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EUROPEAR RESEARCH PROGRESS REPORT

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EC-FAIR1-CT96-1803

Quality improvement of pears by predictive and adaptive technology

Content

General outline of the project

Practical problem
The main objective of the project is the optimization of Conference pear quality and the reduction of losses during storage, by preventing the development of disorders resulting in Brown Heart in Conference pears. Brown Heart in Conference pears is the browning of the flesh, especially the core region, and the subsequent development of cavities. The pears are not suitable for consumption, even with minor symptoms. Losses can add up to 16.5 mln Ecu per year. The causes that underlie the development of the disorder are unknown, although there is a lot of correlative knowledge on the development of Brown Heart. The occurrence is influenced by weather factors, orchard factors (location, nutrition), picking date, post-harvest treatments and storage conditions. However, relationships found in one country cannot simply be applied for other countries or growing seasons.

Goal of the research
To clarify and quantify physiological processes involved in the browning of pear tissue, and the development of technology which enables a rapid measurement and decision about the post-harvest treatments and storage conditions.

Short description of the approach
Because pear tissue has a very low porosity, small changes in metabolic rates or diffusion rates within the tissue can lead to very low internal oxygen levels. Increased fermentation can lead to an increase of toxic metabolites like acetaldehyde. This may reduce cell viability and induce cell death, leading to the Brown Heart disorder. Another explanation is that the combination of oxidative and fermentative processes are not sufficient to maintain cell viability. The reason for a difference in occurrence of Brown Heart between Northern and Southern European countries might be a the influence of different climate and/or agronomical factors on pear growth and development. This could result in different metabolic rates, resistance to gas diffusion, energy metabolism, energy needs for maintenance costs, etc. To check this hypothesis data is needed on climate and orchard conditions during the growing season and gas exchange rates, diffusion resistance and pear quality throughout the storage season, carried out in different European countries. Once a physiological explanation is found, storage conditions have to be adapted in order to prevent disorders. For this purpose predictive models are needed, which use information collected during the growth of the pear, or directly after harvest. Recently several models are developed describing gas exchange based on enzyme kinetics, that could form the basis for the models to be developed within the project. The models mentioned describe the relation between gas (O_2 and CO_2) concentrations and O_2 consumption and CO_2 and ATP production rates.

Project participants

Contractor	1. ATO	2. IRTA	3. IVTPA	4. FPO	5. VCBT	6. UHOH
Subcontractor	Auctions	Cooperation TRECOOP Lleida	Technical service cooperation	Auction CHZ		Marktge-meinshaft Bodensee

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Schedule of meetings

Number	Day	Month	Year	Host	Location
1	3	6	1997	VCBT	Leuven (B)
2	12/13	1	1998	UHOH	Weingarten (D)
3	15/16	6	1998	IVTPA	Milan (I)
4	11/12	1	1999	ATO	Wageningen (NL)
5	14/15	6	1999	IRTA	Llerida (E)
6	19/30	5	2000	FPO	Wilhelminadorp (NL)
7	*	7	2001	ATO	Wageningen (NL)

* Will be planned close before the 8th International Controlled Atmosphere Research Conference, to be held in the Rotterdam, The Netherlands.

Schedule of deliverables

Year	**Month**	**Report**	**Access to deliverable**
1996	October	Technical annex	*Public*
1997	June	*Start of the project*	
1998	July	Individual Progress reports	Confidential
1998	August	Consolidated Progress report	Confidential
1998	August	Abstract	*Public*
1999	July	Individual Progress reports	Confidential
1999	August	Consolidated Progress report	Confidential
1999	August	Abstract	*Public*
2000	July	Individual Progress reports	Confidential
2000	August	Consolidated Progress report	Confidential
2000	August	Abstract	*Public*
2001	July	Individual Progress reports	Confidential
2001	August	Consolidated Progress report	Confidential
2001	August	Abstract	*Public*
2001	October	Final report	*Public*

ANNEX I

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Abstract

From 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project		
Total cost:	1600,3 kECU	*EC contribution:*	1045 kECU (65,3%)
Commencement date:	01-06-1997	*Duration:*	4 years
Completion date:	31-05-2001		
EC contact:	DG VI/F.II.3, Fax: +32 - 2 296 3029		

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Partners (all contractors):

IRTA (Technological Research Institute on Agricultural Products), Spain
IVTPA (Research Institute for Agricultural Products Technologies), Italy
FPO (Research Station for Fruit Growing), The Netherlands
VCBT (Flemish Centre for Storage of Horticultural products), Belgium
UHOH (Höhenheim University, Research Station on Agricultural Production and Ecology), Germany

I. Objectives

Main objective of the project is optimization of Conference pear quality and the reduction of losses during storage, by preventing the development of disorders. Key element in the project is the development of technology which enables a rapid measurement and decision about post-harvest treatments and optimal storage conditions. The main advantage of such a technology is that it leads to an accurate advice based on actual product physiology directly after harvest and before storage. Models will be developed for advice on optimal storage en pre-storage treatments, and for predictions on maximum storability. Specific post-harvest treatments will be developed to increase storability, or reduce the risk of the brown heart disorder. It is expected that the physiological and biochemical measurements within the project will elucidate the main processes that need to be controlled for an effective treatment in practical situations.

II. Description of work

Ten tasks can be distinguished:

1. Cultivation: Pears to be used within this project are produced in all the participating countries of the project. The management of the orchards is under control of the participants. Orchards with a known history, varying in susceptibility for Brown Heart, are selected.
2. Harvest: Pears are picked at several (3) dates every year, to introduce a variance of development stages and pear characteristics at every location. Harvest dates are based on maturity and determined by all partners by comparable methods.
3. Storage: Pears will be stored to judge the development of brown heart and to have material for experiments throughout the year. The storage conditions will be standardised (for statistical and comparative reasons). These conditions include one which is optimal for long term storage (in years with low incidence of Brown Heart) and one known to induce brown heart. This ensures to have Brown Heart occurring every year of the project.
4. Climate and orchard factors: Growing conditions, like climate, fertilisation and tree load, can influence fruit characteristics. A variation in climate is introduced within the project by growing pears in different countries. The meteorological data will be related with pear characteristics and the occurrence of Brown Heart. The measured climate variation is on a macro scale. Also within trees a variation in climate can occur. Therefore pears grown at different locations within trees are separately judged by one partner. Variations in orchard management are incorporated by mineral applications (Ca, K, P, Mg) and variations in fruit load.
5. Postharvest treatments: Several treatments will be tested directly after harvest in order to reduce the risk of brown heart during storage. The treatments are based on different temperature and oxygen scenarios. Two types of treatments can be distinguished; A. minimizing the occurrence of brown heart, B. rapid test for the sensitivity for brown heart.
6. Gas exchange: Internal gas composition is determined by both metabolic rates (oxygen uptake, carbon dioxide production) and the resistance against diffusion of those gasses. The objective of this task is to monitor changes in internal gas composition and the primary cause of such changes (metabolic rates or diffusion resistance). Internal concentrations are compared between the various locations and harvest dates, and related to the intensity of the brown heart disorder.
7. Destructive measurements: Different types of measurements are considered essential in understanding the nature of the disorder of brown heart. Task 7 is divided into three subtasks:: a. Quality evaluation, b. Biochemical studies, c. Mineral composition.
8. Non-destructive measurements: Non-destructive detection techniques will be tested to enable the detection of Brown Heart in a preliminary stage. A non-destructive method would also facilitate the use of the same pears for several measurements in experiments.
9. Model development: In this task the data and information of the other tasks are combined and integrated. The statistical analysis on the original data will be performed by all partners in addition to the tasks. Guidelines will be given. Modelling will take place on the analyzed data. Two models will be constructed. The first model is a correlative model based on the relationship between climate, nutrition and Brown Heart data. The second model uses physiological processes like metabolic rates and energy fluxes. This model will be based on existing models that use enzyme kinetics (both

oxidative and fermentative pathways) and diffusion characteristics. The model will calculate and predict the flow of O_2, CO_2 and ATP.

10. Validation: Functioning of the models will be validated by comparing predictions on disorders and actions to be taken with results obtained by commercial partners in large scale facilities.

