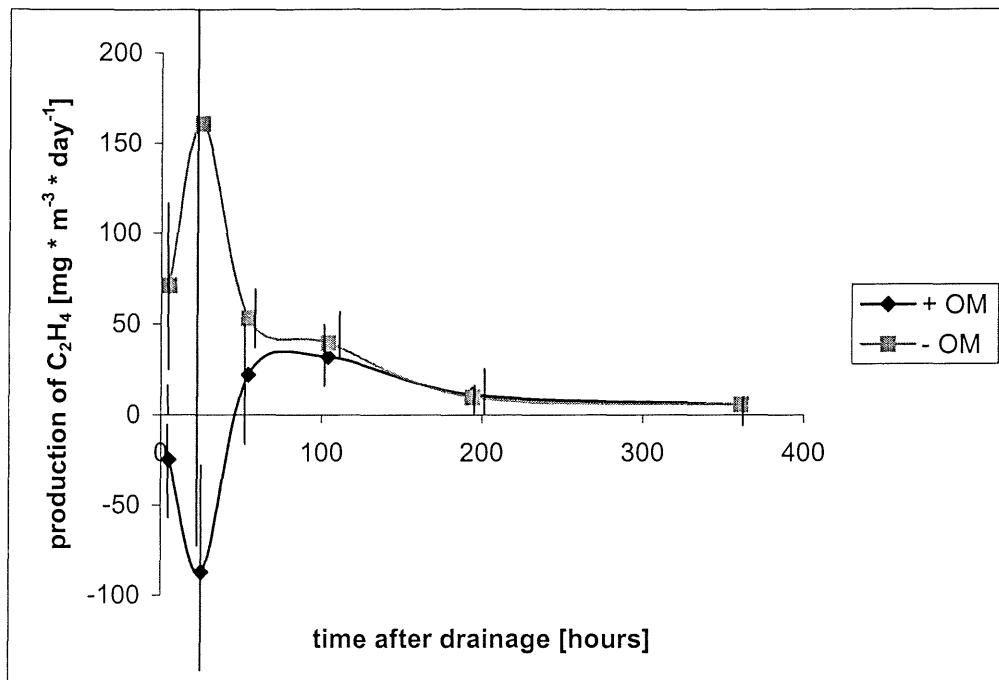


Figure 5 *Production of ethylene as calculated with equation (3) at  $z=2.5$  for application of cotton milling waste at 5 ton/ha (+OM) and zero treatment (-OM), standard deviation is presented as bars*

Crust formation did not result in significant lower flux densities at  $z=0$  compared with no crust formation. The flux densities were calculated by using equation (1). Figure 6 shows the results of comparing the  $C_2H_4$  flux density at the top of the column where  $z=0$ . Maximum release of soil ethylene occurred at about 48 hours after stopping drainage, so about 3.5 days after wetting of the soil.

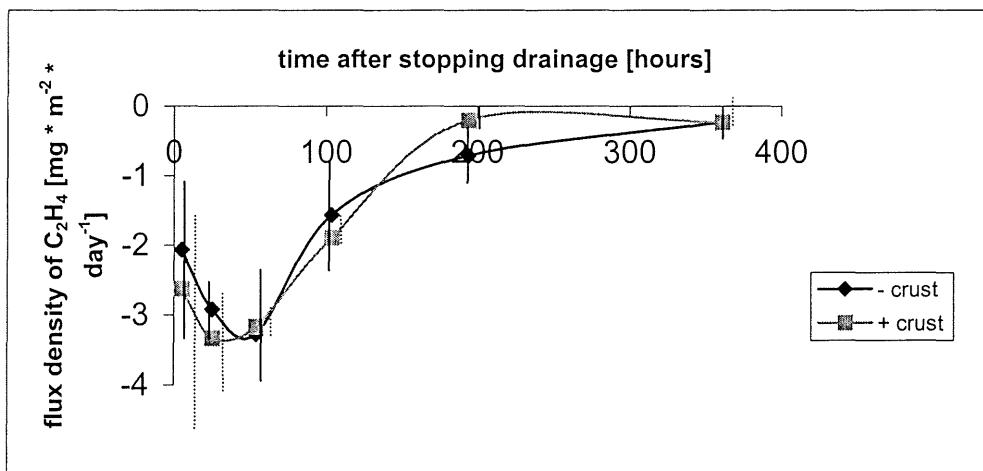


Figure 6 *Flux density of  $C_2H_4$  at  $z=0$  for crust and no crust simulation together with standard deviation bars dashed for +crust, continuous for - crust*

## 2.2.5 Discussion

Ethylene concentrations in the columns were dependent on depth rather than on organic matter application or crust formation. Regarding the time of maximum  $C_2H_4$  production this experiment can be compared with the experiment using the 120-ml serum bottles. Figure 2 shows a maximum production at the very beginning of incubation. The production rate after 5 hours was about 12 nmol  $kg\ dry\ soil^{-1}\ hour^{-1}$ . Conversion to the unit used in Table 13 the production was equal to  $14.5\ mg\ m^{-3}\ day^{-1}$ . After about 100 hours the production of the experiment in which the potential production was determined, was equal to  $0.67\ mg\ m^{-3}\ day^{-1}$ , while production after 450 hours occurred at a rate of about  $0.15\ mg\ m^{-3}\ day^{-1}$ . Sampling the soil columns 5 hours after wetting was practically impossible. The first sampling time was 5 hours after stopping drainage of the soil columns so 40 hours after wetting of the soil. According to Table 13 the average production rate of  $z=2.5$  and  $7.5$  at 40 hours after wetting the soil was  $22\ mg\ m^{-3}\ day^{-1}$ . The maximum calculated production rate for the column experiment at  $z=2.5\ cm$  occurred 89 hours after wetting the soil, while at  $z=7.5\ cm$  the maximum production rate occurred 60 hours after wetting the soil. In the soil column at  $z=2.5\ cm$  the production rate 40 hours after wetting the soil was  $22/1.7=13$  times higher than the experiment in which the potential  $C_2H_4$  production was determined. The difference in production rates between  $z=2.5$  and the 120-ml serum bottles increased in time. From this it may be concluded that the experiment in which the 120-ml bottles were used not resulted in potential ethylene production levels. Microbiological research is necessary to determine the producers of ethylene under a set of soil conditions. Strict anaerobic micro-organisms that do not produce ethylene might have had the upper hand in the serum bottles thus limiting ethylene production. The calculated production rates of the soil columns in Table 13, however, show a high variance, so conclusions may be premature.

The differences in  $C_2H_4$  concentrations between  $z=2.5, 7.5$  and  $12.5$ , see Table 10, are likely caused by the process of diffusion, and decomposition of  $O_2$ . Glinski and Stepniewski (1985) reported that degradation of ethylene took place 50 times faster under oxic than under anoxic conditions. It is therefore interesting to know the rate of chemical oxidation of ethylene by  $O_2$  in order to characterise the decomposition of  $C_2H_4$ . A crust may not only hamper  $C_2H_4$  diffusion out of the soil but also  $O_2$  diffusion into the soil. The latter process is promoting anaerobic conditions that may lead to a slower breakdown of  $C_2H_4$  in the soil.

The calculated diffusion coefficients  $D_g$  with equation (4) are based on a low air filled porosity caused by an imposed  $^b\rho_d$  of  $1.80\ g/cm^3$ . An undisturbed moist soil sample had a bulk density of  $1.90\ g/cm^3$ . If the moisture content is 0.10, the  $^b\rho_d$  would equal  $1.80\ g/cm^3$ , so the imposed  $^b\rho_d$  is not an unrealistic value. The porosity  $\phi_p$  decreased from 0.373 in the top 5 cm to 0.302 between  $z=10.0$  and  $z=17.5\ cm$ . Similarly the measured air filled porosity decreased from 0.181 to 0.095. The flux density of  $C_2H_4$  in water was not taken into account, though in a period of rain showers the top layer may become saturated again and causes a diffusion barrier. The simulated crust formation should be compared with crust formation under field conditions at the onset of the rainy season. Also gas sampling under field conditions is necessary to obtain more realistic values for ethylene concentrations and consequently diffusion parameters can be calculated more precisely.

## 2.3 EFFECT OF ETHYLENE ON GERMINATION OF *STRIGA* SEEDS

### 2.3.1 Introduction

To connect concentrations of ethylene found in re-wetted soils with its role as a germination stimulant of *Striga*, an experiment was designed to determine the germination response of *Striga* to various C<sub>2</sub>H<sub>4</sub> concentrations. A similar experiment was executed by Egley and Dale in 1970. They tested the germination of *Striga lutea L.* after 14 to 21 days conditioning period at 29°C 1 and 4 days after the start of exposure to ethylene. The germination results are summarised in Table 14. A concentration of 0.01 ppm caused already a germination of 15%, while 0.1 ppm was enough to reach maximum germination of the *Striga* seeds. The concentrations used in this experiment were based on the research of Egley and Dale (1970) and on actual C<sub>2</sub>H<sub>4</sub> concentrations found in re-wetted soils.

Germination of *Striga* seeds takes place only after a germination stimulant has been recognised by the *Striga* seed, normally exuded by the host plant (Logan and Stewart, 1991).

Table 14 *Germination of Striga lutea L. at different ethylene concentrations after conditioning for 14-21 days, found by Egley and Dale (1970)*

| Ethylene concentration<br>[ppm] | 0 | 0.001 | 0.01 | 0.1 | 1  | 10 | 100 |
|---------------------------------|---|-------|------|-----|----|----|-----|
| % germination                   | 8 | 5     | 15   | 97  | 95 | 93 | 96  |

### 2.3.2 Materials and methods

To determine the effect of various C<sub>2</sub>H<sub>4</sub> concentrations on the germination chance and rate of *Striga hermonthica* seeds, these seeds were exposed to various concentrations of C<sub>2</sub>H<sub>4</sub>. The seeds used were collected in 1996 in Samayana, Mali. The seeds were first sterilised during 5 minutes using a bleach water solution containing sodiumhyperchlorite (NaClO) (Weast, 1974). The active chlorite was 4 g per litre. After sterilising, the seeds were cleaned with demi-water. Then the seeds were conditioned for 14 days on moist filter paper. The conditioning temperature was kept at 30°C. After this period the seeds were exposed to different treatments, according to Table 15. From previous germination tests and from literature it was concluded that germination without a stimulant does not occur and no zero treatment was included therefore. Ethylene at concentrations of 0.01, 0.1, 1, 10 and 100 ppm was added by injection through a septum. These concentrations were based on the research carried out by Egley and Dale (1970) and the measured C<sub>2</sub>H<sub>4</sub> concentrations in the soil columns, see Tables 9 and 10. GR-24 was introduced to obtain the maximum potential germination of the seeds used in this experiment. All treatments had three replications.

A preliminary test with the same seeds resulted in 79.5% germination 2 days after GR-24 was added. More details about GR-24 are found in Appendix XI. GR-24 was added as a solution in water and acetone to glass fibre punches.

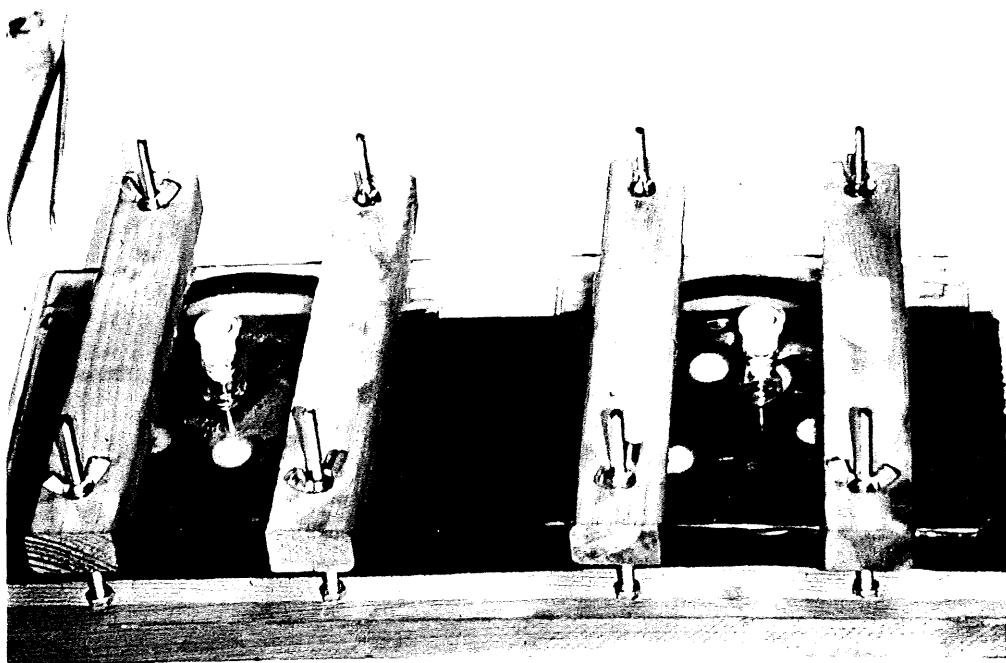
Table 15 *Outline of the treatments used in the germination experiment*

| treatment #                     | 1    | 2   | 3 | 4  | 5   | 6     | 7  |
|---------------------------------|------|-----|---|----|-----|-------|----|
| concentration of ethylene [ppm] | 0.01 | 0.1 | 1 | 10 | 100 | GR-24 | 0* |

\* previously determined

After conditioning the seeds were shortly dried to make it possible to manipulate them. Seeds were placed on moist ( $H_2O$ ) glass fibre punches that were put in airtight petri dishes. These petri dishes were made airtight by using grease and a rubber ring, see Picture 6. Ethylene concentrations were established by replacing a volume of air of the petri dish by a known concentration of ethylene, see Appendix XI for more details. The dishes were stored at 30°C in the dark. At the time of scoring the germination, the dishes were opened. After closing the dishes again the concentration of ethylene was re-established. Germination was scored at 0, 1, 2, 3 and 4 days after exposure to the germination stimulant, using a binocular. A seed was scored as germinated if the germination tube had protruded through the seed coat (Okonkwo, 1987).

