PLANT RESEARCH INTERNATIONAL
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Th.A. Dueck & C.J. van Dijk

Report 204

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Plant Research International B.V., Wageningen
September 2008

Report 204

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Summary

Recommendations in the current risk assessment of gasolines and naphthas state that the vapour phase phytotoxicity of certain blocks of hydrocarbon to plants requires investigation. Therefore a number of hydrocarbons representing these blocks were tested. Undecane was chosen as the first of these hydrocarbons . A fumigation experiment was performed in which a number of plant species representing the European flora was exposed to a range of undecane concentrations. Pressurised nitrogen was bubbled through warmed liquid undecane to bring it into the gas phase. It was then injected into the air-stream entering the plant fumigation chambers at constant concentrations for a period of 12 weeks. The mean measured concentrations were 0.01, 0.13, 0.32, 1.09, 2.73 and 8.68 ppmV undecane.

The main objective of this study was to quantify the effects of undecane on plants and from them, derive no-observed-effect-concentrations (NOECs) for each plant species. However, even at the highest concentration of undecane used in this study, no significant effects on the measured endpoints were found, and thus NOECs could not be calculated for any of the species tested.

An analysis of variance indicated that the regression line for some of the endpoints differed significantly from zero, indicating a significant trend as a result of exposure to undecane. However, this does not indicate a significant treatment effect. A trend was observed for the following endpoints:

- Reduction of shoots biomass of *Phaseolus*, *Brassica* and *Solanum*
- Reduction of root biomass *Solanum*
- Reduction of length of ears in *Plantago*.

The concentration of undecane in tissues of *Solanum* increased with increasing concentrations of undecane in the air, confirming exposure to undecane.

1 Introduction and aims of the study

Recommendations in the current risk assessment of gasolines and naphthas state that the vapour phase toxicity of certain hydrocarbon blocks to plants requires investigation. In order to mimimise extended testing of the many hydrocarbons of relevance, this project proposes to test a number of representative hydrocarbons and via quantitative structure relationships, estimate effect characterizations of untested hydrocarbons.

Undecane was chosen as the first of these hydrocarbons to be tested. The vapour phase exposure test was performed in the summer and autumn of 2006. It was based on an earlier test protocol for DBP (Dueck *et al.*, 2003) and complimented by experience with other air pollutants and air pollution fumigation studies at these facilities.

The objective of the study was to quantify the effects of undecane vapour on plants and derive no-observed-effect-concentrations (NOECs) for each plant species.

2 Materials and Methods

The relevant methodology for the vapour phase testing of undecane will be briefly stated below.

2.1 Test substance

The test substance n-undecane, further referred to as undecane is an alkane hydrocarbon with the chemical formula $CH_3(CH_2)_9CH_3$. It was obtained from Sigma Aldrich, purity 99%.
The technical details are:

Chemical identity:	n-undecane
Chemical formula:	$C_{11}H_{24}$
Molecular weight:	156.3 g mol^{-1}
Appearance:	clear fluid
CAS-No:	1120-21-4
Melting point:	-25°C
Boiling point:	196°C
Vapour pressure (25°C):	0.63 mm Hg
log Koa	3.84
log Kow	5.74

2.2 Exposure facilities and treatment applications

The fumigation experiment was performed in six closed fumigation chambers located in a climate room (15 m^2) at Plant Research International B.V., Wageningen in The Netherlands. The fumigations consisted of a control and five exposure treatments. The airtight fumigation chambers were made from hardened glass set in an aluminium framework (0.85 x 1.0 x 0.9 m; 765 litre), with a stainless steel floor. Tubing for outgoing air was made of teflon. Both incoming and outgoing air streams were passed through an activated charcoal filter. Incoming air entered each chamber under a perforated floor at an air exchange rate of 0.5 m^3 min^{-1} and was continuously recirculated at a rate of 4 m^3 min^{-1} before being blown off into the outdoor air. A lower atmospheric pressure was maintained in the fumigation chambers to avoid contamination in the climate room. Temperature, air humidity and light intensity were recorded inside the chambers. Wind speed and turbulence in the fumigation-chambers was maintained at 0.5 m s^{-1} with additional ventilators to ensure gas exchange.

Pressurised nitrogen, controlled by thermal mass-flow controllers, was lead through commercially available liquid undecane in a vaporiser unit, which was maintained at 23°C. The saturated air stream was mixed with a small amount of ambient air and passed through stainless steel tubing, insulated and warmed to 30°C to avoid condensation in the tubing, before being injected into the fumigation chamber under the perforated floor. Thus, controlled concentrations of atmospheric undecane were produced and injected into the air for 24 h day^{-1}.

2.3 Fumigation treatments

Prior to the actual test, a range-finding experiment was performed from June 21st to July 4th in order to estimate undecane concentrations that were non-lethal, but were still considered to result in undesired effects on plant growth and functioning. Three plant species (*Brassica campestris, Solanum nigrum and Chenopodium album*) were exposed to undecane. Plants were taken from the greenhouse and placed in the fumigation chambers for one day to acclimatize. An initial undecane concentration was chosen (1.6 ppmV) to which plants were exposed for several days. The concentration was then increased in steps until a visual qualitative effect was observed at 6 ppmV.

Based on the results of the range-finding tests the fumigation system was set up for six target exposure levels: control, 0.1, 0.3, 1.0, 3.0 and 10 ppmV[1] undecane. The experimental exposure period was chosen to continue for a period of 12 weeks, or less for a particular species if it flowered and produced seed earlier.

2.4 Measurement of undecane in the test chambers

Sampling. Air samples were taken with an automated gas sampling device. The samples were taken with a flow of 10 ml min^{-1}, controlled with programmable mass flow controllers. Teflon tubing from chamber to the trapping tube was flushed for 5 min. prior to trapping . Samples were pumped through tubes filled with 200 mg Tenax TA with different time intervals (10-20 min) to maintain the concentration within the range of the calibration curve. Each fumigation chamber was sampled twice weekly in duplicate.

Sample Analysis. Analysis was performed by thermal desorption-GC-MS. Samples were desorbed via an Ultra auto sampler (Markes International Ltd, UK) by heating the tube for 4 minutes to 250 °C with a helium flow of 30 ml min^{-1} and focussed on a unity injector (Markes International Ltd, UK) on a trap containing a multibedsorbent at 10 °C. Compounds were injected into the capillary column (RTX-MS, 30 m, 0.25 µm id, 1.4 µm df) by rapidly heating the trap to 250 °C at 12 °C s^{-1}, using a column flow of 1 ml min^{-1} and a split flow of 19 ml min^{-1}.

The GC (trace GC ultra, Interscience, the Netherlands) was programmed at 60 °C for 2.5 minutes, then to 280 °C with a ramp of 20 °C $minute^{-1}$ followed by 1.5 minutes at 280 °C. Quantification was performed by spiking a blank Tenax tube with different quantities of undecane to acquire a calibration curve. The tubes were analysed in the same way as the sampled tubes. Samples were measured on a DSQ mass spectrometer (Interscience, The Netherlands) in full scan mode (mass 35-300) at 952 Amu sec^{-1}. Mass 156 was used for quantification.