III. State of progress

Climate data were collected by IRTA, IVTPA, FPO, VCBT and UHOH, resulting in 180 independent data sets after two years (task 4). An advice on a postharvest method to reduce the risk was generated by FPO and confirmed by ATO. The method is based on delaying the application of Controlled Atmosphere conditions to harvested pears for a specific period (task 5). In addition to this a rapid test to predict susceptibility of pears for the disorder was developed by FPO (task 5). The test is based on a temperature/CO_2 treatment, and was validated in the second year. Methodology on gas exchange and diffusion resistance measurements was tested and optimised, and was used by three partners; ATO, VCBT and UHOH (task 6). Protocols were distributed by ATO, and implemented by VCBT and UHOH. Results indicate that there is not an obvious relation between internal CO2 concentrations (as calculated from metabolic rates and diffusion resistance) and increased brown heart at late harvest dates. Also respriation measurements during the procedure to prevent the disorder (delaying CA) indicate that increased internal CO_2 concentrations are not the primary cause of the problem. Instead the results underline another hypothesis, that the disorder will occur after a shortage of metabolic energy (ATP). Apparently a subsequent change in temperature and oxygen does not lead to (temporary) shortage in ATP. Energy metabolism will be a primary focus in the third year (as planned).
For task 7 comparable results on ascorbate/vitamin C content in the pears were found by ATO, IRTA and UHOH. Pears more susceptible to the disorder showed lower ascorbate levels, such as pears from a late harvest, pears after longer storage periods and pears stored at higher CO_2 concentrations. Ascorbate levels continued to decrease during a treatment with high CO_2. Comparable results were also found by IRTA and ATO on ethane production, which was increased at high CO_2. After extensive research on polyphenol-oxidases, ATO and IRTA concluded that there was no direct relation between PPO content (or activity) and the risk for brown heart. On the other hand, ascorbate levels, and possible other compounds of the oxygen scavenging system as well, are very promising for predicting the disorder and will very likely play a key role in the physiological model. When all the results thusfar are integrated, the central hypothesis as formulated in the technical annex can be described in more detail as a string of processes (see Annex II).

IV. Achievements

For task 3 a method was developed to reduce the risk for brown heart that is tested in practice by Dutch auctions this year. We believe that the results on ascorbic acid will result in an effective tool for predictions on the occurrence of brown heart.

V. Future actions

All tasks are performed as planned, except for task 7. In the technical annex the work described in task 7 (biochemical measurements) primarily focussed on the role of PPO, the enzyme involved in the browning reaction. After extensive research it was concluded that a process prior to PPO action must be monitored in order to find a way of control to prevent the browning disorder. Very likely a control point is the amount of a compound known for its role in oxygen radical scavenging, vitamin C. Variations in harvest date, gas conditions and ripening showed to influence both the vitamin C content and the occurrence of the brown heart disorder. Therefore it was concluded that more effort should be attributed to measurements on vitamin C, also in relation to orchard factors, such as the position of the pear in the tree. Less effort can be attributed to analysis that tries to (cor)relate mineral composition to the susceptibility of the pear to brown heart, since no clear results were obtained from these measurements. The preliminary models developed in year 2 will be validated and extended in year 3 (task 9).

ANNEX II

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Consolidated Progress Report for the period

From 01-06-98 to 31-05-99

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SCIENTIFIC SYNTHESIS

Introduction

Main objective of the project is optimisation of Conference pear quality and the reduction of losses during storage, by preventing the development of disorders. Key element in the project is the development of technology, which enables a rapid measurement and decision about post-harvest treatments and optimal storage conditions. The main advantage of such a technology is that it leads to an accurate advice based on actual product physiology directly after harvest and before storage. Three pathways are be the main objectives of the project:

1. development of a predictive model based on registered variances in orchard and weather conditions of a broad group of European countries;
2. development of a predictive model based on a rapid assessment of pear physiology (respiration rate, fermentation rate, resistance to gas diffusion);
3. testing and implementation of post-harvest treatments, which improve the storability of the harvested pears, leading to pears with a better quality.

The models will be used for advice on optimal storage en pre-storage treatments, and for predictions on maximum storability. Specific post-harvest treatments will be developed to increase storability, or reduce the risk of the brown heart disorder. It is expected that the physiological and biochemical measurements within the project will elucidate the main processes that need to be controlled for an effective treatment in practical situations. An additional part of the project is a fourth objective:

4. the development of a non-destructive detection system for Brown Hearts. A non-destructive method would facilitate the use of the same pears for several other measurements.

Tasks

Ten tasks can be distinguished to achieve the objectives. Within the first year of the project, action was taken for tasks 1 to 7. After every task it is mentioned for which of the four objectives it will be necessary:

Task	Goal	Description
1	1,2,3,4	Cultivation of pears;
2	1,2,3,4	Harvest of pears;
3	1,2,3,4	Storage of pears;
4	1	Registration and variation in weather and orchard conditions;
5	3	Post-harvest treatments;
6	2,3	Gas exchange and diffusion measurements;
7	1,2,3	Destructive measurements (quality evaluation, biochemical studies);
8	4	Non-destructive measurements (detection of disorders);
9	1,2	Model development;
10	1,2	Validation of models in practice.

Results

Climate data were collected by IRTA, IVTPA, FPO, VCBT and UHOH, resulting in 180 independent data sets after two years (task 4). An advice on a postharvest method to reduce the risk was generated by FPO and confirmed by ATO. The method is based on delaying the application of Controlled Atmosphere conditions to harvested pears for a specific period (task 5). In addition to this a rapid test to predict susceptibility of pears for the disorder was developed by FPO (task 5). The test is based on a temperature/CO_2 treatment , and was validated in the second year. Methodology on gas exchange and diffusion resistance measurements was tested and optimised, and was used by three partners; ATO, VCBT and UHOH (task 6). Protocols were distributed by ATO, and implemented by VCBT and UHOH. Results indicate that there is not an obvious relation between internal CO2 concentrations (as calculated from metabolic rates and diffusion resistance) and increased brown heart at late harvest dates. Also respriation measurements during the procedure to prevent the disorder (delaying CA) indicate that increased internal CO_2 concentrations are not the primary cause of the problem. Instead the results underline another hypothesis, that the disorder will occur after a shortage of metabolic energy (ATP). Apparently a subsequent change in temperature and oxygen does not lead to

(temporary) shortage in ATP. Energy metabolism will be a primary focus in the third year (as planned).
For task 7 comparable results on ascorbate/vitamin C content in the pears were found by ATO, IRTA and UHOH. Pears more susceptible to the disorder showed lower ascorbate levels, such as pears from a late harvest, pears after longer storage periods and pears stored at higher CO_2 concentrations. Ascorbate levels continued to decrease during a treatment with high CO_2. Comparable results were also found by IRTA and ATO on ethane production, which was increased at high CO_2. After extensive research on polyphenol-oxidases, ATO and IRTA concluded that there was no direct relation between PPO content (or activity) and the risk for brown heart. On the other hand, ascorbate levels, and possible other compounds of the oxygen scavenging system as well, are very promising for predicting the disorder and will very likely play a key role in the physiological model. When all the results thusfar are integrated, the central hypothesis as formulated in the technical annex can be described in more detail as a string of processes:

a. A decrease in O_2 and an increase in CO_2 leads to a decrease in respiration;
b. At low O_2 leads an increase in CO_2 also to a decrease in fermentation;
c. Reduced respiration and fermentation results in reduced ATP production;
d. Reduced ATP production leads to less ATP available for maintenance processes, such as maintaining the oxygen scavenger pool (antioxidants like ascorbate);
e. Possibly also a direct relation exists between increased CO_2 and decreased levels of ascorbate;
f. This can eventually result in membrane damage by oxygen radicals;
g. Membrane damage can be detected by increased ethane production;
h. With increased membrane damage, PPO enzymes and substrate are no longer compartmentated;
i. Browning reactions occur;
j. Cells are no longer viable, necrosis (hollow areas) can be observed.

Difficulties and changes in the second year
No problems occurred in the second year of the project.

Discussion - Conclusion
Thusfar activities were carried out as planned. Task 5 already lead to a method very succesful in reducing the risk for the brown heart disorder. Nevertheless the research within task 7 will be intensified, especially on vitamin C and energy metabolism, in order to explain the positive results of the prevention method and to ensure that the positive results thusfar are dependable and not limited to two specific growing seasons.