Statistical analysis consisted of comparing the germination fractions of the treatments. The germination fractions were distributed binomial using analyses of deviance. Each treatment was tested with all other treatments for significant differences at  $\alpha=0.05$ .



Picture 6 *Set-up of the petri dishes in which the moist punches containing the *Striga* seeds are located*

### 2.3.3 Results

Ethylene induced germination at all established concentrations. Concentrations of  $C_2H_4 \leq 0.1$  ppm did not result in maximum germination. Maximal germination at  $C_2H_4$  concentrations  $\geq 1$  ppm was reached between 25 and 49 hours of exposure. Ethylene concentrations of 1, 10 and 100 ppm resulted in a higher germination of *Striga* seeds than GR-24. This gives rise to the conclusion that ethylene is a better germination stimulant than GR-24 for *Striga* germination. Between 25 and 49 hours of exposure the % germination among the seeds exposed to  $C_2H_4$  concentrations  $\geq 1$  ppm was significant higher than the seeds exposed to lower  $C_2H_4$  concentrations. Results are presented in Table 16 and Figure 7. Significant differences in germination % in Table 16 at the same exposure time are expressed by only different letters. If two treatments at a certain exposure time have only different letters it means they are significant different. All germination fractions are presented in Appendix XI. Statistical analysis can be found in Appendix XII.

Table 16 Percentage of germinated *Striga hermonthica* seeds exposed to GR-24 and various ethylene concentrations during 96 hours

| germination stimulant | exposure time [hours] |      |      |      |
|-----------------------|-----------------------|------|------|------|
|                       | 25                    | 49   | 71   | 96   |
| $C_2H_4$ 0.01 ppm     | 14 a                  | 29 a | 28 a | 26 a |
| $C_2H_4$ 0.1 ppm      | 12 a                  | 35 a | 37 a | 37 a |
| $C_2H_4$ 1 ppm        | 35 ab                 | 71 c | 72 b | 72 b |
| $C_2H_4$ 10 ppm       | 22 ab                 | 73 c | 73 b | 72 b |
| $C_2H_4$ 100 ppm      | 38 b                  | 76 c | 76 b | 75 b |
| GR-24                 | 30 ab                 | 63 b | 67 b | 66 b |

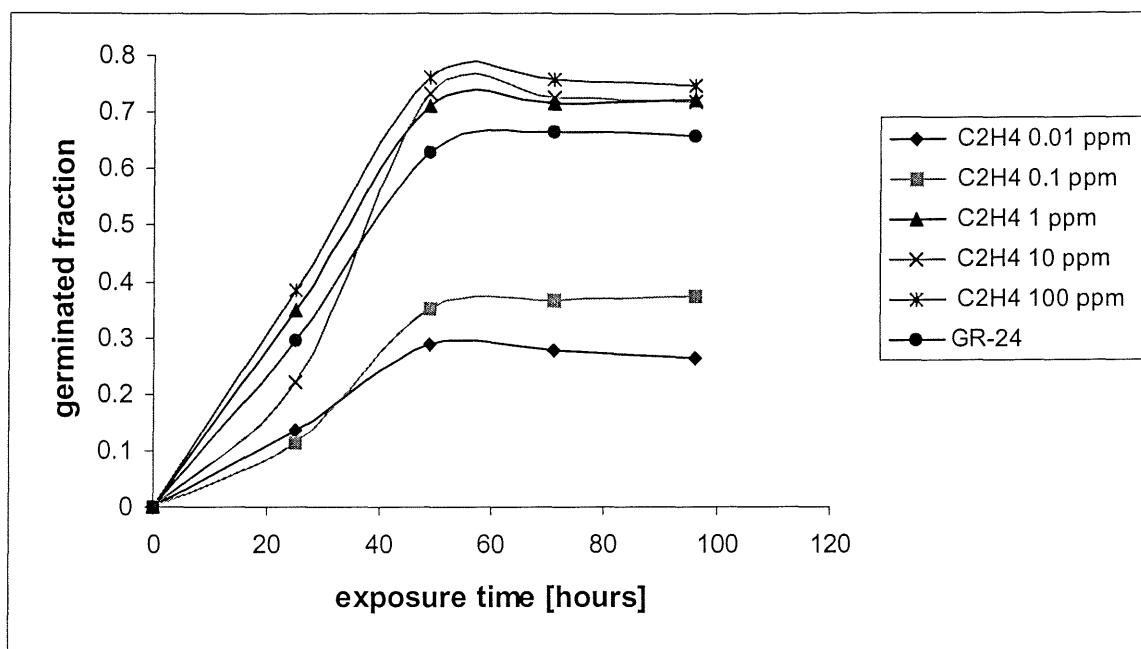


Figure 7 Germinated fractions of *Striga* seeds of the various  $C_2H_4$  concentrations as a function of exposure time

### 2.3.4 Discussion

The experiment revealed that the *Striga* seeds germinated within two days when ethylene concentration was  $\geq 1$  ppm and when the seeds were preconditioned for 14 days. Field practices in sorghum cropping involve landpreparation almost immediately after the first rains. *Striga* seeds have to connect with a host plant within a few days after germination, if not they will die off (Van Ast, 1998). Figure 8 shows the % germination of *Striga* seeds versus the incubation time.

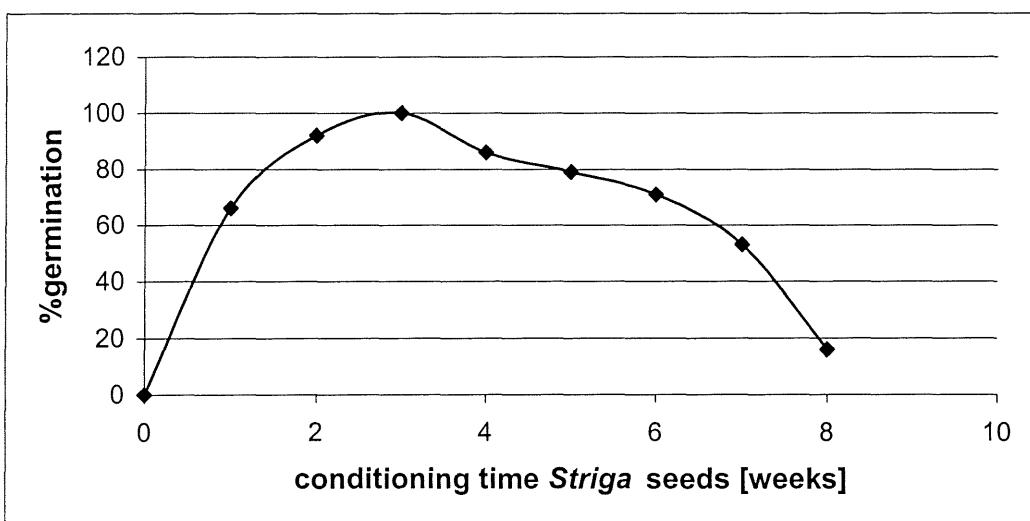


Figure 8 Percentage germination of *Striga* seeds versus the incubation time at 30 °C (Jansen, 1996)

After two weeks conditioning at 30°C 90% of the seeds are in the state are able to germinate. More research is necessary to test the effect of ethylene at shorter conditioning times.

### 3 DISCUSSION AND CONCLUSIONS

To make predictions and recommendations whether natural produced ethylene can be managed to cause suicidal germination of *Striga* seeds, an integration of the experiments should be made. This integration is focussing on occurring ethylene levels and chance that ethylene concentration can cause suicidal germination of *Striga* seeds. From the onset of the rainy season, if the soil becomes wet and the temperature is  $\geq 25^\circ$ , *Striga* seeds are in the phase of conditioning. The conditioning period takes at least 10-14 days before an optimal response to germination stimulants occurs. In connection with the latter hypothesis, Ogborn (1987) proved both in the field and in vitro, that the germination of *Striga* is depressed when the seeds are conditioned in the presence of a germination stimulant.

The experiments with soil, incubated in 120-ml serum bottles and put in columns, show that production of ethylene is maximal at the beginning of the moistening and decreases in time. The impeding effect of conditioning of *Striga* seeds exposed to natural produced ethylene may occur but it is uncertain at what levels of ethylene this hampering effect may arise. In Figures 9 and 10 the decreasing ethylene concentrations in time obtained from the column experiment are plotted versus time. Because the major fraction of *Striga* seeds occur in the top 5 cm of the soil, the  $C_2H_4$  concentration at  $z=2.5$  needs special attention. The 1 ppm level that already causes maximal germination, see chapter 2.3.3, is crossed at  $z=2.5$  at about 8 days after wetting the soil. The vertical lines in Figures 9 and 10 mark this time. A *Striga* seed becomes responsive to  $C_2H_4$  after at least several days of conditioning, see Figure 8. In Figure 10 this period is expressed by arrow A. Maximal response to  $C_2H_4$  occurred in between 2 days after exposure, see Figure 7, and is showed by arrow B. After germination the *Striga* remains threatening for the crop to four days after germination (Van Ast, 1998), expressed by arrow C.

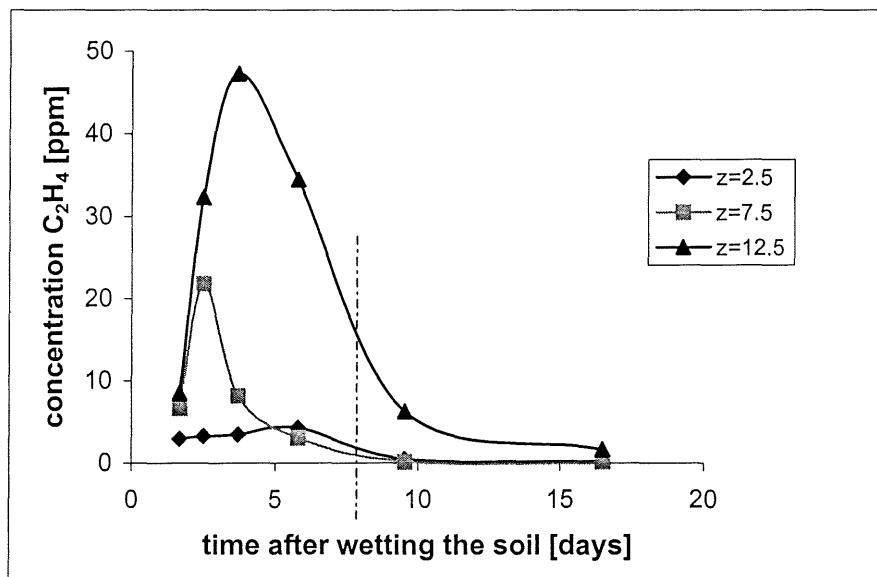


Figure 9 Ethylene concentrations per depth after wetting the soil

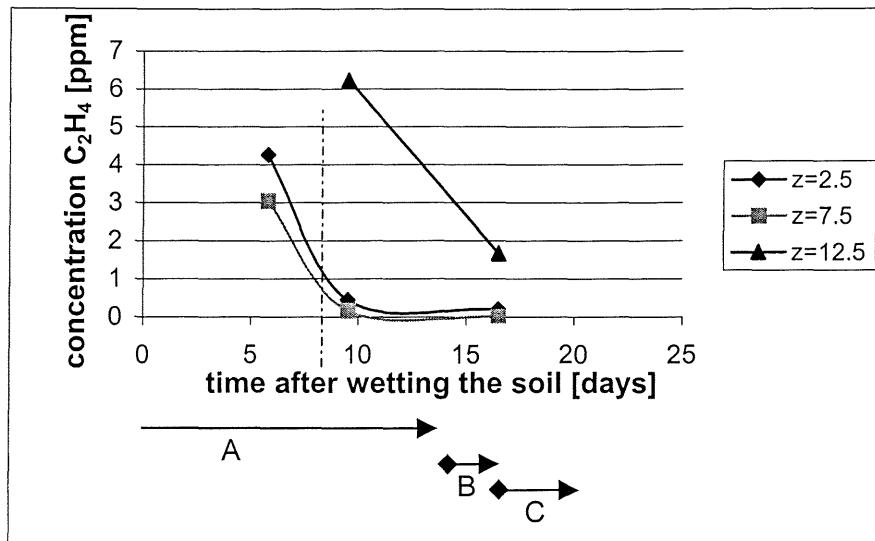


Figure 10 Ethylene concentrations in the supposed conditioning phase A, responsive phase B, and threatening phase C

According to Figure 9 before 10 days the  $C_2H_4$  concentration is lower than the concentration that is necessary to cause maximal suicidal germination of the *Striga* seeds, see Table 16.