2.5 Choice of plant species and endpoints

The plant species chosen for the experiment were representative of the European flora and included plant species representative for crops, trees and natural vegetation (Table 1).

Plant growth is considered to be the most important response (endpoint) to air pollutant exposures in relation to consequences at the population level. For annual species, generative reproduction is especially important and this developmental phase is usually accomplished within 8-12 weeks. Perennial species will not always have flowered within that time period, but will have realized the largest proportion of vegetative growth.

During the fumigation period, daily observations were made with respect to plant appearance. Every 7-10 days all plants were taken out of the fumigation chambers and scored for injury and general appearance, i.e. chlorose, necrose, leaf morphology, number of flowers. Furthermore, the relative amount of chlorophyll was estimated by measuring light transmission through the leaves with a handheld Minolta SPAD50 meter. Individual representative leaves on each plant were chosen for these measurements.

[1] 1ppmV undecane = 6.39 mg m^{-3} at 25°C and 760 mm Hg

Table 1. Plant species selected for the hydrocarbon toxicity test and relevant effect-parameters.

	Plant species	Special characteristic	Relevant effect-parameters
Crops	*Phaseolus vulgaris* (bean)	Nitrogen fixing dicot	shoot and root biomass, pod weight, number of pods
	Brassica campestris (cabbage)	Waxy leaves	shoot and root biomass
Trees	*Picea abies* (Norway spruce)	Evergreen	visual injury, biomass current year branches
Natural Vegetation	*Trifolium repens* (white clover)	Nitrogen fixer	shoot and root biomass, number of flowers
	Solanum nigrum (black nightshade)	Ruderal species	shoot and root biomass, number of berries
	Holcus lanatus (common velvet grass)	Common grass	shoot and root biomass, number of tillers
	Plantago lanceolata (narrowleaf plantain)	Ruderal species	shoot and root biomass, height and number of flowers

At harvest, the relevant effect-parameters mentioned in Table 1, shoot and root biomass, vegetative and generative (flower and seed) production, were taken and dried at 95°C to a constant weight and weighed.

2.6 Measurement of undecane in plant tissue

Sampling. On November 13, 2006 an additional fumigation experiment was begun with *Solanum nigrum* plants exposed to 1, 3 and 9 ppmV undecane. Four plants from each treatment were harvested on November 20, November 27 and on December 4 2006. Harvested leaves were placed in 50 ml polypropylene tubes (Greiner) and immediately frozen in liquid nitrogen. Samples were stored at -80°C.

Sample Analysis.: Plant tissue was finely ground in liquid nitrogen with a IKA-A11 blender. Frozen powdered leaf material (500 mg) was placed in a cooled (liquid nitrogen) glass tube to which 3 ml hexane was added. Limonene (0.4 µg/ml) was used as an internal standard. The resultant sample was then mixed in a vortex (1700 g) four times at room temperature for 10 seconds. The hexane fraction was saved and dehydrated over a sodium sulphate column. Undecane was then analysed on a HP-GCMS with GSgeur15 (a standard program for the analysis of volatiles).

GC:	HP 5890 Series II
MS:	HP 5972
Column:	ZB-5, 30 m, internal diameter 0.53
Temp. progr.:	15°C/min 45 – 280°C
Injector temp.:	250°C
Injection volume:	2 µl

2.7 Statistical analysis

The experimental design entailed a large number of treatments without replication in order to characterize dose-response relationships, and ultimately, NOECs. This implied using a regression approach as the basis for data analysis rather than ANOVA to test for differences between treatments.

Data for the response variables were averaged for each exposure level (fumigation chamber) prior to analysis. Treatment means for each species and response parameter were subjected to regression analyses with a logistic model (Genstat, 1993) to derive response curves. The best fit from a non-linear regression approach was used and applied in the calculation of regression equations using the formula:

$$y = C/\{1 + \exp[-B(\ln(x) - \ln(M)]\}$$

Where C is the calculated response at x=0 and x is the mean pollutant concentration (ppmV) during the exposure period relevant to each species. After estimating M (the pollutant concentration at which biomass is 50% of the control) and B (scale parameter), the Effective Concentrations at 10% (EC10) below C were to be calculated. Following this, No Observable Effect Concentrations (NOECs) were to be calculated for each species according to the formula:

$$NOEC = M - \{\ln(C/yc-1)\}/B$$

where yc is the lower limit of the 95% confidence limit of the asymptote (C). The NOECs and EC10 would then have been calculated using the module Fitnonlinear of the statistical package Genstat.

However, due to the lack of treatment effects, the analysis did not produce the necessary parameters indicated above, and thus, no significant dose-response relationship could be identified.

We then used a standard variation analysis (Genstat, 1993) to test whether or not a trend was present over the range of treatment concentrations. The trend uses the experimental data to estimate if the regression line differs significantly from zero. The presence of a trend however, does not necessarily indicate an adverse treatment related effect.

3 Results

3.1 Range finding test

Three plant species (*Brassica campestris, Solanum nigrum and Chenopodium album*) were exposed to ambient air (control) and to 1.6 ppmV undecane for a period of 7 days. When no effects were observed, the concentration was increased to 6 ppmV and maintained for a period of 14 days.
Four days after increasing the concentration *Chenopodium album* appeared to have slightly darker green colour, an effect that appeared on *Brassica campestris* after 13 days. *Solanum nigrum* plants on the other hand, showed a decrease in biomass production after only 4 days. Secondary shoots were present, but were shorter than in the control and the plants had a darker green colour as well.

In consistence with our experience from earlier studies with gaseous air pollutants (cf. Dueck *et al.*, 2003), we estimated that these concentrations were in the range of effective concentrations. These qualitative effects were assumed to result in measurable effects during a chronic exposure. Based on this range-finding test, a concentration higher than 6 ppmV was selected as the highest concentration to be used in the test, for which adverse effects were anticipated.

Following the range-finding test, a long-term fumigation experiment of 12 weeks was performed using six target exposure levels: control, 0.1, 0.3, 1.0, 3.0 and 9 ppmV undecane.

3.2 Undecane concentrations during plant exposure

Figure 1 shows the twice weekly mean undecane concentrations in the fumigation chambers over the exposure period. The mean measured concentrations of undecane in the exposure chambers and the exposure duration for each species are given in Table 2. Individual concentration measurements are given in Appendix I. The slight differences in mean concentrations for the individual species are due to differences in the length of exposure for each species.