Future actions
The most important action that will be started from the next period onwards is modelling (task 9). Two preliminary models will be developed, one based on climate data and on based on gas exchange characteristics and gas composition in the pear. Both ATO and VCBT will be involved. Other tasks are performed as planned, except for task 7 where a slight change was made after the first year.

Action requested from the Commission
No action from the commission is requested.

METHODOLOGY AND RESEARCH TASKS

Task 1 – Cultivation of pears

Participants: IRTA, IVTPA, FPO, VCBT, UHOH
Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-month: 9.4
No. of man-month devoted already to the task: 5.7

Objectives
In every year of the project pears will be grown in different climate conditions.

Methodology
The pears to be used within this project will be produced in all the participating countries of the project. The management of the orchards is under control of the participants. Orchards with a known history, varying in susceptibility for Brown Heart, were selected. All partners were involved in the selection procedure.

Deliverables
IRTA, IVTPA, FPO, VCBT and UHOH have grown their own pears in sufficient quantities to meet experimental needs. These partners included various orchards, which in combination with a number of harvest dates will generate a high amount of independent data-sets (for task 9, modelling).

Links
Material (pears) from this task has been used for the other tasks.

Progress second 12 months
In the next table the number of orchards as mentioned in the technical annex and the actual number of orchards is given. In the second year the number of data-sets increased from 92 to 180.

Overview of the origin of the data-sets of climate factors and occurrence of Brown-Heart collected by different partners during 1997 and 1998.

Partner	1997				1998			
	Orchard	Nr of locations	Nr of harvests	Nr of subsets	Orchard	Nr of locations	Nr of harvests	Nr of subsets
FPO	Numansdorp	4	5	1	Numansdorp	3	5	1
	Wilhelminadorp	3	5	1	Wilhelminadorp	3	5	1
	Kapelle	1	5	1	Kapelle	1	5	1
	Dodewaard	2	5	1	Dodewaard	1	5	1
VCBT					Velm	1	3	1
					Zellik	1	3	1
UHOH	Bavensdorf	1	3	3	Bavensdorf	1	3	3
	Salem-Mittelsweil.	1	3	3	Salem-Mittelsweil.	1	3	3
IVTPA	Malaguti/Rinaldi	2	3	1	Malaguti	1	3	2
IRTA	Giminels	1	3	3	Giminels	1	2	3
	Albatarrech	1	3	3	Albatarrech	1	2	3
					Mollerusa	1	2	3

Difficulties:
No problems occurred in the 1998 growing season. Cropping behaviour of the pear trees was good and regularly. The cultivation program was carried out as planned.

Task 2 – Harvest of pears

Participants: ATO, IRTA, IVTPA, FPO, VCBT, UHOH
Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-month: 23.6
No. of man-month devoted already to the task: 11.8

Objectives

Harvest date is strongly related to the risk for Brown Heart. Therefore it is important to define strict rules for all partners about the moment of harvest. First it is important that every year pears are picked at several dates, f.i. three dates, to introduce a variance of development stages and pear characteristics at every location. Secondly the dates themselves have to be based on maturity and determined by all partners by comparable methods.

Methodology

Maturity is determined by the Streif index (firmness/(sugars*starch)) or by firmness readings alone. ATO, IRTA, IVTPA, FPO, VCBT and UHOH will carry out their own harvest. FPO used only firmness to estimate optimum harvest date. A firmness of 6.3 kg with 7 mm plunger (penetrometer) is considered to be representative for the optimum harvest period.

Deliverables

All partners harvested pears at various harvest dates, including the optimal harvest date.

Links

Pears were used for the other tasks.

Progress second 12 months

Maturity of pears was measured in order to determine optimal harvest dates. Maturity was determined by the Streif index (firmness / (soluble sugars * starch)) or on firmness alone. Interesting to know is whether the selected 'optimal harvest dates' were on pears of comparable maturity. Except for the pears from Italy (with a high starch index) this seems to be the case. Comparable results were also found in the first 12 months. A summary of the measurements of all partners is given in the next table.

Partner	Harvest date	Firmness (kg/0.5cm^2)	Soluble Sugars (Brix value)	Starch (colour index)	Streif index	Vitamin C (mg/100g)
ATO	10-09-98	6.67	11.2	5.2	0.115	6.7
IRTA	24-08-98	6.70	12.4	*	x	4.5
IVTPA	17-08-98	6.47	12.3	13.5	0.039	0.5[a]
FPO	09-09-98	6.44	11.7	4.8	0.115	7.4
VCBT	31-08-98	7.62	12.1	6.0	0.105	*
UHOH	07-09-98	5.78	12.9	5.4	0.083	5.4

*a = uncertain, * = not measured, x = could not be determined*

As for the 1997 results there are large differences in harvest date and Streif index between the partners. When these results are compared with the results of 1997, the 1998 values for the Streif index are remarkably lower for all partners.

Task 3 – Storage of pears

Participants:	ATO, IRTA, IVTPA, FPO, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	38
No. of man-month devoted already to the task:	19

Objectives

Pears will be stored to judge the development of the brown heart disorder and to have material for experiments throughout the year. The storage conditions are partly standardised (for statistical and comparative reasons). In addition to the standard storage conditions each partner is free to select additional storage conditions. Standard storage conditions are -0.5 °C and 95% relative humidity in combination with 2% O_2 and 0.7 or 5% CO_2. These conditions include a condition which is optimal for long term storage (in years with low incidence of Brown Heart) and a condition which is known to induce Brown Heart. In this way Brown Heart is expected to develop every year, but at different degrees. This ensures to have Brown Heart occurring every year of the project. Samples from the storage facilities will be taken (at least) every 6 weeks. These samples will be used for the measurements described in task 7 and 8.

Methodology

The standard pre-storage procedure can be described as follows: the cooling down procedure will be started as soon as possible after harvest, and after 48 hours the minimum temperature should be reached. From then on the temperature is constantly monitored. One week after the start of the storage the storage rooms are flushed with N_2 until a concentration of 4% O_2 is reached. The rest of the O_2 in the storage room is removed by respiration of the pears, until 2% O_2 is reached. From then on the gas concentrations are continuously monitored and controlled.

Deliverables

All partners have stored their own pears in sufficient quantities to meet experimental needs, at at least two different CA storage regimes.

Links

Stored pears will be used for several types of measurements (task 6, 7 and 8) throughout the storage season.

Progress second 12 months

All partners have stored the pears at at least two storage conditions; standard CA and a condition with a higher risk for brown heart. The storage season ranged between 7 to 8 months.

Difficulties:

No brown heart was observed by IRTA and IVTPA, not even in the high CO_2 storage condition. Therefore the conclusion was drawn that no longer the 5% CO2 condition should be used for storage, since it was not dependable to generate brown-heart for research purposes. Instead it was advised to always store pears from a very late harvest.

Task 4 – Orchard and climate factors

Participants:	IRTA, IVTPA, FPO, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	18.7
No. of man-month devoted already to the task:	9.4

Objectives

1. A variation in climate is introduced within the project by growing pears in different countries. The meteorological data will be related later with pear characteristics and the occurrence of Brown Heart. The measured climate variation is on a macro scale.
2. Within trees a variation in climate can occur. Therefore pears grown at different locations within trees are separately judged by FPO.

Not all differences in brown heart can be related to climate, since also differences between orchards in the same climate zone are found. Therefore two specific influences at the orchard level are investigated:

3. The influence of mineral application (fertilisation): The concentration of calcium, potassium, phosphorus and magnesium will be varied by IRTA and FPO.
$. The influence of fruit load will be investigated by FPO and UHOH.

Methodology

IRTA, FPO and UHOH collect meteorological data in every orchard mentioned in task 1. IVTPA and VCBT can collect data from a meteorological station within a few kilometres from the orchards. Meteorological parameters will be recorded each year both during the preharvest period until the harvest day, and will include temperature (minimum, maximum, mean), rainfall, radiation and hours of sunshine. Fertilisation will match the normal procedures of the partners within this project. However, only fertilisation of the leaves will be applied. The most important goal is to create a variation in the mineral content of the fruits. IRTA applied a N fertilisation and FPO applied N, K and Ca fertilisation.

In order to vary the sink/source ratio, two types of management were carried out. To reduce the leave biomass, trees can be pruned. To reduce the number of fruits per tree (tree load), trees can be thinned early in the season. In both cases estimations of leaf biomass (Leaf Area Index) and tree load have to be made. FPO influenced tree load by removing 33% or 66% of the pears of trees with a full load of pears. They also compared with trees showing roughly 33% or 66% natural losses of fruits. UHOH made a distinction between trees showing a natural difference in crop load, resulting in three groups (0.12, 0.18 or 0.25 pears/cm^2 leaf area).