Comparing the  $C_2H_4$  concentrations found in the column experiment and those required at the time of maximal conditioning, the ethylene concentrations are too low to cause the eradication of all *Striga* seeds. However, this laboratory experiment only gives an indication of  $C_2H_4$  concentrations in the soil under certain conditions. Conditions in the field may differ because of different precipitation patterns, crust formation, different temperatures, soil management, and fertilisation. Field conditions may be initially drier so ethylene production may start later on than found in the column experiment.

The other option of managing ethylene to cause suicidal germination is injecting it into the soil. This technique, however, is too expensive for most small-scale farmers but it can probably be used for cleaning up small infestations (Van Ast, 1998). Ogborn (1987) stated that it is impossible to develop a simple recommendation, which would enable farmers to apply the germination stimulant after the correct amount of rain has been recorded to condition the *Striga* seed. In the future, it may be possible to develop a water-soluble capsule for this purpose. He also stated that at present, there is no method of application, which can be used. The application of ethylene can be made with fair accuracy after the seed has been conditioned and the consequences of an error are not so serious because the ethylene is not persistent (Ogborn, 1987). Considering the fertility status of many African soils the use of ethylene to eradicate *Striga hermonthica* is by no means economic if no fertiliser or manure is applied, especially in soils with a very low fertility. In such soils productivity is more limited by fertility than by *Striga* (Kuiper, 1997).

Finally, the experiments carried out were meant to get an impression of factors affecting the ethylene production and the production and diffusion rates of ethylene in soils. These pilot experiments should be quantitatively tested under field conditions.

Recommendations for field trials are:

- keeping fallow of cultivated land for one season and compare with land cultivated during the same period;
- testing the effect of landpreparation, tillage, ploughing, and rolling the soil to amplify crust formation;
- organic matter amendments for the long-term effect.

All trials involve testing the effect of the treatment on *Striga* seed population.

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## Appendix I

### Design preliminary test for experiment mentioned in chapter 2.1

| bottle nr.  | organic matter |                              |
|-------------|----------------|------------------------------|
|             | source         | application rate<br>[ton/ha] |
| 1-2         | -              | -                            |
| 3-4-7-8     | CMW            | 1                            |
| 5-6-9-10    | CSC            | 1                            |
| 11-12-15-16 | CMW            | 5                            |
| 13-14-17-18 | CSC            | 5                            |

Temperature was constant at T=30C

75.00 gram soil was mixed with 25.00 gram water as ice.

Serum bottles were shaken continuously.

### raw data preliminary experiment

| date      | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> | date     | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> |
|-----------|------------|---|----------|------------|---|
| 31-May-99 | S100*      | 656575                                  | 1-Jun-99 | 13         | 8478                                    |
|           | S100       | 539746                                  |          |            | 7486                                    |
|           | S1000*     | 5500154                                 |          |            | 6675                                    |
|           | S100       | 612870                                  |          |            | 6797                                    |
|           | S100       | 589429                                  |          | 14         | 4914                                    |
|           | S100       | 589612                                  |          |            | 5670                                    |
|           | S100       | 579741                                  |          |            | 4980                                    |
|           | S100       | 577626                                  |          |            | 5571                                    |
|           | S100       | 568040                                  |          | 1          | 5101                                    |
|           | S100       | 575042                                  |          |            | 4775                                    |
|           | S100       | 576808                                  |          |            | 5448                                    |
|           | S100       | 557120                                  |          |            | 5210                                    |
|           | S100       | 469719                                  |          | 2          | 5784                                    |
|           | S100       | 497411                                  |          |            | 5593                                    |
| 1-Jun-99  | S100       | 503129                                  |          |            | 5879                                    |
|           | S100       | 496304                                  |          |            | 6235                                    |
|           | S100       | 491711                                  | 2-Jun-99 | S10        | 52554                                   |
|           | S10        | 60236                                   |          |            | 56656                                   |
|           | S10        | 58336                                   |          |            | 55633                                   |
|           | S10        | 55859                                   |          | 11         | 14533                                   |
|           | S10        | 52768                                   |          |            | 9618                                    |
|           | S10        | 54137                                   |          |            | 11589                                   |
|           | S10        | 55372                                   |          |            | 10421                                   |
|           | 11         | 6731                                    | 12       |            | 11214                                   |
|           |            | 6065                                    |          |            | 18121                                   |
|           |            | 5392                                    |          |            | 9491                                    |
|           |            | 5751                                    |          |            | 10946                                   |
|           | 12         | 7405                                    |          | 13         | 11187                                   |
|           |            | 5913                                    |          |            | 9247                                    |
|           |            | 5301                                    |          |            | 9620                                    |
|           |            | 7792                                    |          |            |   |

\* S1000, S100 and S10 represent standard samples of 1000 ppm, 100 ppm and 10 ppm ethylene

| date     | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> | date     | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> |
|----------|------------|---|----------|------------|---|
| 2-Jun-99 | 14         | 13433<br>10069<br>9631<br>1             | 3-Jun-99 | 10         | 16266<br>14209<br>15806<br>15           |
|          |            | 11074<br>11623<br>11396                 |          |            | 19814<br>20267<br>16                    |
|          | 2          | 17164<br>14497<br>13505                 |          |            | 24109<br>18165<br>17                    |
|          | 7          | 10830<br>10406<br>10535                 |          |            | 24591<br>24689<br>18                    |
|          | 8          | 4585<br>4534<br>4192                    |          | 3          | 15549<br>15578<br>4                     |
|          | 9          | 10110<br>9818<br>11098                  |          | 5          | 19953<br>15148<br>19362                 |
|          | 10         | 10823<br>10736<br>11126                 |          | 6          | 18164<br>17413<br>S10                   |
| 3-Jun-99 | S10        | 74070                                   |          | S10        | 73132<br>74027                          |
|          | S10        | 72554                                   |          | S10        | 74495                                   |
|          | S10        | 71614                                   |          | S10        | 74866                                   |
|          | S10        | 70527                                   | 4-Jun-99 | S10        | 65840                                   |
|          | 11         | 15346<br>15243<br>16240                 |          | S10        | 71093<br>68329<br>1                     |
|          | 12         | 15301<br>15332<br>14733                 |          | 1          | 15076<br>14883<br>2                     |
|          | 13         | 16241<br>15160<br>15479                 |          | 2          | 17938<br>17064<br>3                     |
|          | 14         | 18622<br>14895<br>14548                 |          | 3          | 18078<br>17024<br>4                     |
|          | S10        | 68927                                   |          | 4          | 20734<br>20229                          |
|          | S10        | 73511                                   |          | 5          | 22293                                   |
|          | S10        | 72514                                   |          | 5          | 21708                                   |
|          | 1          | 15484<br>15125<br>14975                 |          | 6          | 19801                                   |
|          | 2          | 20665<br>17812<br>17653                 |          | 6          | 18956                                   |
|          | 7          | 20715<br>18298<br>15637                 |          | 7          | 20250                                   |
|          | 8          | 9762<br>10786<br>10315                  |          | 7          | 20376                                   |
|          | 9          | 13533<br>13873<br>13994                 |          | 8          | 11724<br>11162<br>9                     |
|          |            |   |          | 9          | 14202<br>14180                          |
|          |            |   |          | S10        | 76098                                   |
|          |            |   |          | S10        | 67521                                   |
|          |            |   |          | 10         | 19733                                   |
|          |            |   |          | 10         | 17719                                   |
|          |            |   |          | 10         | 19316                                   |
|          |            |   |          | 10         | 19110                                   |
|          |            |   |          | 11         | 18260                                   |
|          |            |   |          | 11         | 17246                                   |
|          |            |   |          | 12         | 17979                                   |
|          |            |   |          | 12         | 19806                                   |

| date     | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> | date      | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> |
|----------|------------|---|-----------|------------|---|
| 7-Jun-99 | 12         | 17368                                   | 11-Jun-99 | 15         | 29924                                   |
|          | 13         | 16672                                   |           | 15         | 29080                                   |
|          | 13         | 19241                                   |           | 16         | 28980                                   |
|          | 13         | 15960                                   |           | 16         | 28975                                   |
|          | 14         | 16263                                   |           | 17         | 32154                                   |
|          | 14         | 14558                                   |           | 17         | 29032                                   |
|          | 15         | 22210                                   |           | 17         | 26968                                   |
|          | 15         | 22350                                   |           | 18         | 27805                                   |
|          | 16         | 21994                                   |           | 18         | 22576                                   |
|          | 16         | 19236                                   |           | 18         | 21820                                   |
|          | 16         | 18768                                   |           | S10        | 64488                                   |
|          | 17         | 23558                                   |           | S10        | 67099                                   |
|          | 17         | 23528                                   |           | S10        | 70857                                   |
|          | 18         | 19205                                   |           | 4          | 20604                                   |
|          | 18         | 18214                                   |           | 18         | 21600                                   |
|          | S10        | 75026                                   |           | 11         | 33646                                   |
|          | S10        | 72623                                   |           | S10        | 41232                                   |
|          | S10        | 70197                                   |           | S10        | 37541                                   |
|          | S10        | 72023                                   |           | S10        | 33882                                   |
|          | S10        | 68772                                   |           | S1000      | 1568371                                 |
|          | 1          | 23296                                   |           | S10        | 35677                                   |
|          | 1          | 23429                                   |           | S1000      | 4154991                                 |
|          | 2          | 23893                                   |           | S1000      | 4169704                                 |
|          | 2          | 28091                                   |           | S1000      | 4213642                                 |
|          | 3          | 37852                                   |           | S1000      | 3978582                                 |
|          | 3          | 28083                                   |           | S10        | 42822                                   |
|          | 3          | 30731                                   |           | S10        | 39235                                   |
|          | 4          | 19760                                   |           | 2          | 20880                                   |
|          | 4          | 21278                                   |           | 2          | 20984                                   |
|          | S10        | 78599                                   |           | 3          | 20015                                   |
|          | S10        | 71697                                   |           | 3          | 19066                                   |
|          | 4          | 20339                                   |           | 4          | 17942                                   |
|          | 5          | 34050                                   |           | 4          | 17699                                   |
|          | 5          | 34347                                   |           | 5          | 17330                                   |
|          | 6          | 28618                                   |           | 5          | 17522                                   |
|          | 6          | 26107                                   |           | 5          | 16728                                   |
|          | 7          | 33503                                   |           | 6          | 16239                                   |
|          | 7          | 28751                                   |           | 6          | 16824                                   |
|          | 7          | 26800                                   |           | 7          | 21870                                   |
|          | 7          | 30365                                   |           | 7          | 21763                                   |
|          | 8          | 23791                                   |           | S1000      | 4149033                                 |
|          | 8          | 19795                                   |           | S1000      | 4071735                                 |
|          | 9          | 25100                                   |           | S10        | 34707                                   |
|          | 9          | 21226                                   |           | S10        | 37149                                   |
|          | 10         | 25179                                   |           | 11         | 23273                                   |
|          | 10         | 26054                                   |           | 11         | 23425                                   |
|          | 11         | 36393                                   |           | 13         | 19142                                   |
|          | 11         | 34744                                   |           | 13         | 18348                                   |
|          | 12         | 33913                                   |           | 15         | 26685                                   |
|          | 12         | 35819                                   |           | 15         | 26503                                   |
|          | 13         | 30271                                   |           | 16         | 30444                                   |
|          | 13         | 22061                                   |           | 16         | 30606                                   |
|          | 13         | 23224                                   |           | S1000      | 4079290                                 |
|          | 14         | 20281                                   |           | S1000      | 4049419                                 |
|          | 14         | 20413                                   |           |            |   |