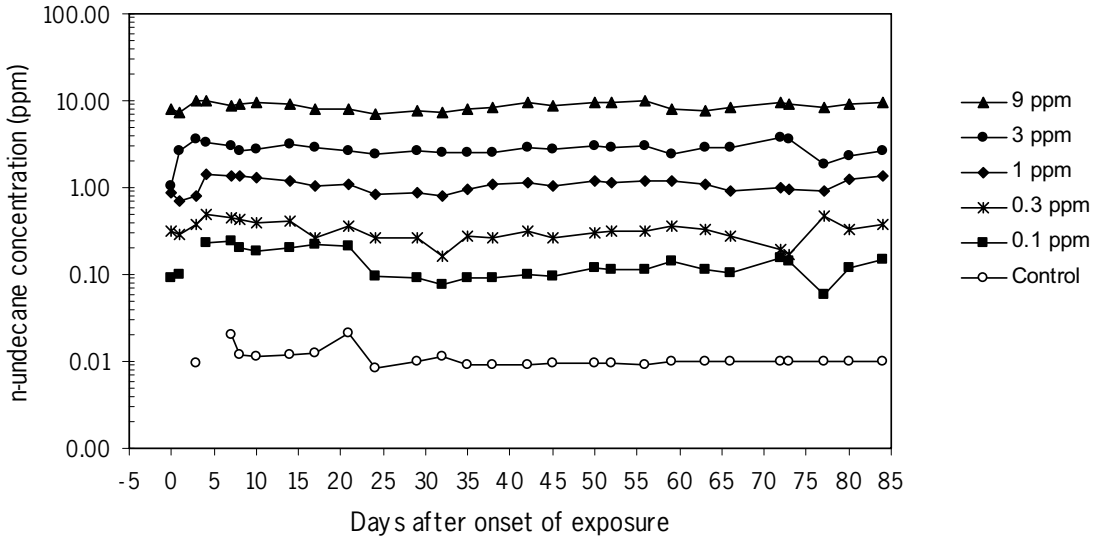

Figure 1. Undecane concentrations (ppmV) in each of the fumigation chambers during the total exposure period of 84 days. Target concentrations are given in the legend. Undecane concentrations are shown on log scale, which is frequently used in fumigation studies.

The results of adjustments in order to meet the desired target concentrations can be seen in Fig. 1. A very low background concentration of undecane (contamination) was detected in the control treatment where a mean concentration of 0.01 ppmV was measured. Periodic measurements indicated a concentration of 0.03 ppmV in ambient air outdoors and 0.06 ppmV in the climate room housing the fumigation chambers. Air from the climate room was directly vented outdoors. An explanation for contamination in the control chamber is that they received outdoor air (0.03 ppmV) which was then filtered. The active charcoal filter was obviously unable to remove all of the undecane, resulting in 0.01 ppmV undecane in the fumigation chamber. The other target concentrations were met reasonably well, with measured concentrations being slightly higher than desired at the lower target concentrations, and being somewhat lower than desired at the higher target concentrations.

Table 2. *Duration and measured undecane concentrations (ppmV, mean ± SE) to which plants species were exposed during the experiment.*

Species	Exposure	Undecane concentration (ppmV)					
	(days)	control	0.1	0.3	1	3	9
Phaseolus vulgaris	36	0.01±0.001	0.11±0.02	0.29±0.05	1.08±0.14	2.75±0.21	8.66±0.99
Brassica oleracea	42	0.01±0.004	0.15±0.06	0.33±0.09	1.07±0.22	2.67±0.54	8.44±0.92
Solanum nigrum	61	0.01±0.001	0.11±0.03	0.30±0.07	1.06±0.16	2.73±0.41	8.64±0.96
Picea abies	84	0.01±0.003	0.13±0.05	0.32±0.08	1.09±0.19	2.73±0.51	8.68±0.90
Trifolium repens	84	0.01±0.003	0.13±0.05	0.32±0.08	1.09±0.19	2.73±0.51	8.68±0.90
Holcus lanatus	84	0.01±0.003	0.13±0.05	0.32±0.08	1.09±0.19	2.73±0.51	8.68±0.90
Plantago lanceolata	84	0.01±0.003	0.13±0.05	0.32±0.08	1.09±0.19	2.73±0.51	8.68±0.90

3.3 Concentrations of undecane in plant tissue

The chromatogram analyses confirmed the presence of undecane in the samples. The difference in peak area from leaves exposed to undecane in the air followed exposure concentrations, increasing by a factor 3 each time (see Appendix II for individual values) . A trace of undecane was detectable in hexane itself, but the concentration was a factor 10 lower than in the plant material. However, the concentration of undecane in plant tissues did not increase with time, but remained more or less constant. It even appeared to decrease slightly with time. This might suggest a rapid equilibrium, but is contradicted by the fact that the tissue concentrations decrease with time, especially at the highest treatment. It appears that the plant is rapidly 'loaded' or becomes saturated with undecane, after which the tissue concentration is diluted as the plant grows.

Table 3. *Undecane concentrations (µg g-1 fresh weight, mean ± SE) in plant tissue from Solanum nigrum after exposure to undecane in the air for 1, 2 and 3 weeks.*

Undecane (ppmV)	Exposure time		
	1 week	2 weeks	3 weeks
1	1.48 ± 0.11	1.06 ± 0.02	1.30 ± 0.03
3	2.88 ± 0.11	2.68 ± 0.11	2.53 ± 0.05
9	10.73 ± 0.14	8.45 ± 0.37	7.72 ± 0.45

3.4 Effects of undecane on individual plant species

Because no dose-response relationships were found for any of the endpoints, the results presented in this report are based on the standard variation analysis, indicating the presence or absence of a (negative) trend in the treatment means. The main results per species are presented here along with a general description and conclusions. The full data set are shown in Appendix III.

Solanum nigrum (black nightshade)

In the course of the undecane treatments, the statistical analysis indicated a trend in the biomass production of both shoots and roots of *Solanum* (Figure 2). Biomass production showed a negative trend with increasing undecane concentrations. The degree of variation between individual plants per treatment was very small (see also Appendix 3 for all values), even resulting in a negative trend for the root biomass as well. Part of the shoot biomass is made up of reproductive organs, i.e. the production of berries containing seeds. The variation between treatments is higher for berry production than for the rest of the shoot, so that no significant trend was observed for berry production alone, even though it appeared to be reduced by exposure to undecane at the upper end of the test concentrations (Figure 3).

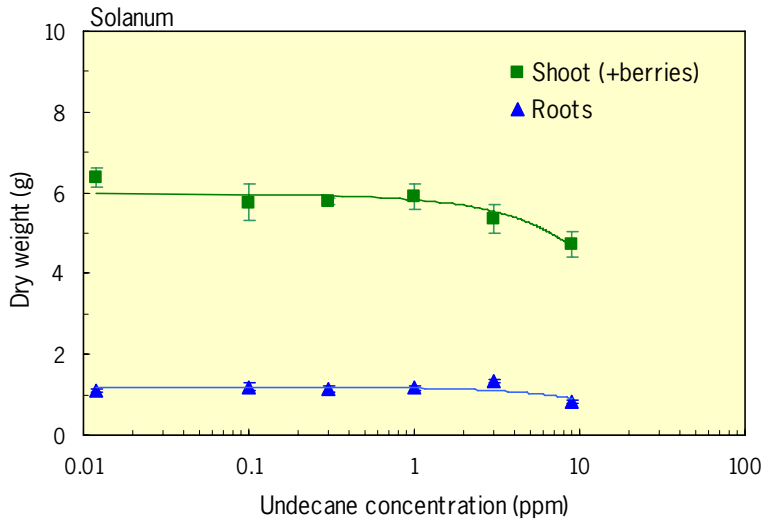

Figure 2. Mean (± SE) shoot and root biomass (g) of Solanum nigrum after 61 days exposure to undecane.