Deliverables

Meteorological data were collected. They indeed seem relevant in predicting the brown heart disorder (see task 9). Mineral treatments have been applied, causing relevant changes in mineral content within the pears. Nevertheless, no significant correlations between mineral content degree of the disorder were found. Mineral applications were therefore terminated after year 2. Position in the tree is relevant indeed, as confirmed by IVTPA. This knowledge can be useful for other picking strategies (to be worked out by FPO). Tree load is also important as a factor for causing brown heart. Both FPO and UHOH found that pears from trees with a low tree load have a much higher chance for brown heart. This can also lead to other picking strategies (separation from pears from such trees) and storage conditions for such a batch of pears.

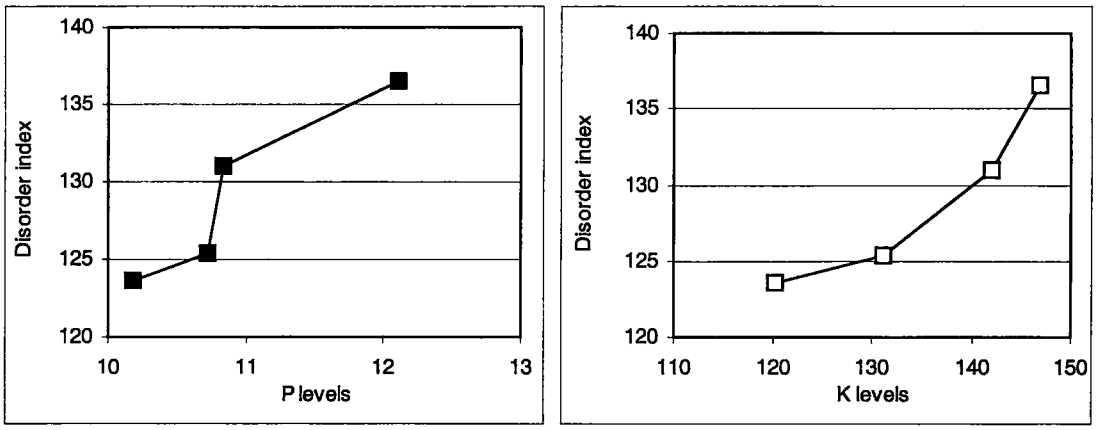
Links

Data from the meteorological measurements were send to ATO and used for the modelling task (task 9).

Progress second 12 months

Climate data
Data were generated by all partners except ATO. The relevance of the various climate parameters will be discussed at task 9.

Mineral application
The second season only FPO continued with mineral applications. As in 1997, they found that extra nutrition with K, Ca or N did not result in significant changes in nutrient levels in the fruit one month before harvest or at harvest (pick 3). The treatments had also no effect on the incidence of brown heart. In conclusion it seems that mineral application is not a major cause for brown heart. When, however, not fertilisation but mineral content is related to disorders, a correlation with P and K levels can be observed (see next graph).



Position in the tree
In the first year FPO found significant differences in brown heart between pears from different locations within the tree. This was confirmed this year by FPO and IVTPA. Pears from high positions in the tree showed brown heart at earlier harvest dates than pears from other locations (including the optimal harvest date). FPO found that at late harvest dates all positions show a high risk for brown heart.

Tree load
After the first year FPO and UHOH concluded that both fruit load in the tree affected the incidence of brown heart after storage. This was confirmed in the second year. Brown heart incidence was least in normal bearing trees. Removing 2/3 of the fruits resulted in a clear increase in brown heart and cavities, especially in the late pickings. A low amount of fruits by natural processes (flowering, fruit set, June drop) stimulated the incidence of cavities even more. This was found after storage from pears harvested in 1997 and in 1998.

Improvements
- The climate data were not always complete (already from anthesis)
- More locations with consequently more variation in climate will improve the ability of the modelling techniques to find the causes for the occurrence of Brown-Heart.
- Similar measurements of radiation (spectrum) among all partners would allow including radiation into the model.

Task 5 – Postharvest treatments

Participants:	ATO, IRTA, IVTPA, FPO, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	16
No. of man-month devoted already to the task:	8

Objectives

Several treatments were tested directly after harvest in order to reduce the risk of losses due to brown heart during storage. Two types of treatments can be distinguished:

A. minimising the occurrence of Brown Heart: The aim of this part of the work is to determine the preventive effect of various pre-storage treatments on Brown Heart.

B. rapid test for the sensitivity for Brown Heart: The aim of this part of the work is the development of a rapid test in order to determine the susceptibility of pears to Brown Heart before storage.

Methodology

A. IRTA (partner 2) will lay special emphasis on a time delay prior to cooling. Pre-cooling is a possible way to prevent Brown Heart. After harvest (fruits were picked at commercial harvest maturity, H2), fruits were kept for 0, 1, 2, 4 and 6 days at 25°C and then stored in conventional CA chamber. After 2, 4 and 6 months of storage the effect of this pre-maturation procedure on B.H incidence and quality was established.

A. ATO and FPO focussed on delaying CA conditions (quick cooling, late CA instead of quick cooling and quick CA). Fruits from 7 orchards and all harvest dates were used. After 7 days cooling at -0.5 °C, storage was continued at -0.5 °C, 2% O_2 and 0.5 or 5.0% CO_2. Fruits from pick 3, 4 and 5 was placed at cooling conditions for 0, 2, 7, 21 or 50 days before being moved to -0.5 °C, 2% O_2 or 0.5% CO_2. Fruits were stored till March 30 1998. Incidence of brown heart, and fruit quality were measured after warming-up during one day.

B. FPO used high CO_2 concentrations, since these are clearly recognised as a major factor influencing Brown Heart and will therefore be used. The effects of high CO_2 concentrations at various temperatures on pear quality and the rapid development of Brown Heart levels will be measured. Pears from pick 2 (insensitive) and pick 4 (sensitive) from 7 orchards were placed at combinations of the following conditions: 2, 5 or 9 days at 18°C and 17, 14 or 9 days at 4 °C, 2 % O_2 and 0.5 or 5% CO_2. The total duration of the test was always 19 days.

Deliverables

The deliverables mentioned in the technical annex include two methods: a method to asses the risk for brown heart (high CO_2 treatment) and a method to reduce the risk during storage (delayed CA). Both methods are extensively tested now.

Links

The methodology to reduce the risk for brown heart is currently being used at several auctions throughout the Netherlands, in order to reduce the risk for brown heart.

Progress second 12 months

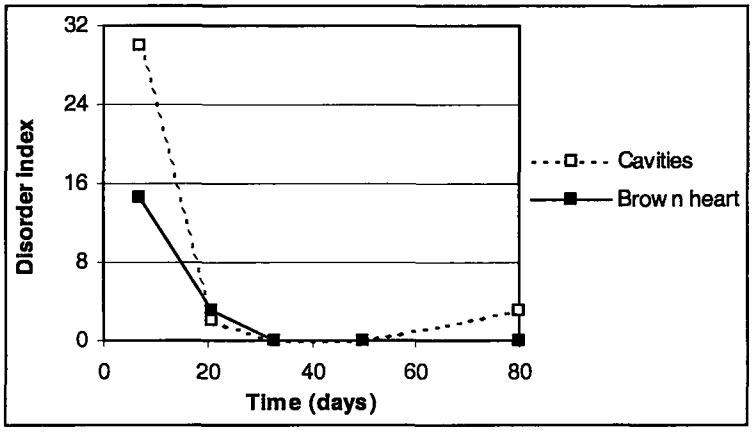
A. IRTA.

Fruits treated with CO_2 show higher firmness values during the storage period. SSC and acidity remain lower in the CO_2-treated fruits. A slight decrease in acetaldehyde and ethanol levels was observed, particularly for the treatment at 15 % of CO_2. No significant changes in the activity of ADH and PDC were found. Immediately after the treatment some significant changes in the oxidative metabolism were observed: a decrease in LOX and CAT activity and an increase in SOD activity and APX activity. After storage the CO_2-treated fruits generally presented less capability to produce peroxides (lower activity in SOD, lower activity in LOX) and this result likely explain why the membranes of these fruits presented

less peroxidative damage and lower electrolyte leakage. No physiological disorders were observed this year during storage. As a consequence the relationship between the CO_2 treatments and BH incidence could not be established. Nevertheless, the results on quality parameters (delay in ripening, higher firmness values) and on the biochemical parameters (less electrolyte leakage, short-term activation of SOD, without prejudicial effect on LOX and fermentative metabolism) indicate that these treatments could be useful to prevent senescence disorders in pears..