| date      | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> | date      | bottle nr. | peak area C <sub>2</sub> H <sub>4</sub> |
|-----------|------------|---|-----------|------------|---|
| 14-Jun-99 | 1          | 21455                                   | 18-Jun-99 | 14         | 1532                                    |
|           | 7          | 19080                                   |           | 16         | 3261                                    |
|           | 9          | 20023                                   |           | S10        | 3375                                    |
|           | 9          | 19713                                   |           | 1          | 2259                                    |
|           | 10         | 21984                                   |           | 2          | 1431                                    |
|           | 11         | 20400                                   |           | 3          | 2027                                    |
|           | 11         | 21019                                   |           | 4          | 1756                                    |
|           | 12         | 36998                                   |           | 5          | 935                                     |
|           | 12         | 35588                                   |           | 6          | 1091                                    |
|           | 13         | 13042                                   |           | 7          | 1756                                    |
|           | 13         | 12567                                   |           | 8          | 1637                                    |
|           | 14         | 14368                                   |           | 9          | 1801                                    |
|           | 14         | 14909                                   |           | 10         | 1574                                    |
|           | 15         | 23910                                   |           | 11         | 1201                                    |
| 15        | 23620      |   |           | 12         | 2800                                    |
|           | 16         | 29847                                   |           | 13         | 1209                                    |
|           | S10        | 38233                                   |           | 14         | 959                                     |
|           | S10        | 34438                                   |           | 15         | 2116                                    |
|           | S10        | 35700                                   |           | 16         | 2976                                    |
|           | 17         | 18740                                   |           | 17         | 1116                                    |
|           | 17         | 16729                                   |           | 18         | 1162                                    |
|           | 13         | 12479                                   |           | S10        | 3346                                    |
|           | S1000      | 345465                                  | 28-Jun-99 | 2          | 2444                                    |
|           | S10        | 6355                                    |           | 9          | 1857                                    |
|           | S10        | 3775                                    |           | 17         | 897                                     |
|           | 13         | 1311                                    |           | 1          | 2148                                    |
|           | 13         | 1304                                    |           | 12         | 3076                                    |
|           | 1          | 20245                                   |           | 8          | 1299                                    |
|           | 1          | 22724                                   |           | 6          | 1103                                    |
|           | S10        | 37417                                   |           | 18         | 1382                                    |
|           | S10        | 37348                                   |           | 10         | 1670                                    |
| 18-Jun-99 | S10        | 2787                                    |           | 13         | 1601                                    |
|           | S10        | 2811                                    |           | 3          | 2366                                    |
|           | 1          | 2404                                    |           | 5          | 751                                     |
|           | 2          | 1552                                    |           | 11         | 1079                                    |
|           | 3          | 2084                                    |           | 16         | 4597                                    |
|           | 4          | 1994                                    |           | 4          | 2199                                    |
|           | 5          | 1201                                    |           | 15         | 2377                                    |
|           | 6          | 1366                                    |           | 7          | 1913                                    |
|           | 7          | 2001                                    |           | 14         | 1584                                    |
|           | 8          | 1684                                    |           | S10        | 3043                                    |
|           | 9          | 1734                                    |           | S10        | 3113                                    |
|           | 10         | 2157                                    |           | S10        | 2959                                    |
|           | 11         | 1554                                    |           |            |   |
|           | 12         | 3601                                    |           |            |   |
|           | 13         | 1593                                    |           |            |   |
|           | 14         | 1342                                    |           |            |   |
|           | 15         | 2004                                    |           |            |   |
|           | 16         | 2758                                    |           |            |   |
|           | 17         | 1768                                    |           |            |   |
|           | 18         | 1284                                    |           |            |   |
|           | S10        | 3094                                    |           |            |   |
|           | 1          | 2146                                    |           |            |   |
|           | 11         | 1592                                    |           |            |   |
|           | 12         | 2862                                    |           |            |   |

#### volume headspace and water content in bottles used in preliminary experiment

| bottle nr. | volume         | bottle nr. | water        |
|------------|----------------|------------|--------------|
|            | headspace [ml] |            | content [ml] |
| 3          | 62.35          | 6          | 26.40        |
| 4          | 61.54          | 18         | 23.46        |
| 7          | 62.99          | 1          | 22.97        |
| 10         | 65.67          | 9          | 21.02        |
| 11         | 61.99          | 15         | 25.03        |
| 12         | 64.15          | 2          | 24.73        |
| 16         | 63.72          | 13         | 22.09        |
| 17         | 61.75          | 14         | 20.95        |
|            |                | 5          | 27.41        |
| mean       | 63.02          | mean       | 23.78        |
| standard   | 1.42           | standard   | 2.28         |

For calculation of the accumulated production of ethylene in nmol/kg dry soil see chapter 2.1.3. Take in account that T was 30 C. Table 1 presents the final data of the preliminary experiment.

final results preliminary experiment

| incubation<br>time [hours] | 0                                | 24   | 48   | 72   | 96    | 168   | 264   | 336   | 432   | 504   | 672   |
|----------------------------|----------------------------------|------|------|------|-------|-------|-------|-------|-------|-------|-------|
| treatment                  | C2H4 accumulation [nmol/kg soil] |      |      |      |       |       |       |       |       |       |       |
| -/- (AN)                   | 0                                | 34.9 | 83.1 | 80.8 | 78.1  | 120.9 | 193.6 | 191.2 | 227.3 | 188.9 | 261.0 |
| CMW 1 (AN)                 | 0                                |      |      | 77.2 | 89.7  | 127.0 | 167.7 |       | 238.6 | 190.9 | 254.7 |
| CSC 1 (AN)                 | 0                                |      |      | 89.5 | 104.0 | 157.9 | 161.2 |       | 159.9 | 108.8 | 110.0 |
| CMW 1                      | 0                                |      | 47.1 | 67.7 | 76.1  | 126.3 | 199.0 | 171.9 | 219.1 | 174.0 | 182.1 |
| CSC1                       | 0                                |      | 66.7 | 69.7 | 80.0  | 119.6 |       | 189.2 | 232.6 | 172.8 | 199.8 |
| CMW 5 (AN)                 | 0                                | 39.8 | 75.3 | 73.1 | 86.8  | 170.8 | 209.7 | 258.3 | 287.6 | 206.7 | 237.7 |
| CSC 5 (AN)                 | 0                                | 38.7 | 63.9 | 72.8 | 75.9  | 107.9 | 166.9 | 119.7 | 174.5 | 107.7 | 174.9 |
| CMW 1                      | 0                                |      |      | 98.7 | 102.4 | 144.4 | 262.8 | 243.7 | 300.4 | 263.2 | 398.1 |
| CSC 5                      | 0                                |      |      | 97.7 | 100.0 | 127.6 |       |       | 179.1 | 115.4 | 127.9 |

## Appendix II

Determination dry bulk density  ${}^b\rho_d$  of the disturbed soil.

| tare  | tare + 100 ml<br>soil | weight 100 ml<br>soil [g] |
|-------|-----------------------|---------------------------|
| 45.36 | 216.92                | 171.56                    |
| 45.61 | 216.8                 | 171.19                    |
| <hr/> |                       |                           |
|       | tare + 25 ml soil     |                           |
| 56.32 | 95.26                 | 155.76                    |
|       | mean                  | 166.17                    |

$$\text{bulk density } {}^b\rho_d = 166.17 \text{ g/100 ml} = 1.66 \text{ kg/dm}^3$$

$$\text{solid phase in vol \%}, \phi_s = 1.66/2.65 = 0.626$$

$$\text{porosity } \phi_p = 1-0.626 = 0.374$$

Per sample 75.0 g of dry soil is used. To reach saturation : Per 100 ml soil should be added 37.4 ml water. Per 100 g of soil  $37.4/1.66 = 22.5$  g water was added. Per 75 g of soil  $0.75 \times 22.5 = 16.9$  g water was added.

### organic matter amendments

supposed tillage depth is 5 cm, application rates are 1 and 5 ton/ha

application of 1 ton/ha involves 1000 kg OM incorporated in  $0.05 \times 100 \times 100 = 500 \text{ m}^3$  soil  
 $500 \text{ m}^3$  soil equals  $500 \times 1.66 = 830$  ton soil.

Per 75 g of soil the application rate is  $75/830000 = 0.090$  g

An application rate of 5 ton/ha involves amending 75 g of soil with 0.452 g of OM.

## Appendix III

### Solubility of $\text{C}_2\text{H}_4$ in water

According to Seidell (1941), the solubility of gaseous  $\text{C}_2\text{H}_4$  in water compared with solubility in air  $K_{w,a}$  is described by the following equation  $K_{w,a} = 1 \cdot 10^{19} T^{-8.076}$ ,  $T$  in [K], see Figure 1. Table 1 shows the original data and Figure 1 shows the curve fitted through the data points. The solubility of ethylene in water at 25°C has been calculated by the Bunsen Absorption Coefficient  $\beta$ . This coefficient represents the volume of gas (reduced to 0° and 760 mm Hg pressure) absorbed by 1 volume of the liquid when the pressure of the gas itself without the tension of the liquid amounts to 760 mm Hg.

Table 1  $K_{w,a}$  values at different temperatures

| Temperature (°C) | $K_{w,a}$ |
|------------------|-----------|
| 0                | 0.226     |
| 5                | 0.191     |
| 10               | 0.162     |
| 15               | 0.139     |
| 20               | 0.122     |
| 25               | 0.108     |
| 30               | 0.098     |
| 37.5             | 0.078     |

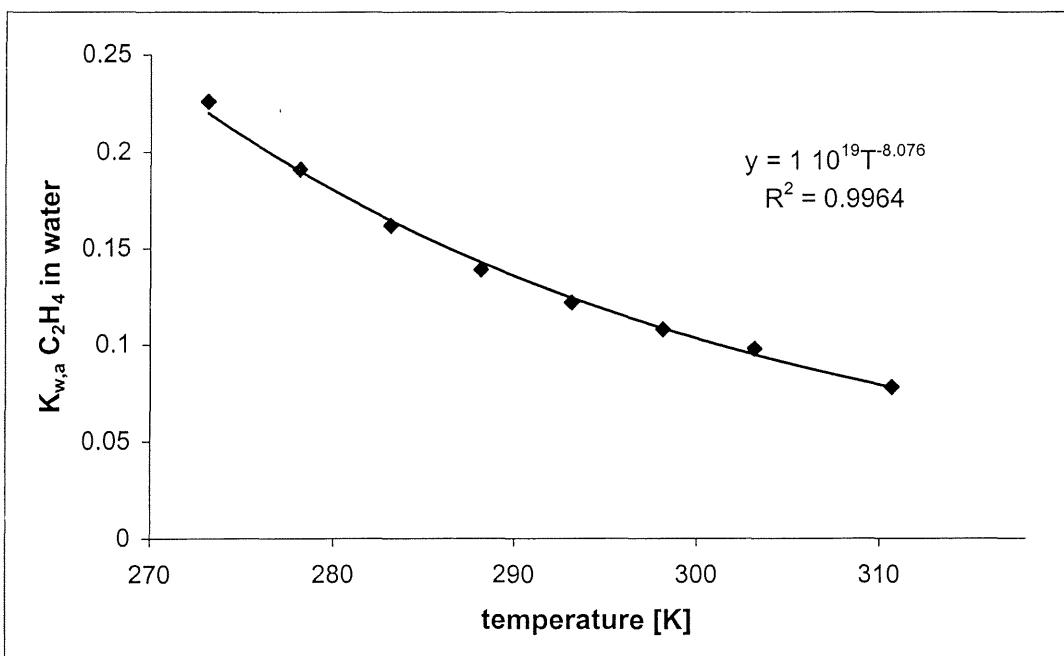


Figure 1 Solubility of ethylene as a function of temperature

## Appendix IV

| trt # | sample<br>nrs | temperature<br>[C] | organic matter |                               |
|-------|---------------|--------------------|----------------|-------------------------------|
|       |               |                    | source         | application<br>rate [ton/ha]* |
| 1     | 1-4           | 35                 | -              | -                             |
| 2     | 5-8           | 35                 | BS             | 1                             |
| 3     | 9-12          | 35                 | CMW            | 1                             |
| 4     | 13-16         | 35                 | CSC            | 1                             |
| 5     | 17-20         | 35                 | BS             | 5                             |
| 6     | 21-24         | 35                 | CMW            | 5                             |
| 7     | 25-28         | 35                 | CSC            | 5                             |
| 8     | 29-32         | 25/45              | -              | -                             |
| 9     | 33-36         | 25/45              | BS             | 1                             |
| 10    | 37-40         | 25/45              | CMW            | 1                             |
| 11    | 41-44         | 25/45              | CSC            | 1                             |
| 12    | 45-48         | 25/45              | BS             | 5                             |
| 13    | 49-52         | 25/45              | CMW            | 5                             |
| 14    | 53-56         | 25/45              | CSC            | 5                             |
| 15    | 57-60         | 35                 | -              | -                             |
| 18    | 69-72         | 35                 | CMW            | 5                             |
| 19    | 73-76         | 35                 | -              | -                             |