In summary, although no effect was observed on the shoot and root biomass, a negative trend was observed, indicating that a (not significant) influence of increasing concentrations of undecane. No significant trend was observed for the berry production, even though it seemed (visually) to be enhanced at the lower concentrations and then declined again with increasing concentrations of undecane.

Plantago lanceolata (narrowleaf plantain)

A curious result was observed by *Plantago* (Figure 4), with the amount of whole plant biomass produced being highest at the extreme concentrations (control and 9 ppmV undecane). The reason for this is not clear, as the degree of variation between individual plants was relatively small and no outliers occurred within the data set. No significant trend could be observed, which means that the whole plant biomass was not affected by undecane.

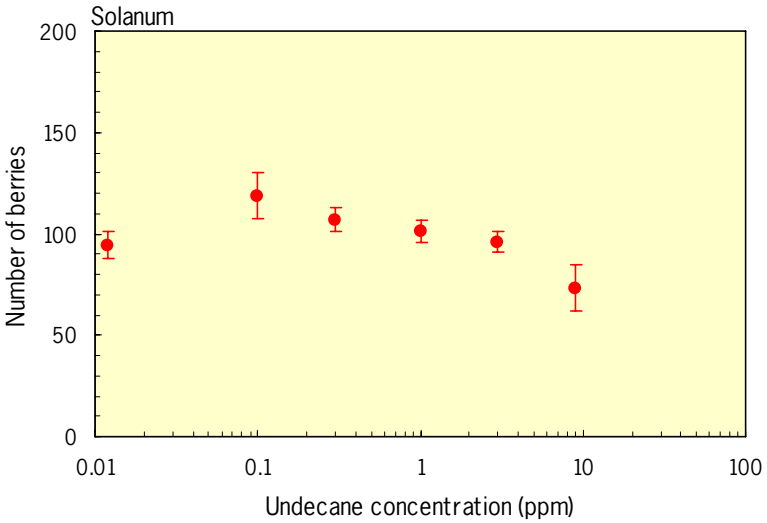

Figure 3. *Mean (± SE) number of berries of Solanum nigrum after 61 days exposure to undecane.*

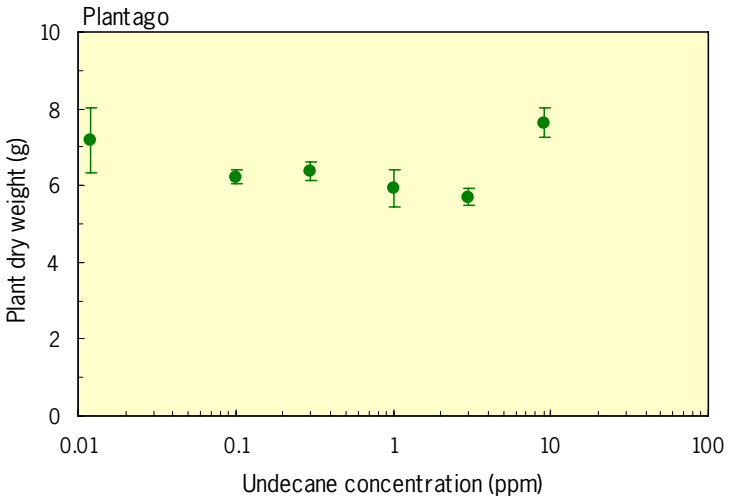

Figure 4. *Mean (±SE) plant biomass (g) of Plantago lanceolata after 84 days exposure to undecane.*

Although the reproductive organs of *Plantago* appeared to be negatively affected by undecane, no trend in the number of *Plantago* ears in relation to undecane concentrations was observed (Figure 5). A significant trend in the mean length of individual ears however, was indicated, reducing ear length with increasing undecane concentrations (Figure 6). This suggests that while the vegetative production (whole plant biomass) was not negatively affected, the generative reproduction is affected by undecane. This might well have consequences for the genetical composition of field populations of *Plantago lanceolata*.

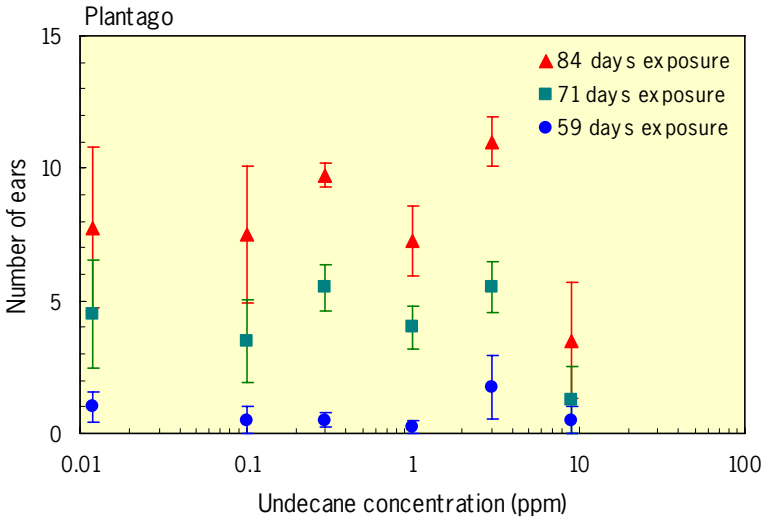

Figure 5. *Mean (± SE) number of ears of Plantago lanceolata after 59, 71 and 84 days exposure to undecane.*

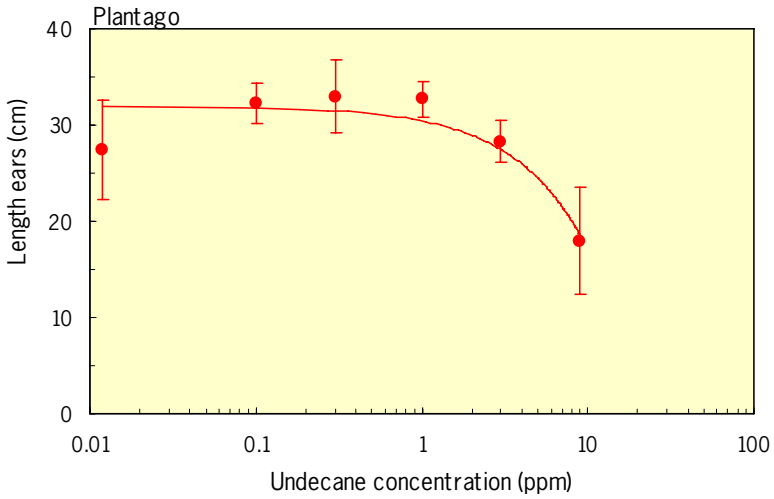

Figure 6. *Mean (±SE) length of ears of Plantago lanceolata after 84 days exposure to undecane.*

Summary: No trend could be observed for the biomass production or number of ears in Plantago, but was present for length of ears. Ear length tended to be significantly reduced with increasing concentrations of undecane.

Picea abies (Norway spruce)

Picea abies remained unaffected by all test concentrations of undecane (Figure 7).