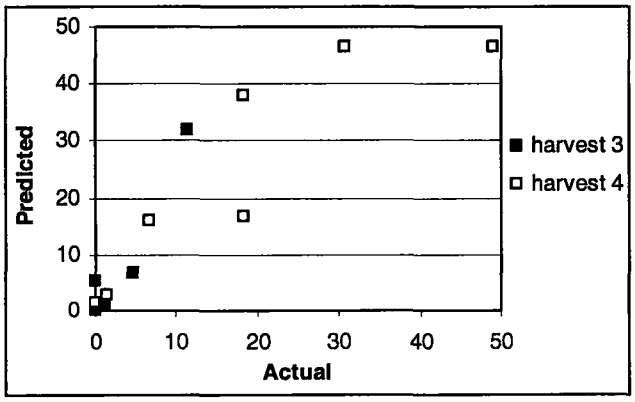
A.FPO.

This year, as in the preceding year, the incidence of disorders is very much reduced in fruit from late harvest dates by postponing the start of CA conditions more than 7 days. Exactly 7 days of delay, however, causes more brown heart than no delay at all. When all results are combined, the minimal delay period seems to be around 4 weeks. This is also shown in the next graph.



B. FPO.

The aim of a stress test is to indicate sensitive lots of fruit before CA conditions are started i.e. within 21 days. This means that any test should take not more than 19 days in order to evaluate the result and support the decision. Several stress scenarios tested in the first year were repeated in the second year. Brown heart found after one of the stress scenario's showed a good correlation with actual brown heart levels after storage in both the first as the second year. This scenario consisted out of 4 days at 18 °C and ambient air, followed by 14 days at 4 °C, 2% O_2 and 0.5% CO_2 . Results for harvest 3 and 4, the only relevant commercial harvest dates, are shown in the next graph.



Difficulties

IRTA: Since IRTA did not detect brown heart at all, the relevance of the high CO_2 pre-treatments could not be established.

Task 6 – Gas exchange measurements

Participants:	ATO, IVTPA, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	77
No. of man-month devoted already to the task:	50

Objectives

An increase of the metabolic gas CO_2 in the atmosphere surrounding pears is known to cause the brown heart disorder. This task is focussing on the accumulation of internal CO2 by measuring metabolic rates (oxygen uptake, carbon dioxide production) and the resistance against gas diffusion. The objective of this task is to monitor changes in internal gas composition and the primary cause of such changes (metabolic rates or diffusion resistance). Internal concentrations are compared between the various locations and harvest dates, and related to the intensity of the brown heart disorder.

Methodology

Gas exchange rates

Pears are placed in cuvettes and are subjected to a range of gas conditions (combinations of O_2 and CO_2). Gas exchange is measured by analysing the change in gas composition in a temporarily closed cuvette. With this head-space technique an exact number for free gas volume must be known. Additional measurements are: the volume of the cuvette, the volume of the pears and the pressure inside the cuvette. When these values are known gas exchange can be calculated in nmoles/kg.s.

Diffusion resistance

The method of measuring diffusion resistance as described by Peppelenbos and Jeksrud (1998) was slightly adjusted. The inert gas neon was used, but instead of measuring the diffusion of neon into the fruit the diffusion of neon out of the fruit was measured. First the fruit was stored in a cuvette with a high concentration of neon (5000-6000 ppm) for one night, assuming that by that time an equilibrium between the concentration inside the fruit and in the cuvette was reached. The final concentration was measured. Then the fruit was transferred to another cuvette, and the increase in neon was measured after short specific time intervals (linear increase in neon, Banks 1985). This linear method, however, can only be carried out when a quick gas chromatograph is available.

Deliverables

Methodology on gas exchange and diffusion resistance measurements was tested and optimised and is used by four partners; ATO, IVTPA, VCBT and UHOH. ATO and UHOH are also testing alternative ways for measuring gas diffusion to validate the chosen method. IVTPA is directly measuring internal gas conditions to validate the entire methodology.

Links

Protocols were distributed by ATO, and implemented by IVTPA, VCBT and UHOH.

Progress second 12 months

Differences in gas exchange, diffusion resistance and, if possible, internal gas concentrations will be related to disorders caused by (late) harvest dates, the delayed CA treatment and long term storage. Also differences between countries will be discussed.

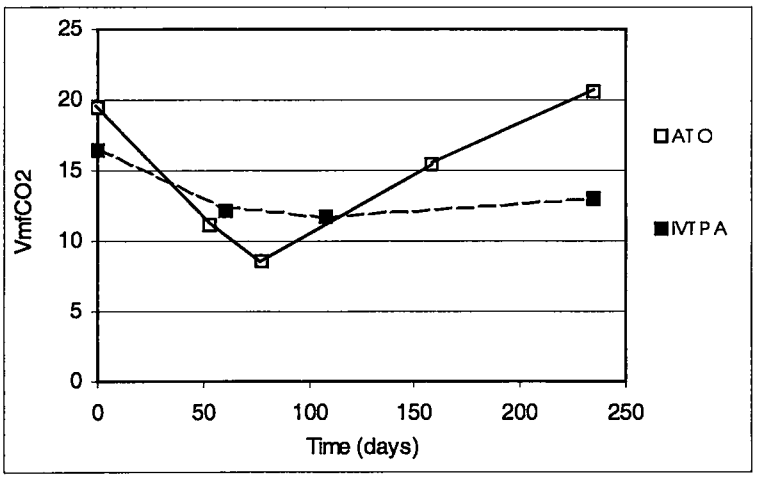
Harvest date

Per country different results were found. With later harvest dates ATO found no differences in metabolic rates, while IVTPA found a decrease and UHOH an increase in metabolic rates. In the first year, ATO also found an increase in respiration rate with harvest date. For diffusion resistance all

partners found no differences between harvest dates.
Conclusion: there does not seem to be an obvious relation between internal CO2 concentrations (as calculated from metabolic rates and diffusion resistance) and increased brown heart at late harvest dates.

Delay CA treatment
ATO and IVTPA found that pears subjected to a delay in CA show increased respiration and fermentation rates as compared to pears directly subjected to CA. With a comparable diffusion resistance (ATO) or increasing diffusion resistance (IVTPA) this leads to increasing internal CO_2 concentrations. Because the delay treatment in great extent prevents the brown heart disorder, internal CO_2 is not the explanation. Instead this result underlines another hypothesis, that the disorder will occur after a shortage of metabolic energy (ATP). After harvest pears are subjected to low temperature and low oxygen. Both factors largely decrease metabolic rates and ATP production. At the same time the pear tissue has to adapt to the different environment. Apparently a subsequent change in temperature and oxygen does not lead to (temporary) shortage in ATP.

Storage
During long term storage no big differences in respiration were observed by ATO, IVTPA and UHOH. ATO and IVTPA, however, found the lowest fermentation rates after a period of 3 to 4 months of storage (see next graph). After this period, fermentation rates increased again.



Differences between countries
When the results of different countries are compared, the modelling results are comparable. No big differences in gas exchange parameters were found that can explain differences in brown heart. The only small difference is for the response of respiration to increased CO2, described in KmCO2. Pears grown in the Netherlands (ATO) show in general lower values (= a greater CO2 influence) than pears grown in Italy (IVTPA). Since pears in the Netherlands have a greater risk for brown heart, this could be something to focus on next year.

Model results		**Parameter**				**Goodness of fit (R^2)**	
Partner	**Period**	**VmO2**	**KmO2**	**KmCO2**	**VmfCO2**	**O_2 model**	**CO_2 model**
ATO	harvest	15-19	0.3-0.8	18-24	15-25	81-89	43-63
	delay	17-22	0.3-1.4	12-19	11-20	84-91	58-77
	storage	18-25	0.9-1.8	8-15	9-20	77-91	51-68
IVTPA	harvest	14-27	0.3-0.9	18-36	16-18	75-99	-
	delay	10-18	0.2-0.5	9-42	11-19	67-97	37-58
	storage	9-19	0.4-0.9	14-35	12-13	70-78	42
VCBT	storage	24	2.2	8	16	-	-

Difficulties
UHOH: The respiration and diffusion measurement for sampling date 4 (end of storage after 6 months) couldn't be carry out because of technical problems with the GC. Due to bad separation of argon and oxygen peak with the used molsieve A column we had to change elements of the instrument.