note: the treatment order is different from Table 4, chapter 2.1.2.2

| serie               | sample# | $V_{hs}$ | moisture content,<br>$V_w$ [ml] |         |            |
|---------------------|---------|----------|---------------------------------|---------|------------|
|                     |         |          | serie                           | sample# | $V_w$ [ml] |
| 25/45               | 55      | 71.40    | 25/45                           | 35      | 16.17      |
| 25/45               | 44      | 72.82    | 25/45                           | 49      | 15.52      |
| 25/45               | 51      | 72.33    | 25/45                           | 67      | 15.2       |
| 25/45               | 52      | 71.23    | 25/45                           | 31      | 18.63      |
| 25/45               | 36      | 71.34    | 25/45                           | 66      | 15.1       |
| 25/45               | 43      | 71.42    | 25/45                           | 30      | 15.96      |
| 25/45               | 64      | 71.36    | 25/45                           | 33      | 15.52      |
| 25/45               | 34      | 71.99    | 25/45                           | 37      | 15.98      |
| 25/45               | 54      | 71.72    | 25/45                           | 42      | 15.99      |
| 25/45               | 48      | 70.93    | 25/45                           | 65      | 15.48      |
| 25/45               | 38      | 71.74    | 25/45                           | 46      | 16.21      |
| 35                  | 23      | 72.31    | 25/45                           | 40      | 15.77      |
| 35                  | 72      | 72.30    | 25/45                           | 47      | 16.13      |
| 35                  | 73      | 70.25    | 25/45                           | 53      | 15.79      |
| 35                  | 2       | 72.23    | 25/45                           | 62      | 16.16      |
| 35                  | 71      | 71.33    | 25/45                           | 63      | 16.15      |
| 35                  | 11      | 72.54    | 25/45                           | 39      | 15.78      |
| 35                  | 3       | 71.71    | 25/45                           | 61      | 16.41      |
| 35                  | 58      | 71.11    | 35                              | 21      | 15.42      |
| 35                  | 14      | 72.19    | 35                              | 27      | 15.56      |
| 35                  | 8       | 72.41    | 35                              | 5       | 15.67      |
| 35                  | 16      | 71.60    | 35                              | 10      | 16.01      |
| 35                  | 9       | 71.57    | 35                              | 60      | 15.99      |
| 35                  | 28      | 70.98    | 35                              | 20      | 17.22      |
| 35                  | 6       | 71.16    | 35                              | 15      | 15.66      |
| 35                  | 75      | 70.79    | 35                              | 7       | 15.77      |
| 35                  | 26      | 72.07    | 35                              | 74      | 17.33      |
| 35                  | 12      | 71.23    | 35                              | 70      | 16.4       |
| 35                  | 22      | 72.05    | 35                              | 13      | 16.05      |
|                     |         |          | 35                              | 19      | 16.48      |
| mean:               |         | 71.66    | 35                              | 76      | 17.75      |
| standard deviation: |         | 0.60     | 35                              | 17      | 15.4       |
|                     |         |          | 35                              | 57      | 15.58      |
|                     |         |          | 35                              | 18      | 15.65      |
| mean:               |         |          |                                 |         | 16.06      |
| standard deviation: |         |          |                                 |         | 0.73       |

peak areas for 35 serie

incubation started June 17 at 15:15

| sample # | treatment # | incubation time |      |      |       |      |
|----------|-------------|-----------------|------|------|-------|------|
|          |             | 22              | 145  | 194  | 290   | 461  |
| 1        | 1           | 919             | 1794 | 1619 | 1739  | 1357 |
| 2        | 1           | 881             | 1716 | 1592 | 1979  | 688  |
| 3        | 1           | 861             | 1735 | 1669 | 1617  | 0    |
| 4        | 1           | 765             | 1687 | 1607 | 1799  | 1697 |
| 5        | 2           | 936             | 1759 | 1583 | 1875  | 1990 |
| 6        | 2           | 938             | 1707 | 1590 | 1900  | 1189 |
| 7        | 2           | 905             | 1700 | 1545 | 1788  | 1968 |
| 8        | 2           | 1044            | 1568 | 1566 | 2284  | 309  |
| 9        | 3           | 871             | 1690 | 1552 | 2091  | 1955 |
| 10       | 3           | 945             | 1669 | 1706 | 2421  | 1428 |
| 11       | 3           | 844             | 1715 | 1655 | 1810  | 1743 |
| 12       | 3           | 927             | 1533 | 1501 | 1580  | 1216 |
| 13       | 4           | 797             | 1756 | 1640 | 3860  | 967  |
| 14       | 4           | 951             | 1784 | 1650 | 2094  | 1321 |
| 15       | 4           | 853             | 1671 | 1568 | 1412  | 1485 |
| 16       | 4           | 869             | 1664 | 1641 | 1871  | 489  |
| 17       | 5           | 1314            | 1736 | 1672 | 2436  | 2490 |
| 18       | 5           | 1194            | 1926 | 1674 | 2553  | 1909 |
| 19       | 5           | 1137            | 1865 | 1568 | 2552  | 1935 |
| 20       | 5           | 1213            | 1713 | 1554 | 2568  | 2537 |
| 21       | 6           | 849             | 1613 | 1603 | 1750  | 2087 |
| 22       | 6           | 753             | 1826 | 1879 | 1984  | 2623 |
| 23       | 6           | 965             | 1951 | 1838 | 1870  | 1131 |
| 24       | 6           | 977             | 2004 | 1832 | 3000  | 3231 |
| 25       | 7           | 885             | 1411 | 1355 | 2376  | 195  |
| 26       | 7           | 928             | 1637 | 1622 | 2590  | 809  |
| 27       | 7           | 865             | 1514 | 1459 | 11982 | 1737 |
| 28       | 7           | 899             | 1563 | 1481 | 2441  | 1331 |
| 57       | 15          | 2573            | 3476 | 3273 | 3870  | 4120 |
| 58       | 15          | 1726            | 2646 | 2587 | 2789  | 3259 |
| 59       | 15          | 1647            | 2203 | 2102 | 2493  | 2075 |
| 60       | 15          | 2078            | 3124 | 2966 | 3223  | 3314 |
| 69       | 18          | 637             | 1353 | 1132 | 2576  | 2086 |
| 70       | 18          | 512             | 1531 | 1487 | 1976  | 1619 |
| 71       | 18          | 492             | 1429 | 1337 | 1866  | 1497 |
| 72       | 18          | 620             | 1362 | 1214 | 1831  | 1064 |
|          |             |                 | 27   | 76   | 172   | 504  |
| 73       | 19          |                 | 0    | 0    | 218   | 353  |
| 74       | 19          |                 | 0    | 0    | 0     | 260  |
| 75       | 19          |                 | 0    | 0    | 0     | 0    |
| 76       | 19          |                 | 0    | 0    | 0     | 0    |

S10            3114    3339    2906.5    3071    2963

S10 represents the peak area of the 10 ppm standard sample

**peak areas for 25/45 serie**

incubation started June 16 at 12.05

| sample # | treatment # | incubation time |      |      |      |      |      |
|----------|-------------|-----------------|------|------|------|------|------|
|          |             | 29              | 145  | 194  | 290  | 461  | 625  |
| 29       | 8           | 965             | 1459 | 2216 | 2148 | 1927 | 1950 |
| 30       | 8           | 569             | 1984 | 2151 | 2303 | 2495 | 2179 |
| 31       | 8           | 671             | 1009 | 1742 | 1784 | 2111 | 1806 |
| 32       | 8           | 1069            | 2254 | 2436 | 2276 | 2485 | 1911 |
| 33       | 9           | 930             | 1450 | 2125 | 1956 | 1957 | 1735 |
| 34       | 9           | 1063            | 2044 | 2039 | 2115 | 2418 | 2122 |
| 35       | 9           | 745             | 1416 | 1881 | 1898 | 1983 | 1951 |
| 36       | 9           | 666             | 1832 | 1991 | 2042 | 2348 | 2055 |
| 37       | 10          | 1095            | 2169 | 2407 | 2317 | 2548 | 2338 |
| 38       | 10          | 917             | 1973 | 2135 | 2036 | 4035 | 2109 |
| 39       | 10          | 992             | 2014 | 2138 | 2030 | 2355 | 1601 |
| 40       | 10          | 930             | 1933 | 2124 | 1961 | 2052 | 1890 |
| 41       | 11          | 1525            | 1790 | 1933 | 1934 | 2102 | 1567 |
| 42       | 11          | 1034            | 1374 | 2045 | 2044 | 1963 | 1450 |
| 43       | 11          | 1071            | 1690 | 1903 | 1912 | 1926 | 1395 |
| 44       | 11          | 968             | 1307 | 1868 | 1704 | 2083 | 1768 |
| 45       | 12          | 1040            | 1672 | 1924 | 1885 | 2038 | 1816 |
| 46       | 12          | 991             | 1827 | 1864 | 1909 | 2575 | 1989 |
| 47       | 12          | 1006            | 1582 | 1704 | 1731 | 1842 | 1769 |
| 48       | 12          | 1305            | 1457 | 1853 | 1845 | 1942 | 1895 |
| 49       | 13          | 1022            | 2038 | 2075 | 2195 | 2407 | 2293 |
| 50       | 13          | 940             | 1848 | 2074 | 2122 | 2183 | 1605 |
| 51       | 13          | 862             | 1595 | 2083 | 2055 | 2111 | 871  |
| 52       | 13          | 1000            | 2122 | 2934 | 2200 | 2286 | 1539 |
| 53       | 14          | 802             | 1508 | 1589 | 1751 | 2225 | 1048 |
| 54       | 14          | 873             | 1191 | 1682 | 1694 | 1833 | 1740 |
| 55       | 14          | 991             | 1356 | 1745 | 1659 | 2037 | 927  |
| 56       | 14          | 833             | 1113 | 1710 | 1631 | 2037 | 1795 |
| 65       | 17          | 614             | 1398 | 1578 | 1633 | 2181 | 1725 |
| 66       | 17          | 645             | 1157 | 1638 | 1709 | 1916 | 1579 |
| 67       | 17          | 684             | 1150 | 1782 | 1900 | 1953 | 1526 |
| 68       | 17          | 643             | 1424 | 1623 | 1613 | 1742 | 1692 |

S10            2992    3150    3377.5    3038    3196    2880

S10 represents the peak area of the 10 ppm standard sample

**Results of applying General Linear Models Procedure to calculated accumulated ethylene production [nmol/kg dry soil]**

Factor analysis

Factor analysis determined whether the factors temperature (T) regime, type of organic matter or interaction between those, and anaerobic conditions (pO<sub>2</sub>) had significant influence on the C<sub>2</sub>H<sub>4</sub> production. The outcomes are presented as F-probabilities. Factor analysis was carried out per sampling time, according to Table 5 of chapter 2.1.4.

*Factor analysis for temperature type of organic matter, anoxic conditions, and interaction between these two*

| T regime           | BS    | CMW    | CSC    | T * BS | T*CMW | T*CSC | PO <sub>2</sub> |
|--------------------|-------|--------|--------|--------|-------|-------|-----------------|
| <i>t=22 hours</i>  |       |        |        |        |       |       |                 |
| -                  | 0.000 | 0.0031 | 0.9406 | -      | -     | -     | 0.000           |
| <i>t=29 hours</i>  |       |        |        |        |       |       |                 |
| -                  | 0.113 | 0.130  | 0.786  | -      | -     | -     | 0.001           |
| <i>t=145 hours</i> |       |        |        |        |       |       |                 |
| 0.452              | 0.376 | 0.886  | 0.003  | 0.486  | 0.903 | 0.111 | 0.000           |
| <i>t=194 hours</i> |       |        |        |        |       |       |                 |
| 0.012              | 0.599 | 0.750  | 0.002  | 0.235  | 0.820 | 0.112 | 0.000           |
| <i>t=290 hours</i> |       |        |        |        |       |       |                 |
| -                  | 0.298 | 0.714  | 0.000  | -      | -     | -     | 0.000           |
| <i>t=461 hours</i> |       |        |        |        |       |       |                 |
| 0.715              | 0.169 | 0.122  | 0.436  | 0.042  | 0.014 | 0.453 | 0.001           |
| <i>t=626 hours</i> |       |        |        |        |       |       |                 |
| -                  | 0.230 | 0.328  | 0.003  | -      | -     | -     | 0.656           |

## Appendix V

### Protocol column column built-up

Packing the column:

1. PVC pipe with inside  $r = 38.4$  mm was sawn in pieces of 5 (2 times), 7.5 and 12.5 cm. The column was built up at the base with the 7.5-cm part followed by the two parts of 5 cm high and completed with the 12.5-cm part. The complete column was filled with soil up to the 12.5-cm part.
2. From the top of the soil column downward, at three depths gas chambers were put inside the column, namely at 2.5, 7.5 and 12.5 cm. The gas chambers were made of a sawn through syringe with an internal volume of about 0.5 ml and connected with a septum. The holes that were drilled in the PVC had a radius of 2.9 mm. The open end was closed by a piece of linen preventing soil movement inside the gas chamber.
3. When soil was put in the different parts it was tamped down to reach a homogeneous  $^b\rho_d$  of 1.80 g/cm<sup>3</sup>. According to the heights of the parts this resulted in the following soil weights:

$$h = 5.0 \text{ cm} \quad \pi r^2 h \ ^b\rho_d = \pi 3.84^2 5 1.80 = 417 \text{ g}$$

$$h = 7.5 \text{ cm} \quad \pi r^2 h \ ^b\rho_d = \pi 3.84^2 7.5 1.80 = 625 \text{ g}$$

The volume of the gas chamber was neglected for this calculation.