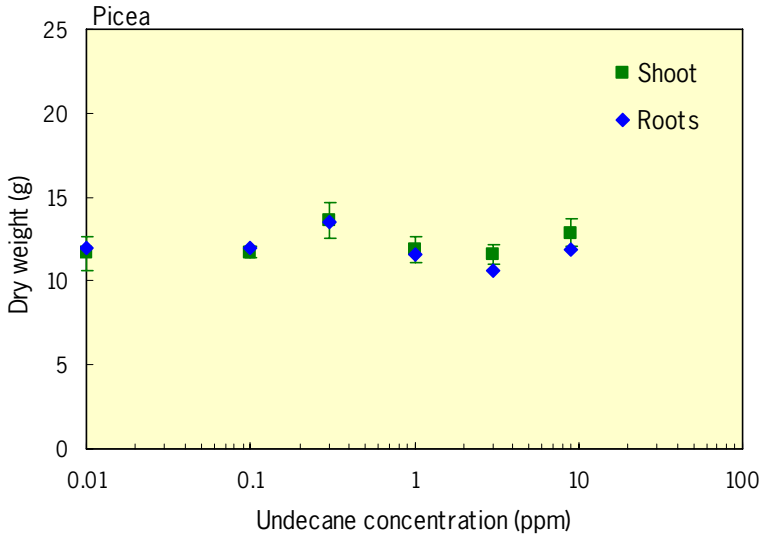

Figure 7. *Mean (± SE) shoot and root biomass (g) of Picea abies after 84 days exposure to undecane.*

Summary: Undecane had no effect on Picea at any concentration

Brassica campestris **(cabbage)**

Only the shoot biomass showed a negative trend in relation to increasing concentrations of undecane (Figure 8), a similar effect to that observed in shoots of *Solanum nigrum* . The exposure to undecane appears to affect the leaf area (Figure 9), but no significant trend was observerd, which would have indicated a reduction with increasing concentrations of undecane. Less shoot biomass, especially leaf area reduces the plant's photosynthetic capacity and its rate of growth.

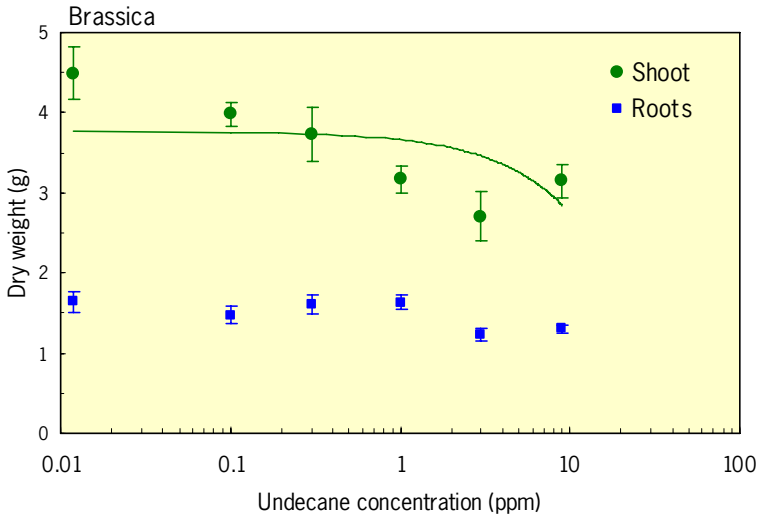

Figure 8. *Mean (± SE) shoot and root biomass (g) of Brassica campestris after 43 days exposure to undecane.*

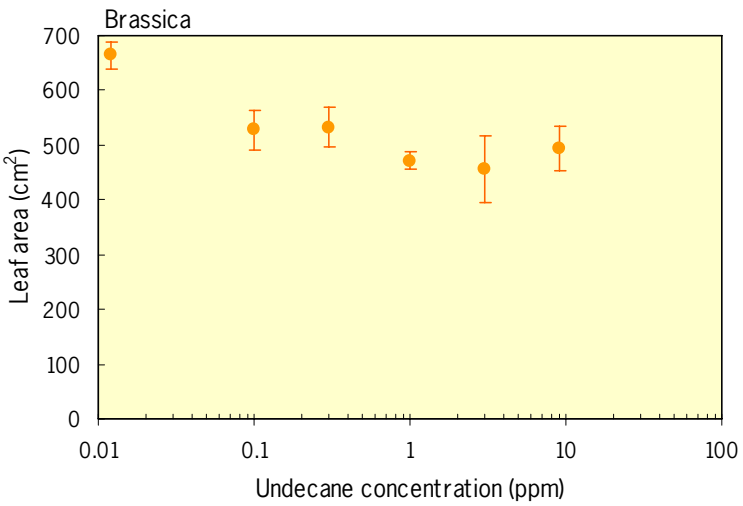

Figure 9. *Mean (±SE) leaf area of Brassica campestris after 43 days exposure to undecane.*

Summary: The shoot biomass of Brassica showed a significant negative trend with increasing undecane concentrations, which seemed to be reflected in the leaf area data, the leaf area showed no trend in relation to increasing undecane.

Phaseolus vulgaris **(bean)**

As was the case for *Brassica*, exposure to increasing concentrations of undecane resulted in a significantly negative trend in shoot biomass production of *Phaseolus.* This became more pronounced at the lower end of the concentration range (Figure 10). This likely results from a reduction in leaf area (Figure 11), even though no significant trend could be shown. Neither the roots nor the pods (reproductive organs) indicated an effect of undecane.

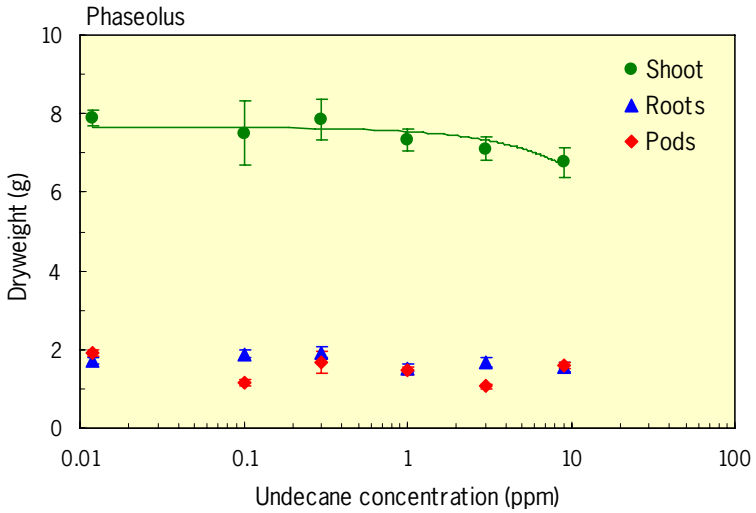

Figure 10. *Mean (± SE) shoot, root and pod biomass (g) of Phaseolus vulgaris after 36 days exposure to undecane.*

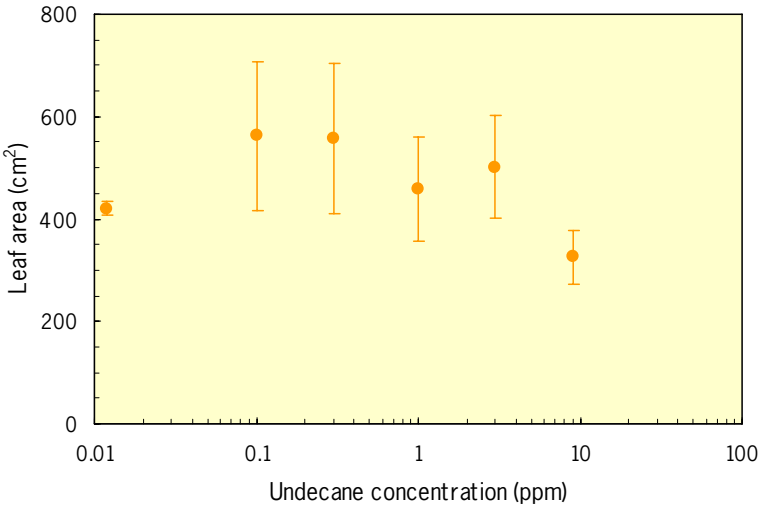

Figure 11. *Mean (± SE) leaf area of Phaseolus vulgaris after 36 days exposure to undecane.*

Summary: The shoot biomass of Phaseolus showed a significant negative trend with increasing undecane concentrations.