Task 7 – Destructive measurements

Participants:	ATO, IRTA, IVTPA, FPO, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	134.5
No. of man-month devoted already to the task:	72.5

Objectives
Different types of measurements are considered essential in understanding the nature of the brown heart disorder. The measurements will increase essential knowledge on the process and guide the modelling task. Task 7 is divided into three subtasks:: a. Quality evaluation, b. Biochemical studies, c. Mineral composition.

7a. Quality evaluation (all partners)

Objective
The min objective of this task is controlling the occurrence of Brown Heart. For this purpose several fruits are sampled at various moments throughout the storage period. In addition other quality aspects are measured, to ensure that a good treatment against brown heart will not result in a reduction in other quality aspects.

Methodology
Quality changes after monitored during the storage period and after storage by a shelf-life simulation at 20 °C and 60% relative humidity for maximally two weeks. Other quality measurements are colour, firmness and appearance. Colour will be monitored visually, by Minolta or by computer imaging, firmness will be measured by compressive techniques (Instron, Penetrometer), and appearance will be judged by scoring the incidence of bacterial rot and fungal growth.

Deliverables
A relation between the occurrence of brown heart and harvest date, storage condition and region within Europe has been established for one year.

Links
Data from all partners were send to ATO and will also be used in task 9, modelling.

Progress second 12 months
One of the premises of the project, that pears grown in Northern Europe are more susceptible to the disorder, was confirmed in the first and the second year. More brown was found in the Netherlands and Belgium, and less in Spain and Italy. It is concluded that for this project a good range of orchards was selected.
In all orchards the pears from later harvest dates were more susceptible than pears from early harvest dates. The storage condition with elevated CO_2 clearly induced brown heart in all countries, although also with this extreme storage condition a difference was found between Northern and Southern European countries.

7b. Biochemical studies (ATO, IRTA, IVTPA, UHOH)

Objective
Several biochemical pathways are expected to be involved in the development of Brown Hearts in pears, such

as fermentation rate, energy metabolism and PPO action.

Methodology

Vitamin C; ascorbic and dehydroascorbic acid (ATO, IRTA, UHOH): Ascorbic acid and dehydroascorbic acid were separately determined in various tissues of the pear by high-pressure liquid chromatography (HPLC, ATO and IRTA) or a fluorometric technique (UHOH).

Antioxidative potential (UHOH): The antioxidative potential (AP) of lipo- and hydrophile antioxidants in Conference pears was assayed according to the method of Chevolleau et al (1992), and a modified method of Hillebrandt and Schmitz (unpublished). The test solution consisted of 0.5% ß-carotene and 20 mg linolic acid.

Enzymatic antioxidants (IRTA, UHOH): The activity of glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) was measured as follows:

- GR-activity by following the oxidation of NADPH.
- CAT activity by following the consumption of H_2O_2.
- SOD activity by quantifying the ability of enzyme extracts to inhibit light-induced conversion of nitroblue tetrazolium (NBT) to formazan.
- APX activity by the decrease in absorption at 290 nm of an assay mixture containing ascorbate.

Membrane peroxidation / MDA (UHOH): Malondialdehyde content (MDA, a secondary end product of lipid peroxidation) was determined by the thiobarbituric acid (TBA) reaction.

Membrane deterioration / ethane (ATO): Ethane release by intact pears was measured using a photo-acoustic laser device from the University of Nijmegen.

Ethanol, acetaldehyde and ethylacetate (UHOH): The determination of these compounds was done simultaneously by head space GC analysis.

Energy metabolism / NAD(H) and NADP(H) (UHOH): Fruit material without skin and core was frozen in liquid nitrogen, freeze dried and powdered. Samples were homogenised in a solution of KOH in ethanol plus KCl and for the oxidised forms in a solution of trichloracetic acid (TCA) plus KCl. After incubation at 60°C samples were centrifugated, the supernatant was neutralised and photometrically measured at 600 nm. The reaction solution for NAD and NADH contained alcohol dehydrogenase (ADH) (24 Units) and the reaction mixture for NADP and NADPH isocitrat dehydrogenase (18 Units).

Energy metabolism / ATP and ADP (UHOH): ATP and ADP were determined by a bioluminecence technique. This assay is based upon the measurement of a stable level of light produced in a reaction catalysed by the firefly luciferase enzyme. For this measurement a chemical kit from Bio-Orbit was used.

Polyamines (IRTA): Compounds not directly involved in the problem but probably related to it are polyamines (Put, Spd, Spm). They will be determined using a thin-layer chromatography method.

Deliverables

A relation between browning of pears and PPO action and fermentative metabolites was established. Also the relationship between risk for browning and vitamin C levels was shown. No results were obtained on energy metabolism.

Links

UHOH used techniques and protocols for determination of CAT, SOD and APX developed by IRTA.

Progress second 12 months

Vitamin C: ascorbic and dehydroascorbic acid (ATO, IRTA, IVTPA, FPO, UHOH):

Important before reading the results: vitamin C is the sum of two molecules: ascorbic acid and dehydro-ascorbic acid.

FPO found differences in vitamin C between pears grown in different orchards (25%). IVTPA found higher ascorbic acid levels in pears from the top of the tree. FPO, who measured vitamin C, did not find a relation with position in the tree.

Two partners found a decrease in vitamin C with harvest date: ATO (19%) and FPO (23%), while UHOH did not find differences. An increase in ascorbic acid with harvest date was found by IVTPA (56%) and UHOH (35%).

All partners found a decrease in vitamin C (or ascorbic acid) after long term storage: ATO (68%), IRTA (62%), IVTPA, FPO (60%) and UHOH (70%). IRTA found that vitamin C rapidly decreased after the start of the storage, and increased again when CO_2 was low. This was not found by UHOH, who found a continuous decrease in vitamin C.
All partners found that increased levels of CO2 decreased vitamin C content. ATO found that when CO2 is removed after a treatment at high CO2, vitamin C levels increase again. Both ATO and UHOH suggest that monitoring vitamin C levels can be used to predict the risk for brown heart.

Antioxidative potential (UHOH):
At 0% CO_2 an increase in AP is found until three months of storage. From then on AP decreases again. When pears are stored at 5% CO_2, AP increases more rapidly and after 1 month of storage it decreases again. No relation with the onset of disorders can be made yet.

Enzymatic antioxidants (IRTA, UHOH):
IRTA found that increased CO2 causes an increase in APX and LOX, but a decrease in SOD. UHOH found that CAT and APX decreased with harvest date. SOD increased rapidly after harvest. When all the information of two years is combined, no clear picture can be drawn. It seems that the only element in the AsA-GSH regenerating system that is not measured thusfar (deshydroascorbate reductase or DHAR) is the key element to explain the results. Studies in the next year will therefore include DHAR. Interesting question is whether a possible direct influence of CO_2 on vitamin C levels can be explained by an influence of CO_2 on DHAR.

Membrane deterioration (ATO, UHOH):
Increased emission of ethane can already be found by pears with slight symptoms of brown heart (ATO). In contrast to ethane no relation is found in MDA release and the onset of brown heart (UHOH). It will be interesting if a volatile can be detected before any visible signs of the disorder are found.

Ethanol, acetaldehyde and ethylacetate (UHOH):
No differences in fermentation metabolites were found between the various harvest dates. Also during storage at 0% CO_2 these levels did not change. At 5% CO_2, however, both ethanol and acetaldehyde increased significantly.

Energy metabolism (UHOH):
Nad(H) and NADP(H) levels increased with 100% during a storage period of 6 months. Differences between the various storage conditions (including ambient air) were rather small. During long term storage ATP levels were reduced by low oxygen, and especially by the combination of low O_2 with high CO_2. These latter results confirm the idea that energy metabolism is involved in the start of the brown heart disorder.