The internal volume  $V_c$  of the column was equal to:  $V_c = \pi r^2 h = \pi 3.84^2 17.5 = 810.7 \text{ cm}^3$ ; the weight of the soil per column was  $V_c \ ^b\rho_d = 1459.2 \text{ g}$ .

4. Organic matter was applied as cotton milling waste (CMW). For C/N ratio, see Table 2 of paragraph 2.1.2.1. A field application rate of 5 ton/ha resulted in adding 2.32 g of CMW to this part with a surface area of  $\pi r^2 = 46.3 \text{ cm}^2 = 46.3 10^{-4} \text{ m}^2 = 46.3 10^{-8} \text{ ha}$ . The application rate of OM was  $5000 10^3 \text{ g}$ , so  $5000 10^3 46.3 10^{-8}$  resulted in application of 2.32 g per column, for a matter of fact only restricted to the upper most part of 5 cm.

| Column # | Organic matter | Crust |
|----------|----------------|-------|
| 1-3      | -              | -     |
| 4-6      | +              | -     |
| 7-9      | -              | +     |
| 10-12    | +              | +     |

5. The 12 columns were moistened from below in a container that was tilted to an angle of 11° in which the water level could be altered. Six columns with no crust formation on top were covered by the part with a height of 12.5 cm. The other columns with crust formation were artificially smeared using a small teaspoon at saturation and covered with the 12.5-cm part. After saturation of water, the columns were drained for 35 hours by removing a rubber stopper in the bottom of the container. The container remained tilted so that water in the gas chambers drained into the soil. For security the gas chambers

were injected with air to remove lagged water. A preliminary experiment gave an indication of the rate of drainage, see Figure 1.

6. Sampling took place 5, 25, 54, 104, 194, and 361 hours after stopping the drainage. 0.01 ml of air was injected before a gas sample was withdrawn from the gas chamber to prevent sampling liquid water.

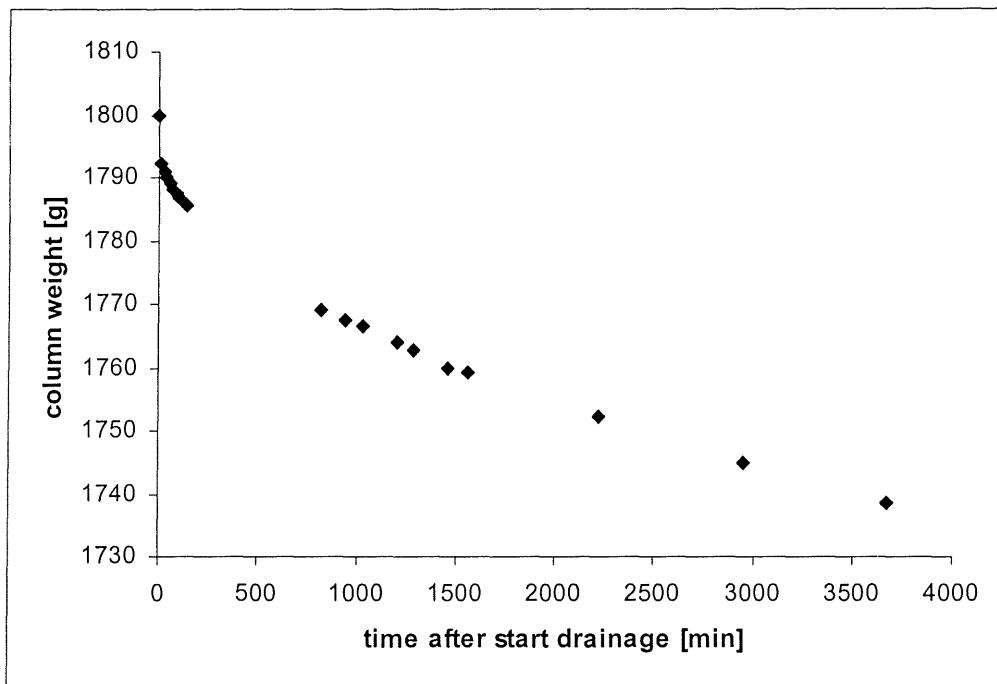


Figure 1 *Drainage rate in a test column with the same  ${}^b\rho_d$  as in the experiment*

## Appendix VI

Measured values for moisture content per layer per column and calculated values for volumetric moisture content, air filled porosity, tortuosity, and diffusion coefficient of ethylene in the soil.

| column# | depth   | tare weight [g] | tare + soil + moisture [g] | tare + dry soil [g] | moisture weight [g] | dry soil weight [g] | total weight of dry soil per column [g] |
|---------|---------|-----------------|----------------------------|---------------------|---------------------|---------------------|---|
| 1       | 0-5     | 14.19           | 431.62                     | 389.61              | 42.01               | 375.42              |   |
| 1       | 5-10    | 13.75           | 486.47                     | 438.33              | 48.14               | 424.58              |   |
| 1       | 10-17.5 | 13.82           | 731.81                     | 660.2               | 71.61               | 646.38              | 1446.38                                 |
| 2       | 0-5     | 13.91           | 435.68                     | 395.05              | 40.63               | 381.14              |   |
| 2       | 5-10    | 10.63           | 467.91                     | 424.37              | 43.54               | 413.74              |   |
| 2       | 10-17.5 | 14.31           | 735.78                     | 667                 | 68.78               | 652.69              | 1447.57                                 |
| 3       | 0-5     | 10.67           | 440.18                     | 397.98              | 42.20               | 387.31              |   |
| 3       | 5-10    | 9.57            | 476.49                     | 428.4               | 48.09               | 418.83              |   |
| 3       | 10-17.5 | 10.67           | 717.21                     | 644.81              | 72.40               | 634.14              | 1440.28                                 |
| 4       | 0-5     | 9.61            | 437.30                     | 391.66              | 45.64               | 382.05              |   |
| 4       | 5-10    | 10.54           | 489.36                     | 438.85              | 50.51               | 428.31              |   |
| 4       | 10-17.5 | 10.55           | 722.25                     | 650.27              | 71.98               | 639.72              | 1450.08                                 |
| 5       | 0-5     | 10.57           | 446.29                     | 401.84              | 44.45               | 391.27              |   |
| 5       | 5-10    | 8.33            | 469.88                     | 423.13              | 46.75               | 414.8               |   |
| 5       | 10-17.5 | 10.64           | 727.76                     | 654.07              | 73.69               | 643.43              | 1449.5                                  |
| 6       | 0-5     | 10.54           | 440.64                     | 393.93              | 46.71               | 383.39              |   |
| 6       | 5-10    | 10.58           | 482.83                     | 434.48              | 48.35               | 423.9               |   |
| 6       | 10-17.5 | 9.61            | 722.44                     | 650.54              | 71.90               | 640.93              | 1448.22                                 |
| 7       | 0-5     | 9.6             | 443.69                     | 399.83              | 43.86               | 390.23              |   |
| 7       | 5-10    | 9.7             | 473.66                     | 428.07              | 45.59               | 418.37              |   |
| 7       | 10-17.5 | 9.76            | 729.64                     | 658.61              | 71.03               | 648.85              | 1457.45                                 |
| 8       | 0-5     | 10.71           | 446.72                     | 401.02              | 45.70               | 390.31              |   |
| 8       | 5-10    | 10.74           | 486.10                     | 436.34              | 49.76               | 425.6               |   |
| 8       | 10-17.5 | 10.73           | 715.00                     | 643.12              | 71.88               | 632.39              | 1448.3                                  |
| 9       | 0-5     | 10.77           | 439.50                     | 395.92              | 43.58               | 385.15              |   |
| 9       | 5-10    | 10.65           | 462.49                     | 416.99              | 45.50               | 406.34              |   |
| 9       | 10-17.5 | 10.67           | 726.61                     | 653.53              | 73.08               | 642.86              | 1434.35                                 |
| 10      | 0-5     | 10.72           | 450.57                     | 403.85              | 46.72               | 393.13              |   |
| 10      | 5-10    | 10.71           | 473.46                     | 426.85              | 46.61               | 416.14              |   |
| 10      | 10-17.5 | 10.64           | 720.35                     | 649.89              | 70.46               | 639.25              | 1448.52                                 |
| 11      | 0-5     | 10.55           | 438.77                     | 392.33              | 46.44               | 381.78              |   |
| 11      | 5-10    | 10.68           | 478.00                     | 428.32              | 49.68               | 417.64              |   |
| 11      | 10-17.5 | 10.56           | 723.67                     | 648.59              | 75.08               | 638.03              | 1437.45                                 |
| 12      | 0-5     | 10.56           | 434.30                     | 388.58              | 45.72               | 378.02              |   |
| 12      | 5-10    | 10.67           | 483.79                     | 433.62              | 50.17               | 422.95              |   |
| 12      | 10-17.5 | 9.28            | 728.36                     | 656.15              | 72.21               | 646.87              | 1447.84                                 |

| column# | porosity | gas phase | depth z [cm] | average gas phase | tortuosity factor | diffusion coefficient in soil [m <sup>2</sup> /s] |
|---------|----------|-----------|--------------|-------------------|-------------------|---|
| 1       | 0.388    | 0.207     | 0-2.5        | 0.207             | 0.107             | 3.4581E-07  |
| 1       | 0.308    | 0.100     | 2.5 - 7.5    | 0.154             | 0.063             | 1.5118E-07  |
| 1       | 0.298    | 0.092     | 7.5 - 12.5   | 0.096             | 0.027             | 4.0505E-08  |
| 2       | 0.379    | 0.204     | 0-2.5        | 0.204             | 0.104             | 3.3065E-07  |
| 2       | 0.326    | 0.138     | 2.5 - 7.5    | 0.171             | 0.077             | 2.0531E-07  |
| 2       | 0.291    | 0.093     | 7.5 - 12.5   | 0.115             | 0.039             | 7.033E-08   |
| 3       | 0.369    | 0.187     | 0-2.5        | 0.187             | 0.089             | 2.5956E-07  |
| 3       | 0.318    | 0.110     | 2.5 - 7.5    | 0.148             | 0.059             | 1.3669E-07  |
| 3       | 0.311    | 0.103     | 7.5 - 12.5   | 0.106             | 0.034             | 5.6478E-08  |
| 4       | 0.378    | 0.180     | 0-2.5        | 0.180             | 0.084             | 2.3674E-07  |
| 4       | 0.302    | 0.084     | 2.5 - 7.5    | 0.132             | 0.049             | 1.0121E-07  |
| 4       | 0.305    | 0.098     | 7.5 - 12.5   | 0.091             | 0.024             | 3.4104E-08  |
| 5       | 0.362    | 0.171     | 0-2.5        | 0.171             | 0.076             | 2.0246E-07  |
| 5       | 0.324    | 0.122     | 2.5 - 7.5    | 0.146             | 0.058             | 1.3264E-07  |
| 5       | 0.301    | 0.089     | 7.5 - 12.5   | 0.106             | 0.033             | 5.4442E-08  |
| 6       | 0.375    | 0.174     | 0-2.5        | 0.174             | 0.079             | 2.1425E-07  |
| 6       | 0.309    | 0.101     | 2.5 - 7.5    | 0.137             | 0.052             | 1.1135E-07  |
| 6       | 0.304    | 0.097     | 7.5 - 12.5   | 0.099             | 0.029             | 4.4703E-08  |
| 7       | 0.364    | 0.175     | 0-2.5        | 0.175             | 0.08              | 2.1841E-07  |
| 7       | 0.318    | 0.121     | 2.5 - 7.5    | 0.148             | 0.059             | 1.3651E-07  |
| 7       | 0.295    | 0.091     | 7.5 - 12.5   | 0.106             | 0.034             | 5.6347E-08  |
| 8       | 0.364    | 0.167     | 0-2.5        | 0.167             | 0.073             | 1.901E-07   |
| 8       | 0.307    | 0.092     | 2.5 - 7.5    | 0.129             | 0.048             | 9.6869E-08  |
| 8       | 0.313    | 0.106     | 7.5 - 12.5   | 0.099             | 0.029             | 4.481E-08   |
| 9       | 0.372    | 0.184     | 0-2.5        | 0.184             | 0.087             | 2.5042E-07  |
| 9       | 0.338    | 0.141     | 2.5 - 7.5    | 0.163             | 0.07              | 1.7808E-07  |
| 9       | 0.302    | 0.091     | 7.5 - 12.5   | 0.116             | 0.04              | 7.2725E-08  |
| 10      | 0.359    | 0.158     | 0-2.5        | 0.158             | 0.066             | 1.6259E-07  |
| 10      | 0.322    | 0.121     | 2.5 - 7.5    | 0.139             | 0.054             | 1.1742E-07  |
| 10      | 0.306    | 0.103     | 7.5 - 12.5   | 0.112             | 0.037             | 6.4584E-08  |
| 11      | 0.378    | 0.177     | 0-2.5        | 0.177             | 0.082             | 2.2724E-07  |
| 11      | 0.320    | 0.105     | 2.5 - 7.5    | 0.141             | 0.055             | 1.2131E-07  |
| 11      | 0.307    | 0.091     | 7.5 - 12.5   | 0.098             | 0.027             | 4.1294E-08  |
| 12      | 0.384    | 0.187     | 0-2.5        | 0.187             | 0.089             | 2.5948E-07  |
| 12      | 0.311    | 0.094     | 2.5 - 7.5    | 0.140             | 0.054             | 1.1846E-07  |
| 12      | 0.297    | 0.089     | 7.5 - 12.5   | 0.092             | 0.025             | 3.5871E-08  |