Holcus lanatus **(common velvet grass)**

Holcus lanatus is the only grass species in the list of tested species. *Holcus* does not appear to be affected in any way by undecane in any of its organs (Figure 12 and 13).

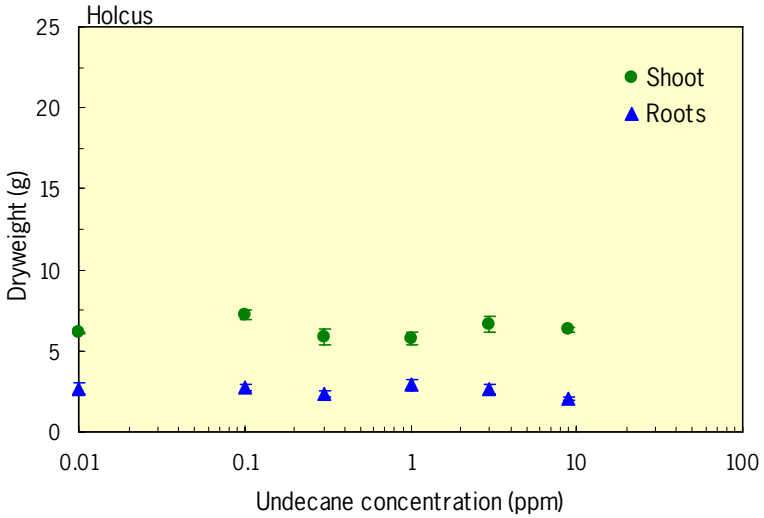

Figure 12. *Mean (±SE) shoot and root biomass (g) of Holcus lanatus after 84 days exposure to undecane.*

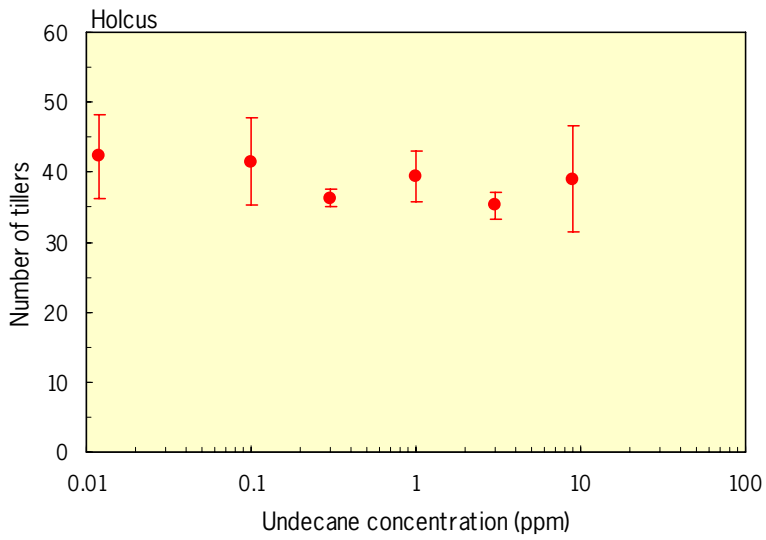

Figure 13. Mean (±SE) number of tillers of Holcus lanatus after 84 days exposure to undecane.

Summary : Undecane had no effect on Holcus.

Trifolium repens (white clover)

Trifolium repens was the only species that did not grow and develop as was expected in the fumigation chambers, including the control treatment. No abnormal morphology was observed, but the growth rate was much lower than expected throughout the experiment. From the plants that were harvested at the end of the fumigation period, both the root and shoot biomass production did not show an effect of undecane on their growth (Figure 14). Undecane did not affect the production of new (vegetative) stolons either (Figure 15).

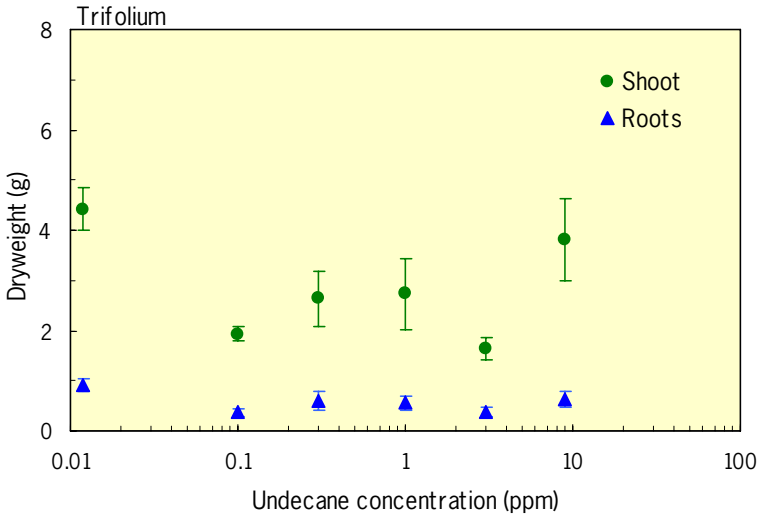

Figure 14. Mean (± SE) shoot and root biomass (g) of Trifolium repens after 84 days exposure to undecane.

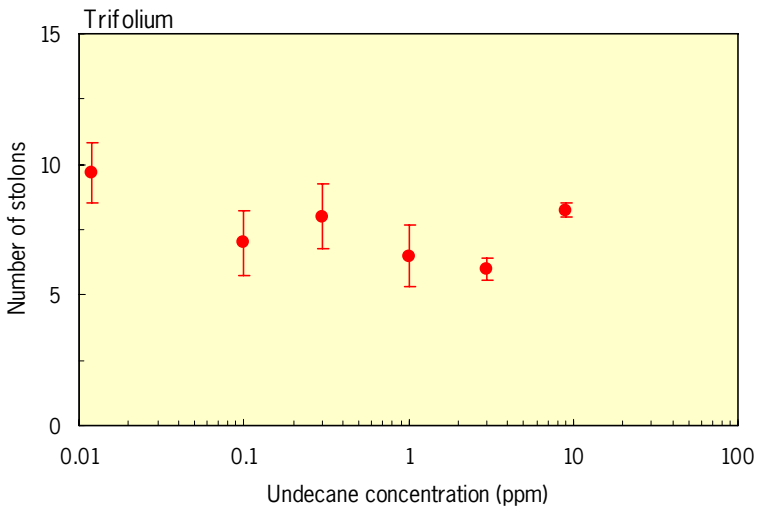

Figure 15. Mean (± SE) number of stolons of Trifolium repens after 84 days exposure to undecane.