Polyamines (IRTA):
One of the measured polyamins, putrescine, clearly accumulated when pears were subjected to high CO_2 levels. Since no brown heart was detected this year in Spanish pears, this compound alone is not dependable as an indicator of tissue disorders.

Difficulties
UHOH: The method for the determination of lipoxygenase activity (LOX) was not reproducible. We have to improve the protocols further. Also the problem with increased temperatures in part of the freezer for storage of fruit samples has to be solved.

7c. Mineral composition (partner 2,3,4,6)

Objective
Even without varying the mineral application during growth, a difference in mineral content can occur

between pears of the different locations and countries. Therefore the mineral content always has to be measured. The main elements that will be focused on are calcium, potassium and magnesium, although some partners also plan to measure nitrogen content.

Methodology
The mineral content will be analysed during the growth of the pears and directly after each harvest.

Deliverables
Mineral content of pears was measured one month before optimal harvest and at every harvest by three partners (IVTPA, FPO, UHOH). Thusfar no clear (cor)relation is found. Measurements will be repeated for one year.

Links
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Progress second 12 months
As in the first year no clear relation between mineral content and brown heart was found by IVTPA, FPO and UHOH. This measurement will not be continued in the next phase of the project.

Task 8 - Non destructive measurements

Participants:	ATO, FPO
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	3.9
No. of man-month devoted already to the task:	2

Objectives
The development of a non-destructive detection technique to retrieve unaffected pears from a batch of pears partly affected with brown heart.

Methodology
Several techniques will be tested, all based on an acoustic technique (Firmalon, PFT). Batches of pears will be measured with the technique and afterwards the degree of browning will be assessed.

Deliverables
The Firmalon method was developed and tested. It can be concluded technique of Firmalon will not be successful in practice (FPO). The PFT method was tested as well and seems promising. Validation will take place in the third year of the project (ATO).

Links
-

Progress second 12 months
Using the PFT technique different signals could be obtained for brown and non-brown pears. Methods were developed that combine several signals. Two types of error still occurred: brown pears not detected and non-brown pears classified as brown. The numbers of these errors, for the best method thusfar, were 4.8% and 11.4 % respectively (% of the total batch of pears). To obtain a useful method in practice these errors should be reduced. Next season task 8 will therefore focus on optimising the signals and the signal analysis.

Task 9 - Modelling

Participants: ATO, VCBT
Duration: 40 months
Current Status: 24 months to completion
Total estimated Man-month: 18
No. of man-month devoted already to the task: 7

Objectives

In this task the data and information of the other tasks are combined and integrated. Modelling will take place on the analysed data. Two models will be constructed. The first model is a correlative model based on the relationship between climate, nutrition and brown heart data. The second model uses physiological processes like metabolic rates and energy fluxes. This model will be based on existing models that use enzyme kinetics (both oxidative and fermentative pathways) and diffusion characteristics.

Methodology

The first model will be build using neural network techniques, in order to identify and clarify relationships. Only one output is needed; the probability of the Brown Heart disorder. To build the model, about 400 independent data-sets are necessary. Based on current estimations (see next table) the final number will be somewhat lower (368). It will be investigated how to increase this number.

Estimation of total number of data-sets for model 1						
Realised in year 1 and 2	**Total**					85
	Total					95
		orchard	**subset**	**harvest**	**years**	
Estimated for year 3 and 4	**IRTA**	2	1	3	2	12
	IVTPA	2	1	3	2	12
	FPO	11	1	5	2	110
	VCBT	3	1	3	2	18
	UHOH	2	3	3	2	36
Grand Total						368

The second model is based on descriptions of actual physical and biochemical processes (respiration rates, diffusion resistances) in combination with the hypothesis of a (temporary) lack of energy to maintain cell viability (see technical annex). Calculation of respiration and fermentation is based on Michaelis-Menten kinetics. By using gas exchange models, ATP production is estimated by combining oxidative and fermentative ATP production.
Both models are used to predict and design optimal storage conditions for pears. In the second, third and fourth year of the project these predicted gas conditions are used in the experimental storage facilities in addition to the standard gas conditions. In the last two years of the project the predictions will also be tested in practice by commercial packing houses and auctions (task 10).

Deliverables

First prediction on the risk of brown heart related to climate factors.

Links

-

Progress second 12 months

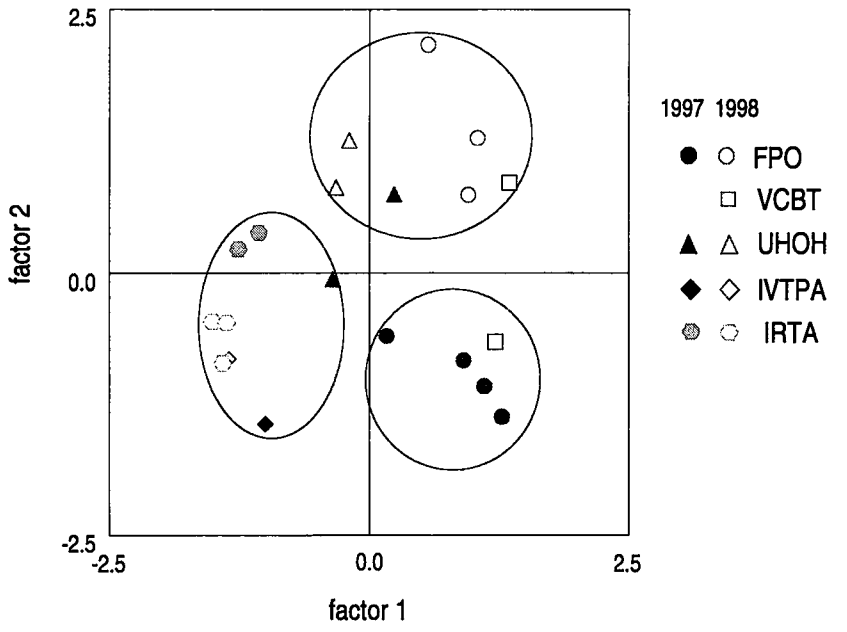
Model 1: Climate and orchard factors

Data-sets of the first two growing seasons were available. In these years, 1997 and 1998, data were collected by all partners. Data from VCBT of 1997 were not included in the data-set because the

storage conditions deviated from those in the other countries. The data-set consisted of two types of variables:

- orchard factors: type and amount of fertilisation, soil type and the age of the trees.
- climate factors: minimum (Tmin), maximum (Tmax) and average (Tavg) daily temperature, relative humidity, total global daily radiation and daily precipitation.

Using the date of flowering and the date of optimal harvest a relative developmental time scale was defined. The time of flowering was defined as stage 0 and the optimal harvest date as stage 1. This allows the comparison at similar developmental stage of fruits from different countries. This is necessary as in for instance in 1997 the duration of the growing season was 160 days in the Netherlands and 140 days in Italy. After storage the occurrence of Brown-Heart was assessed. The original climate data consisted of daily values of minimum, maximum and average temperature, relative humidity, total global radiation and precipitation. To correlate such data with the occurrence of brown-heart it is necessary to transform the data into integrated values that describe the climate over a certain period of time. Several periods of time were selected: the entire growing season and 5 periods each representing a period of a 20% of the season. Temperature sum, radiation sum, precipitation sum over the complete growing season were calculated and for every period the average difference between maximum and minimum temperature (Tdiff), the highest maximum temperature, the lowest minimum temperature and the largest difference between the maximum and minimum temperature that occurred in a period. In this way, every season the climate was described by 63 climate parameters.
Then a Principal Components Analysis (PCA) was performed to obtain the climate parameters that contributed most to the variation in climate between the different orchards. Factors were formed that describe a data-set. Every factor consists of a number of the original variables. The original 63 climate parameters are then reduced to a small set of factors. A number of consecutive PCA's was performed. In every following PCA only those variables were included with a correlation coefficient higher than 0.7 with the one of the factors. In the end three factors emerged that could describe 98.5 of the observed variation in brown heart. The first factor mainly describes the temperature over the entire growing season , the second factor mainly describes the temperature during the first period (0 - 0.2 development), and the third factor the difference between minimum and maximum temperature. After plotting the factors 3 different climate groups can be distinguished: a group of climates including mainly the northern European countries in the first growing season, a group containing these locations in the second growing season, and a group mainly consisting of the southern countries (see next graph).