## Appendix VII

### Calculation scheme for production and diffusion of ethylene at various depths in the column

- (1) date: August 13 (25 hours incubation)
- (2) column : 2  
depth :  $z = 7.5 \text{ cm}$   
temperature at analysing : 298 K
- (3) peak area  $\text{C}_2\text{H}_4$  by gas chromatograph: 2745
- (4) average peak area standard sample 10 ppm: 2724
- (5) concentration  $\text{C}_2\text{H}_4$  in the soil at  $z=7.5 \text{ cm}$  was equal to 10.08 ppm  
The concentration in  $\text{mg}/\text{m}^3$  is could be derived using the equation:  
$$1 \text{ ppmV} = \frac{P}{RT} \frac{Mw}{10^6} 10^3 = 1.145 \text{ mg}/\text{m}^3$$
and was equal to  $11.54 \text{ mg}/\text{m}^3$ .
- (6) moisture weight layer between  $z=5.0$  and  $z=10.0 \text{ cm}$  : 48.35 g  
volume part of column between  $z=5.0$  and  $z=10.0$  :  $\pi 3.84^2 5 = 231.6 \text{ cm}^3$   
dry weight soil layer between  $z=5.0$  and  $z=10.0 \text{ cm}$  : 423.90 g
- (7) porosity  $\phi_p = 1 - \text{dry weight soil [g] / density solid parts of the soil [g}/\text{cm}^3]$  / volume soil  $[\text{cm}^3]$  ;  $\phi_p = 1 - \{(423.90/2.65)/231.6 = 0.310$
- (8) air filled porosity,  $\phi_g$ , is related with  $\phi_p$  with the equation:  $\phi_p = \phi_g + \theta$ ;  $\theta$  can be derived from the moisture content of the layer between  $z=5.0$  and  $z=10.0 \text{ cm}$  and equals 48.35 g.  
The moisture content  $\theta$  is the result of the division: (moisture volume/total volume);  
$$\phi_g \text{ at } z = 5.0 = \frac{\phi_g \text{ of layer } z = 0.0 \text{ to } z = 5.0 + \phi_g \text{ of layer } z = 5.0 \text{ to } z = 10.0}{2}$$
- (9) The tortuosity factor,  $\tau$ , for the layers between  $z=2.5$  and  $z=7.5 \text{ cm}$  and  $z=7.5$  and  $z=12.5 \text{ cm}$  were calculated by linear interpolation by using Table 8, chapter 2.2.3. The values for  $\tau$  were equal to 0.052 and 0.029 respectively.
- (10) The diffusion coefficient was calculated by using equation (2) of chapter 2.2.3.  
$$D_g = \tau \phi_g D_0$$
; the value for  $D_g$  for the layer between  $z=2.5$  and  $z=7.5 \text{ cm}$  was  $1.11 \cdot 10^{-7} \text{ m}^2/\text{s}$ .

(11) With equation (1) of chapter 2.2.3 the flux density was calculated at a depth of  $z=5.0$  cm. The concentration gradient  $dc/dz$  for  $z=2.5$  and  $z=7.5$  was equal to:

$C_{C_2H_4(7.5)} - C_{C_2H_4(2.5)} / 7.5 - 2.5$ ; the  $C_2H_4$  concentration at  $z=7.5$  cm was equal to  $11.54 \text{ mg/m}^3$  and the  $C_2H_4$  concentration at  $z=2.5$  cm was equal to  $5.38 \text{ mg/m}^3$ . The concentration gradient  $dc/dz$  was equal to  $123.25 \text{ mg/m}^4$ . The flux density  $q$  was calculated as:

$$q = -D_g \frac{dc}{dz} \text{ and found equal to } -1.37 \cdot 10^{-5} \text{ mg m}^{-2} \text{ s}^{-1}$$

(12) With the flux density at  $z=5.0$  and  $z=10.0$  the production  $\alpha$  of  $C_2H_4$  was calculated with equation (3) of chapter 2.2.3. Flux density at  $z=10.0$  was equal to  $-3.48 \cdot 10^{-5} \text{ mg m}^{-2} \text{ s}^{-1}$ . The production was found equal to  $-4.22 \cdot 10^{-4} \text{ mg m}^{-3} \text{ s}^{-1}$ . A negative sign before the amount of  $C_2H_4$  production indicates absorption or consumption of the  $C_2H_4$ .

## Appendix VIII

### ACTUAL PRODUCTION OF ETHYLENE IN A SOIL COLUMN AT DEPTHS OF 2.5, 7.5 AND 12.5 cm.

| column#           | depth [cm] | treatment       |       | peak area | C2H4 conc.<br>[ppm] | 5         |                     | 25        |                     |
|-------------------|------------|-----------------|-------|-----------|---------------------|-----------|---------------------|-----------|---------------------|
|                   |            | CMW 5<br>ton/ha | crust |           |                     | peak area | C2H4 conc.<br>[ppm] | peak area | C2H4 conc.<br>[ppm] |
| 1                 | headspace  | -               | -     | 282       | 1.02                | 296       | 1.09                |           |                     |
|                   | 2.5        | -               | -     | 216       | 0.78                | 485       | 1.78                |           |                     |
|                   | 7.5        | -               | -     | 5813      | 21.04               | 11734     | 43.08               |           |                     |
|                   | 12.5       | -               | -     | 2898      | 10.49               | 9308      | 34.17               |           |                     |
| 2                 | headspace  | -               | -     | 349       | 1.26                | 574       | 2.11                |           |                     |
|                   | 2.5        | -               | -     | 361       | 1.31                | 373       | 1.37                |           |                     |
|                   | 7.5        | -               | -     | 3939      | 14.26               | 10316     | 37.87               |           |                     |
|                   | 12.5       | -               | -     | 1688      | 6.11                | 8208      | 30.13               |           |                     |
| 3                 | headspace  | -               | -     | 290       | 1.05                | 440       | 1.62                |           |                     |
|                   | 2.5        | -               | -     | 276       | 1.00                | 874       | 3.21                |           |                     |
|                   | 7.5        | -               | -     | 309       | 1.12                | 550       | 2.02                |           |                     |
|                   | 12.5       | -               | -     | 3979      | 14.40               | 12589     | 46.22               |           |                     |
| 4                 | headspace  | +               | -     | 362       | 1.31                | 636       | 2.33                |           |                     |
|                   | 2.5        | +               | -     | 297       | 1.08                | 904       | 3.32                |           |                     |
|                   | 7.5        | +               | -     | 1679      | 6.08                | 7750      | 28.45               |           |                     |
|                   | 12.5       | +               | -     | 1814      | 6.57                | 7045      | 25.86               |           |                     |
| 5                 | headspace  | +               | -     | 268       | 0.97                | 619       | 2.27                |           |                     |
|                   | 2.5        | +               | -     | 2196      | 7.95                |           | -                   |           |                     |
|                   | 7.5        | +               | -     | 3003      | 10.87               | 9892      | 36.31               |           |                     |
|                   | 12.5       | +               | -     | 2970      | 10.75               | 3777      | 13.87               |           |                     |
| 6                 | headspace  | +               | -     | 507       | 1.84                | 1028      | 3.77                |           |                     |
|                   | 2.5        | +               | -     | 406       | 1.47                | 1280      | 4.70                |           |                     |
|                   | 7.5        | +               | -     | 794       | 2.87                | 2745      | 10.08               |           |                     |
|                   | 12.5       | +               | -     | 2285      | 8.27                | 12011     | 44.09               |           |                     |
| 7                 | headspace  | -               | +     | 0         | -                   | 414       | 1.52                |           |                     |
|                   | 2.5        | -               | +     | 344       | 1.25                | 840       | 3.08                |           |                     |
|                   | 7.5        | -               | +     | 1242      | 4.50                | 6851      | 25.15               |           |                     |
|                   | 12.5       | -               | +     | 1163      | 4.21                | 1738      | 6.38                |           |                     |
| 8                 | headspace  | -               | +     | 377       | 1.36                | 445       | 1.63                |           |                     |
|                   | 2.5        | -               | +     | 289       | 1.05                | 703       | 2.58                |           |                     |
|                   | 7.5        | -               | +     | 1619      | 5.86                | 8127      | 29.83               |           |                     |
|                   | 12.5       | -               | +     | 3529      | 12.77               | 12246     | 44.96               |           |                     |
| 9                 | headspace  | -               | +     | 220       | 0.80                | 448       | 1.64                |           |                     |
|                   | 2.5        | -               | +     | 386       | 1.40                | 698       | 2.56                |           |                     |
|                   | 7.5        | -               | +     | 1440      | 5.21                | 2854      | 10.48               |           |                     |
|                   | 12.5       | -               | +     | 1331      | 4.82                | 10955     | 40.22               |           |                     |
| 10                | headspace  | +               | +     | 278       | 1.01                | 0         | -                   |           |                     |
|                   | 2.5        | +               | +     | 3919      | 14.19               | 2367      | 8.69                |           |                     |
|                   | 7.5        | +               | +     | 1905      | 6.90                | 8835      | 32.43               |           |                     |
|                   | 12.5       | +               | +     | 2631      | 9.52                | 12406     | 45.54               |           |                     |
| 11                | headspace  | +               | +     | 141       | 0.51                | 220       | 0.81                |           |                     |
|                   | 2.5        | +               | +     | 481       | 1.74                | 787       | 2.89                |           |                     |
|                   | 7.5        | +               | +     | 0         | -                   | 337       | 1.24                |           |                     |
|                   | 12.5       | +               | +     | 1680      | 6.08                | 3162      | 11.61               |           |                     |
| 12                | headspace  | +               | +     | 252       | 0.91                | 435       | 1.60                |           |                     |
|                   | 2.5        | +               | +     | 488       | 1.77                | 1236      | 4.54                |           |                     |
|                   | 7.5        | +               | +     | 301       | 1.09                | 1423      | 5.22                |           |                     |
|                   | 12.5       | +               | +     | 2116      | 7.66                | 12185     | 44.73               |           |                     |
| standard 10 ppm → |            |                 |       | 2762.5    |                     | 2724      |                     |           |                     |