Summary: Trifolium did not grow and develop as normally as expected. No effects to undecane were observed on root and shoot biomass or the production of new (vegetative) stolons.

4 Discussion

In summary, the following observations can be made:
Solanum: although there was no significant effect on the shoot and root biomass, a negative trend was observed, indicating an influence of increasing concentrations of undecane. No trend was observed for the berry production, even though it seemed (visually) to be enhanced at the lower concentrations and then declined again with increasing concentrations of undecane.
Plantago: No trend could be observed for the biomass production or number of ears, but was present for length of ears. Ear length tended to be significantly reduced with increasing concentrations of undecane.
Picea: Undecane had no effect on *Picea* at any concentration
Brassica: The shoot biomass of Brassica showed a negative trend with increasing undecane concentrations, which seemed to be reflected in the leaf area data, the leaf area showed no trend in relation to increasing undecane.
Phaseolus: The shoot biomass showed a negative trend with increasing undecane concentrations.
Holcus: Undecane had no effect on *Holcus*.
Trifolium: *Trifolium* did not grow and develop as normally as expected. No effects to undecane were observed on root and shoot biomass or the production of new (vegetative) stolons.

The objective of this study was to quantify the effects of undecane on plants and from them, derive no-observed-effect-concentrations (NOECs) for each plant species. The best fit from a non-linear regression approach was used, and applied for the calculation of regresson equations. However, the parameters necessary for the calculation of dose-response relationships could not be derived.

During the range-finding test, a number of plant species were exposed to undecane for one to two weeks. In consistence with our experience from earlier studies with gaseous air pollutants (cf. Dueck *et al.*, 2003), we estimated that the concentrations used for range-finding were in the range of effective concentrations during a chronic exposure.
Based on this range-finding test, a concentration higher than 6 ppmV was chosen as the highest concentration to be used, and at which effects were assumed certain to appear. This however, did not occur, indicating perhaps that the pollutant concentrations had a brief influence on some morphological parameters, did not affect the growth and production as was expected.

The endpoint data were then subjected to a standard analysis of variance to ascertain if a significant negative trend in the response to undecane could be shown. If a trend was found to be present however, it did not mean that a significant effect had occurred as a result of the exposure to undecane.
The analysis indicated that for some of the individual endpoints a significant trend was indeed present. This applied to the shoots of *Phaseolus*, *Brassica* and *Solanum*, as well as for the roots of *Solanum* and the length of ears in *Plantago*, all of which decreased with increasing concentrations of undecane.

In addition, measurements of tissue concentrations of *Solanum* confirmed the exposures to undecane. When exposed to undecane in the air, the concentrations of undecane in plant tissue was proportional to concentrations in the air (exposure concentrations). The maximum tissue concentration is apparently achieved within the first week of exposure.

5 Conclusions

1. Based on the experimental data, no dose-response relationships could be calculated and thus no NOECs could be derived.

2. Significant negative trends resulting from exposure to increasing concentrations of undecane could be observed for the following endpoints:
   - Reduction of shoot biomass of *Phaseolus*, *Brassica* and *Solanum*
   - Reduction of root biomass *Solanum*
   - Reduction of length of ears in *Plantago*.

   However, no statistically significant adverse effects on plant growth or reproduction were recorded following prolonged exposure to air concentrations of 9ppmV undecane vapour.

3. The concentration of undecane in tissues of *Solanum* was proportional to the exposure concentration of undecane.

References

Dueck, Th.A., C.J. van Dijk, F. David, N. Scholz & F. Vanwalleghem, 2003.
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Appendix I.
Atmospheric undecane concentration

Tabel I-1. Realized concentrations of undecane (ppmV) measured in each of the fumigation chambers during the entire exposure period starting on August 14, 2006. Concentrations were measured twice weekly in duplicate (sample 1 and 2).

Day	Treatment (target concentration, ppmV)											
	Control		0.1		0.3		1		3		9	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1			0.08	0.10	0.29	0.35	0.84	0.93	1.03	1.09	7.90	8.45
2			0.10		0.29		0.71		2.66		7.20	
4	0.01	0.01			0.37		0.81		3.68		9.94	
5			0.22	0.25	0.50	0.49	1.36	1.46	3.28	3.33	9.70	9.91
8	0.02		0.24		0.46		1.39		3.04		8.89	
9	0.01	0.01	0.20	0.21	0.43	0.44	1.35	1.36	2.71	2.68	8.88	9.14
11	0.01	0.01	0.19	0.18	0.40	0.38	1.34	1.29	2.68	2.76	9.22	9.54
15	0.01	0.01	0.19	0.21	0.38	0.46	1.17	1.21	3.16	3.14	8.63	9.34
18	0.01	0.01	0.23	0.21	0.26	0.26	1.03	1.04	2.88	2.97	7.84	8.07
22	0.03	0.01	0.21	0.21	0.35	0.36	1.07	1.07	2.66	2.66	8.05	8.23
25	0.01	0.01	0.09	0.10	0.27	0.27	0.84	0.84	2.34	2.41	7.11	7.19
30	0.01	0.01	0.08	0.10	0.27	0.25	0.89	0.90	2.60	2.68	7.53	8.12
33	0.01	0.01	0.07	0.08	0.15	0.18	0.74	0.84	2.54	2.46	6.52	7.90
36	0.01	0.01	0.10	0.08	0.31	0.25	1.00	0.92	2.57	2.48	7.62	8.06
39	0.01	0.01	0.09	0.09	0.28	0.26	1.09	1.08	2.52	2.56	8.04	8.63
43	0.01	0.01	0.10	0.10	0.31	0.31	1.16	1.16	2.80	2.92	9.32	9.79
46	0.01	0.01	0.09	0.10	0.27	0.27	1.00	1.06	2.74	2.83	8.65	9.24
51	0.01	0.01	0.12	0.12	0.30	0.31	1.16	1.21	2.85	3.07	9.27	10.06
53	0.01	0.01	0.11	0.12	0.30	0.32	1.12	1.20	2.81	2.99	9.42	9.89
57	0.01	0.01	0.11	0.12	0.33	0.31	1.21	1.18	2.99	3.06	9.70	10.25
60	0.01	0.01	0.16	0.13	0.39	0.32	1.31	1.12	2.55	2.35	8.27	7.43
64	0.01	0.01	0.11	0.12	0.34	0.32	1.12	1.11	2.80	2.93	7.69	7.95
67	0.01	0.01	0.11	0.10	0.30	0.26	0.97	0.89	2.86	2.86	8.25	8.54
73	0.01	0.01	0.15	0.16	0.20	0.19	1.02	1.02	3.64	3.82	9.07	9.74
74		0.01		0.14		0.17		0.94		3.59		9.15
78	0.01	0.01	0.06	0.06	0.46	0.49	0.89	0.91	1.83	1.92	8.05	8.54
81	0.01	0.01	0.11	0.13	0.34	0.33	1.24	1.22	2.29	2.40	8.92	9.34
85	0.01	0.01	0.14	0.16	0.36	0.38	1.35	1.39	2.63	2.76	9.10	10.06

Appendix II.
Undecane in plant tissue

Tabel II-1. Undecane concentrations in plant tissue (µg/g fresh weight) of Solanum nigrum in three different treatments. Concentrations were measured in triplicate at one, two and three weeks after onset of exposure.