|           | 54                  | 104       | 194                 | 361       |                     |           |                     |
|-----------|---------------------|-----------|---------------------|-----------|---------------------|-----------|---------------------|
| peak area | C2H4 conc.<br>[ppm] |
| 209       | 0.83                | 0         | -                   | 0         | -                   | 0         | -                   |
| 337       | 1.34                | 0         | -                   | 253       | 0.91                | 201       | 1.01                |
| 6509      | 25.88               | 656       | 2.50                | 260       | 0.93                | 0         | -                   |
| 11500     | 45.72               | 17355     | 66.09               | 7690      | 27.60               | 323       | 1.62                |
| 565       | 2.25                | 2422      | 9.22                | 318       | 1.14                |           | -                   |
| 1183      | 4.70                |           | -                   | 268       | 0.96                | 0         | -                   |
| 3453      | 13.73               | 4386      | 16.70               | 314       | 1.13                | 0         | -                   |
| 13362     | 53.12               | 9713      | 36.99               | 1803      | 6.47                | 237       | 1.19                |
| 540       | 2.15                | 0         | -                   | 0         | -                   |           | -                   |
| 732       | 2.91                | 426       | 1.62                | 0         | -                   | 0         | -                   |
| 425       | 1.69                | 214       | 0.81                | 0         | -                   | 0         | -                   |
| 5936      | 23.60               | 806       | 3.07                | 0         | -                   | 0         | -                   |
| 328       | 1.30                | 0         | -                   | 0         | -                   | 0         | -                   |
| 800       | 3.18                | 888       | 3.38                | 530       | 1.90                | 0         | -                   |
| 959       | 3.81                | 368       | 1.40                |           | -                   | 0         | -                   |
| 14097     | 56.04               | 8985      | 34.22               | 2160      | 7.75                | 232       | 1.16                |
| 563       | 2.24                | 0         | -                   | 0         | -                   | 0         | -                   |
| 1113      | 4.42                | 981       | 3.74                | 0         | -                   | 0         | -                   |
| 1280      | 5.09                | 542       | 2.06                |           | -                   | 0         | -                   |
| 10674     | 42.44               | 5314      | 20.24               | 844       | 3.03                | 0         | -                   |
| 531       | 2.11                | 289       | 1.10                | 0         | -                   |           | -                   |
| 637       | 2.53                | 0         | -                   | 0         | -                   | 0         | -                   |
| 576       | 2.29                | 297       | 1.13                | 0         | -                   | 0         | -                   |
| 11855     | 47.13               | 12515     | 47.66               | 1376      | 4.94                | 2652      | 13.31               |
| 623       | 2.48                | 0         | -                   | 0         | -                   | 0         | -                   |
| 1208      | 4.80                | 765       | 2.91                | 218       | 0.78                | 0         | -                   |
| 3667      | 14.58               | 830       | 3.16                | 0         | -                   | 0         | -                   |
| 10423     | 41.44               | 12681     | 48.29               | 3764      | 13.51               | 306       | 1.54                |
| 403       | 1.60                | 310       | 1.18                | 0         | -                   | 0         | -                   |
| 867       | 3.45                | 438       | 1.67                | 0         | -                   | 0         | -                   |
| 2888      | 11.48               | 547       | 2.08                | 0         | -                   | 0         | -                   |
| 13299     | 52.87               | 6553      | 24.95               | 688       | 2.47                | 0         | -                   |
| 348       | 1.38                | 0         | -                   | 0         | -                   |           | -                   |
| 824       | 3.28                | 705       | 2.68                | 0         | -                   | 0         | -                   |
| 500       | 1.99                | 317       | 1.21                | 0         | -                   | 0         | -                   |
| 12929     | 51.40               | 4978      | 18.96               | 211       | 0.76                | 0         | -                   |
| 0         | -                   | 0         | -                   | 0         | -                   | 0         | -                   |
| 1023      | 4.07                | 788       | 3.00                | 232       | 0.83                | 0         | -                   |
| 3381      | 13.44               | 883       | 3.36                | 0         | -                   | 0         | -                   |
| 15471     | 61.51               | 15058     | 57.34               | 684       | 2.46                | 0         | -                   |
| 0         | -                   | 0         | -                   | 0         | -                   | 0         | -                   |
| 945       | 3.76                | 417       | 1.59                | 0         | -                   | 0         | -                   |
| 430       | 1.71                | 344       | 1.31                | 0         | -                   | 0         | -                   |
| 10622     | 42.23               | 9577      | 36.47               | 430       | 1.54                | 0         | -                   |
| 334       | 1.33                | 0         | -                   | 0         | -                   |           | -                   |
| 744       | 2.96                | 405       | 1.54                | 0         | -                   | 267       | 1.34                |
| 556       | 2.21                | 217       | 0.83                | 0         | -                   | 0         | -                   |
| 12524     | 49.79               | 5153      | 19.62               | 1158      | 4.16                | 210       | 1.05                |
| 2515.333  | →                   | 2626      |                     | 2786      |                     | 1992.167  |                     |
|           | standard 10 ppm     |           |                     |           |                     |           |                     |

*data representative for t=54, t=104, t=194*

|   |          |
|---|----------|
| peak area supposed anoxic sample                | 2247     |
| peak area atmospheric sample                    | 11384.67 |
| slope peak area vs O <sub>2</sub> concentration | 436.1655 |

*data representative for t=361*

|   |          |
|---|----------|
| peak area supposed anoxic sample                | 3936.667 |
| peak area atmospheric sample                    | 12364.75 |
| slope peak area vs O <sub>2</sub> concentration | 402.2951 |

Because the syringe with which O<sub>2</sub> was sampled contained atmospheric O<sub>2</sub> a correction was made. Samples taken from prepared strict anaerobe serum bottles were analyzed for peak area. Also atmospheric samples were injected for the peak area. A sample taken from the column was then corrected for the occurring O<sub>2</sub> and then related with the atmospheric %O<sub>2</sub> that equals 20.95%.

| column # | depth [cm] | 194       |                | 361       |                | % O <sub>2</sub> in sample | C <sub>2</sub> H <sub>4</sub> [ppm] |
|----------|------------|-----------|----------------|-----------|----------------|----------------------------|-------------------------------------|
|          |            | peak area | O <sub>2</sub> | peak area | O <sub>2</sub> |                            |                                     |
| 1        | h.s.       | 9356      | 16.30          | -         | 10853          | 17.19                      | -                                   |
| 1        | 2.5        | 8986      | 15.45          | 0.91      | 9637           | 14.17                      | 1.01                                |
| 1        | 7.5        | 7861      | 12.87          | 0.93      | 9277           | 13.27                      | -                                   |
| 1        | 12.5       | 2155      | 0.00           | 27.60     | 4153           | 0.54                       | 1.62                                |
| 2        | h.s.       | 3822      | 3.61           | 1.14      | 7473           | 8.79                       | -                                   |
| 2        | 2.5        | 5430      | 7.30           | 0.96      | 7034           | 7.70                       | -                                   |
| 2        | 7.5        | 4352      | 4.83           | 1.13      | 6674           | 6.80                       | -                                   |
| 2        | 12.5       | 2346      | 0.23           | 6.47      | 4511           | 1.43                       | 1.19                                |
| 3        | h.s.       | 7450      | 11.93          | -         | 8959           | 12.48                      | -                                   |
| 3        | 2.5        | 7056      | 11.03          | -         | 8458           | 11.24                      | -                                   |
| 3        | 7.5        | 6154      | 8.96           | -         | 8041           | 10.20                      | -                                   |
| 3        | 12.5       | 4413      | 4.97           | -         | 7625           | 9.17                       | -                                   |
| 4        | h.s.       | 7636      | 12.36          | -         | 9384           | 13.54                      | -                                   |
| 4        | 2.5        | 7052      | 11.02          | 1.90      | 8538           | 11.44                      | -                                   |
| 4        | 7.5        | 7123      | 11.18          | -         | 8964           | 12.50                      | -                                   |
| 4        | 12.5       | 2575      | 0.75           | 7.75      | 4275           | 0.84                       | 1.16                                |
| 5        | h.s.       | 3130      | 2.02           | -         | 4731           | 1.97                       | -                                   |
| 5        | 2.5        | 4432      | 5.01           | -         | 7052           | 7.74                       | -                                   |
| 5        | 7.5        | 6392      | 9.50           | -         | 7689           | 9.33                       | -                                   |
| 5        | 12.5       | 2675      | 0.98           | 3.03      | 4576           | 1.59                       | -                                   |
| 6        | h.s.       | 3973      | 3.96           | -         | 6102           | 5.38                       | -                                   |
| 6        | 2.5        | 5744      | 8.02           | -         | 7815           | 9.64                       | -                                   |
| 6        | 7.5        | 6155      | 8.96           | -         | 7722           | 9.41                       | -                                   |
| 6        | 12.5       | 2593      | 0.79           | 4.94      | 4598           | 1.64                       | 13.31                               |
| 7        | h.s.       | 7839      | 12.82          | -         | 10014          | 15.11                      | -                                   |
| 7        | 2.5        | 7217      | 11.39          | 0.78      | 8966           | 12.50                      | -                                   |
| 7        | 7.5        | 3673      | 3.27           | -         | 8572           | 11.52                      | -                                   |
| 7        | 12.5       | 2576      | 0.75           | 13.51     | 4217           | 0.70                       | 1.54                                |
| 8        | h.s.       | 9048      | 15.59          | -         | 9997           | 15.06                      | -                                   |
| 8        | 2.5        | 8379      | 14.06          | -         | 9346           | 13.45                      | -                                   |
| 8        | 7.5        | 8165      | 13.57          | -         | 9410           | 13.61                      | -                                   |
| 8        | 12.5       | 2699      | 1.04           | 2.47      | 6980           | 7.56                       | -                                   |
| 9        | h.s.       | 9281      | 16.13          | -         | 9922           | 14.88                      | -                                   |
| 9        | 2.5        | 8908      | 15.27          | -         | 9446           | 13.69                      | -                                   |
| 9        | 7.5        | 8151      | 13.54          | -         | 8466           | 11.26                      | -                                   |
| 9        | 12.5       | 4847      | 5.96           | 0.76      | 7121           | 7.92                       | -                                   |
| 10       | h.s.       | 11352     | 20.88          | -         | 11636          | 19.14                      | -                                   |
| 10       | 2.5        | 8907      | 15.27          | 0.83      | 9545           | 13.94                      | -                                   |
| 10       | 7.5        | 7472      | 11.98          | -         | 8542           | 11.45                      | -                                   |
| 10       | 12.5       | 3717      | 3.37           | 2.46      | 7140           | 7.96                       | -                                   |
| 11       | h.s.       |           | -              | 13753     |                | 24.40                      | -                                   |
| 11       | 2.5        | 10041     | 17.87          | -         | 11877          | 19.74                      | -                                   |
| 11       | 7.5        | 9616      | 16.89          | -         | 6815           | 7.15                       | -                                   |
| 11       | 12.5       | 6456      | 9.65           | 1.54      | 9743           | 14.43                      | -                                   |
| 12       | h.s.       | 8192      | 13.63          | -         | 8563           | 11.50                      | -                                   |
| 12       | 2.5        | 6915      | 10.70          | -         | 8111           | 10.38                      | 1.34                                |
| 12       | 7.5        | 7866      | 12.88          | -         | 8069           | 10.27                      | -                                   |
| 12       | 12.5       | 2843      | 1.37           | 4.16      | 4559           | 1.55                       | 1.05                                |

Table 4 *t* probabilities of pairwise differences at 71 days exposure

| contrast<br>treatments* | t-prob | contrast     | t-prob |
|-------------------------|--------|--------------|--------|
| 0.01 vs 0.1             | 0.325  | 0.1 vs GR-24 | 0.001  |
| 0.01 vs 1               | 0.000  | 1 vs 10      | 0.873  |
| 0.01 vs 10              | 0.000  | 1 vs 100     | 0.403  |
| 0.01 vs 100             | 0.000  | 1 vs GR-24   | 0.333  |
| 0.01 vs GR-24           | 0.000  | 10 vs 100    | 0.600  |
| 0.1 vs 1                | 0.000  | 10 vs GR-24  | 0.352  |
| 0.1 vs 10               | 0.000  | 100 vs GR-24 | 0.092  |
| 0.1 vs 100              | 0.000  |              |        |

Table 5 *t* probabilities of pairwise differences at 96 days exposure

| contrast<br>treatments* | t-prob | contrast     | t-prob |
|-------------------------|--------|--------------|--------|
| 0.01 vs 0.1             | 0.186  | 0.1 vs GR-24 | 0.001  |
| 0.01 vs 1               | 0.000  | 1 vs 10      | 0.889  |
| 0.01 vs 10              | 0.000  | 1 vs 100     | 0.724  |
| 0.01 vs 100             | 0.000  | 1 vs GR-24   | 0.210  |
| 0.01 vs GR-24           | 0.000  | 10 vs 100    | 0.663  |
| 0.1 vs 1                | 0.000  | 10 vs GR-24  | 0.343  |
| 0.1 vs 10               | 0.000  | 100 vs GR-24 | 0.128  |
| 0.1 vs 100              | 0.000  |              |        |