Treatment (ppmV)	Sample	Undecane (µg/g fw)		
		1 week	2 weeks	3 weeks
1	1	1.50	1.06	1.30
	2	1.65	1.03	1.36
	3	1.28	1.08	1.24
3	1	3.10	2.88	2.61
	2	2.72	2.49	2.45
	3	2.83	2.66	2.52
9	1	11.02	9.18	7.10
	2	10.63	8.21	8.59
	3	10.55	7.98	7.46

Appendix III.
Plant measurements

Tabel III-1. Measurements of individual endpoints for each of the species subjected to a range of undecane concentrations for up to 12 weeks. The presence of a negative trend is indicated for each endpoint..

Treatment (ppmV)	Plant no	Solanum Shoot (g)	Roots (g)	Berries (no)	Plantago Plant (g)	Ears (no) 59 days	Ears (no) 71 days	Ears (no) 84 days	Ears (cm)	Picea Shoot (g)	Roots (g)	Brassica Shoot (g)	Roots (g)	Leaf area (cm2)
Control	1	5.81	1.04	81	4.63	2	4	10	38.7	13.72	13.81	4.99	1.63	647.0
	2	6.97	1.20	110	8.18	0	0	1	15.0	11.07	12.30	5.12	1.88	702.8
	3	6.47	1.07	86	8.28	0	4	5	32.6	12.60	11.62	4.08	1.28	703.5
	4	6.22	1.07	100	7.61	2	10	15	23.5	9.17	10.10	3.77	1.76	602.2
0.1	1	4.67	1.25	108	6.44	0	0	0		12.52	12.10	3.75	1.29	637.7
	2	6.33	0.94	104	5.65	0	2	9	27.8	11.80	10.65	4.04	1.32	498.8
	3	5.38	1.39	110	6.44	0	5	9	36.3	11.55	13.84	4.39	1.77	489.6
	4	6.67	1.19	153	6.35	2	7	12	32.7	10.92	11.43	3.75	1.51	485.3
0.3	1	5.76	0.90	123	6.30	0	4	10	27.4	15.96	13.12	4.74	1.27	587.2
	2	5.73	1.18	104	6.53	1	8	11	43.2	10.75	9.27	3.39	1.81	453.6
	3	5.65	1.37	95	5.74	1	5	9	26.7	14.14	18.99	3.47	1.58	485.2
	4	5.98	1.12	106	6.93	0	5	9	34.7	13.52	12.61	3.31	1.76	604.8
1	1	6.44	1.03	111	6.32	0	4	5	36.8	13.10	11.72	3.02	1.49	428.7
	2	5.56	1.28	85	7.08	1	6	10	32.1	9.86	11.15	3.10	1.48	498.3
	3	6.41	1.11	103	5.38	0	2	5	27.8	13.11	10.89	2.91	1.72	461.8
	4	5.24	1.27	106	4.94	0	4	9	34.0	11.35	12.54	3.64	1.86	496.8
3	1	6.06	1.19	105	5.33	0	4	9	22.6	12.97	12.85	2.27	1.33	317.9
	2	5.38	1.36	95	5.56	2	6	10	31.0	10.30	8.24	3.58	1.05	612.1
	3	5.63	1.40	82	6.34	0	4	12	27.6	10.85	9.50	2.64	1.19	458.0
	4	4.40	1.35	102	5.57	5	8	13	32.1	12.24	12.07	2.35	1.36	438.7
9	1	5.66	0.89	104	8.57	0	0	2	12.0	11.61	9.99	2.66	1.19	373.6
	2	4.41	0.69	76	6.88	2	0	1	6.0	13.16	13.82	3.52	1.39	552.5
	3	4.46	0.91	56	7.95	0	5	10	22.7	15.00	12.95	2.96	1.27	528.7
	4	4.35	0.86	57	7.15	0	0	1	31.0	11.70	10.81	3.46	1.37	517.8
Trend		yes	yes	no	no	no	no	no	yes	no	no	yes	no	no

Treatment (ppmV)	Plant no	Phaseolus				Holcus			Trifolium		
		Shoot (g)	Roots (g)	Pods (g)	Leaf area (cm2)	Shoot (g)	Roots (g)	Tillers (no)	Shoot (g)	Roots (g)	Stolons (no)
Control	1	7.76	1.79	1.86	441.4	6.10	2.47	32	3.75	0.62	7
	2	7.57	1.91	1.96	420.9	6.18	2.06	34			
	3	8.37	1.51	2.11	437.9	5.97	3.62	45	5.38	1.17	11
	4	7.86	1.69	1.74	381.9	6.24	2.59	58	4.13	0.95	11
0.1	1	9.84	2.11	1.27	975.0	7.84	3.10	36	1.74	0.55	10
	2	7.51	1.86	1.29	510.8	6.72	2.11	34	0.70	0.20	5
	3	6.58	1.65	1.03	467.1	7.13	3.17	60	2.13	0.34	5
	4	6.11	1.93	1.00	297.1	7.21	2.68	36	0.91	0.38	8
0.3	1	8.54	1.59	2.46	859.1	6.67	2.45	35	3.32	0.60	7
	2	8.35	2.22	1.69	746.3	4.64	1.76	35	3.77	1.12	10
	3	8.14	1.99	1.48	378.2	6.60	2.66	40	2.01	0.22	5
	4	6.36	1.90	1.11	242.2	5.57	2.35	35	1.45	0.50	10
1	1	7.75	1.41	1.54	444.0	6.59	3.11	45	4.45	0.83	10
	2	7.57	1.79	1.48	746.5	6.27	3.70	36	1.64	0.25	5
	3	7.51	1.44	1.62	277.8	5.08	2.04	31	3.32	0.81	6
	4	6.52	1.45	1.18	363.0	5.05	2.73	46	1.51	0.34	5
3	1	7.87	1.89	1.24	695.0	6.61	2.77	39	1.91	0.59	7
	2	6.54	1.81	1.01	303.5	5.55	2.12	30	1.86	0.41	6
	3	6.79	1.70	0.97	355.1	7.94	2.31	37	1.13	0.37	6
	4	7.24	1.32	1.02	650.9	6.48	3.32	35	0.36	0.11	5
9	1	5.83	1.37	1.55	338.5	6.34	1.80	53	1.50	0.26	8
	2	7.57	1.56	1.71	253.4	6.56	1.96	51	4.31	0.53	8
	3	7.14	1.41	1.72	470.6	6.55	2.16	24	4.18	0.84	8
	4	6.55	1.91	1.40	238.0	5.76	2.35	28	5.26	0.95	9
Trend		yes	no	no	no	no	no	no	no	no	no