The preliminary conclusion from these results is that the occurrence of Brown-Heart is higher when there is a low temperature over the whole growing season, there is a low temperature early in the season and when there is a small difference between the minimum and maximum daily temperature. From the analysis it resulted that especially the first part of the growing period contributed to variation among the countries. Interestingly, this period overlaps with the period of cell division in pears. This may indicate that problems with the occurrence of Brown-Heart during storage may originate from the period of cell division. It should be noted that the current analysis was performed using only a limited data-set including climate parameters over 2 growing seasons. The analysis will improve if more data are included later in the project

Improvements

- More locations with consequently more variation in climate will improve the ability of the modelling techniques to find the causes for the occurrence of Brown-Heart.
- Similar measurements of radiation among all partners would allow including radiation into the model.
- Including harvests later than the optimal harvest will give a better impression on the potential for development of Brown-Heart
- Low CO2 and 2% O2 can be used as standard storage procedure, while storage at 5% can be skipped.
- In the present data-set storage time varies between 176 and 259 days. An equal storage time among partners will lead to a more fair comparison of the occurrence of Brown-heart.

Model 2: physiological parameters

Currently the relation between metabolic rates, reduction in vitamin C levels, energy metabolism and the onset of the brown-heart disorder are quantified. Based on this analysis the measurements directly after harvest will be re-evaluated. Predictions on the risk of brown heart based on such measurements will be generated for the next harvest period (September 2000).

Task 11 - Dissemination

Participants:	ATO, IRTA, IVTPA, FPO, VCBT, UHOH
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	6
No. of man-month devoted already to the task:	3

Objectives

This task was added to ensure a good dissemination of the experimental work. Throughout the project there will be a close contact between partners and possible end-users. These contacts are important to disseminate interesting results, and to have feed-back from practice. It is encouraged to publish results in national (applied) and international (scientific) journals. At the end of the project a symposium will be organised, to give all who are interested the opportunity to take notice of the final results and interpretations.

Deliverables

Papers were published not only in national but in international journals and conferences as well.

Progress first 24 months

Thusfar 15 papers were published, 8 lectures were given and 8 posters presented that were strongly related to the work in this project.

Journals (1=international refereed journal, 2=proceedings, 3=national journal)

1 Larrigaudiere C., I. Lentheric and M. Vendrell. 1998. Relationship between enzymatic browning and internal disorders in controlled-atmosphere stored pears. Journal of the Science of Food and Agriculture, 78(2): 232-237.

1 Lentheric I., E. Pinto, M. Vendrell and C. Larrigaudiere. 1999. Harvest date affects the antioxidative systems in pear fruits. Journal of Horticultural Science and Biotechnology, 74 (6) – in press.

1 Veltman, R.H., C. Larrigaudiere, H.J. Wichers, A.C.R. van Schaik, L.H.W. van der Plas and J. Oosterhaven, 1998. PPO activity and polyphenol content are no limiting factors during brown core development in pears (Pyrus communis L. cv. Conference). Journal of Plant Physiology, 154: 697-702.

1 Veltman, R.H., M.G. Sanders, S.T. Persijn, H.W. Peppelenbos, and J. Oosterhaven. 1999. Decreased ascorbic acid levels and brown core development in pears (Pyrus communis L. cv. Conference). Physiologia Plantarum, 107: 39-45.

1 De Wild H.P.J., Woltering E.J. and Peppelenbos H.W., 1999. Carbon dioxide and 1-MCP inhibit ethylene production and respiration of pear fruit by different mechanisms. J. Exp. Botany, 50 (335): 837-844.

2 Lammertyn J., Aerts M., Verlinden B., De Baerdemaeker J. and Nicolaï B. 1998. Logistic regression for modelling factors influencing core breakdown disorder in Pyrus communis cv. Conference. Proceedings EurAgEng 98, Oslo, Norway, 23-27 August 1998.

2 Peppelenbos H.W., Jeksrud W.K., 1998. A method for the simultaneous measurement of gas exchange and diffusion resistance of fruits and vegetables under various gas conditions. Acta Horticulturae, 464: 333-338.

2 Peppelenbos H.W., Oosterhaven J., 1998. A theorethical approach on the role of fermentation in fruits and vegetables. Acta Horticulturae, 464: 381-386.

2 Peppelenbos, H.W., 1998. Gas exchange models and the prediction of disorders in fruits. Proc. COST915 - Copernicus CIPA-CT94-0120 workshop on Food Quality Modelling. Leuven, 3-6 June 1997, pp 69-74.

3 Lammertyn J. en Schenk A., 1998. Cavities and brown in Conference (in Dutch). De Boer en de Tuinder (108) 39:12.

3 Lammertyn J. en Schenk A., 1998. Storage research on Conference: extreme browning (in Dutch). Belgische fruitrevue (50) 11:5-9.

3 Peppelenbos H.W., 1999. European research on causes and solutions for brown heart (in Dutch). Fruitteelt, 31: 14-15.

3 Schenk A. en Lammertyn J., 1998. Pear cavities (in Dutch). Fruitteeltnieuws, Berichten uit wetenschap en praktijk (11) 18:16-17.

3 Streif, J., 1998. Erntetermine und Hinweise für die Lagerung und Vermarktung von Apfel und Birnen. Kernobstsortiment für die Obstregion Bodensee: 39-45.

3 Veltman R.H., Peppelenbos H.W., 1998. Healthy Conference pears in CA storage thanks to vitamin C (in Dutch). Fruitteelt, 22: 14-15.

Lectures and posters

- Eccher Zerbini, P., Grassi, M., 1998. Influenza dell' irrigazione sulla qualità delle pere Conference conservate in atmosfera a basso ossigeno. Atti IV Giornate Scientifiche S.O.I., Sanremo, 1-3 april.
- Lammertyn, J., Verlinden, B., De Baerdemaeker, J. and Nicolaï,B., 1998. Relation between core breakdown disorder and respiration characteristics of Pyrus communis. XXV Int. Hort. Congress, Brussels, 2-7 August 1998 (Poster).
- Larrigaudiere C., I. Lentheric and M. Vendrell. 1998. Late harvest induced changes in antioxidant defense mechanisms in Conference pears. 11th FESPP (Federation of European Societies of Plant Physiology) congress, Varna (Bulgaria), 7-11 September 1998. (Poster).
- Lentheric I., E. Pinto, C. Larrigaudiere., and I. Recasens. 1998. Effects of N_2 fertilization on storage, quality and brown heart incidence in Conference pears. Madrid98–COST915 conference "Physiological and technological aspects of gaseous and thermal treatments of fresh fruits and vegetables". 15-16 October 1998. (Poster).

- Lentheric I., E. Pinto and C. Larrigaudiere.. 1999. Multivariate analysis as a mean to detect brown heart in Conference pears. Workshop on optimal harvest date, St Remy de provence (France).
- Lentheric I., E. Pinto and C. Larrigaudiere. 1999. Efecto a corto plazo de las concentraciones de CO_2 sobre el metabolismo antioxidativo de la pera Conference. XIII reunión de la sociedad española de fisiología vegetal. 19-22 September 1999 (Poster).
- Peppelenbos, H.W., 1997. Gas exchange models and the prediction of disorders in fruits. COST915 - Copernicus CIPA-CT94-0120 workshop on Food Quality Modelling. Leuven, 3-6 June 1997.
- Peppelenbos, H.W. and Schouten, S.P., 1998. Control of fermentation in harvested plant products. XXV Int. Hort. Congress, Brussels, 2-7 August 1998.
- Rabus, C. und J. Streif: Effect of various preharvest treatments on the development of internal browning disorders in 'Braeburn' apples. 25. International Horticultural Congress, Brüssel, 2-7 August 1998 (Poster).
- Streif, J. and Rabus, C., 1998. Effect of various preharvest treatments on development of internal browning disorders in 'Braeburn' apples. XXV Int. Hort. Congress, Brussels, 2-7 August 1998 (Poster).
- Streif, J.: Lagerung von Apfel und Birne unter besonderer Berücksichtigung von Braeburn Äpfeln und Conference Birnen. Neustadt, 8 January 1999
- Streif, J. Optimaler Erntetermin und Lagerungsbedingungen für Braeburn and Conference Früchte. Bitzfeld-Öhringen, 8 March 1999
- Streif, J. Gasdiffusionsmessungen an Früchten. Annual meeting of the German Society for Quality Research (DGG), Freising-Weihenstephan, 22-3-1999 (Poster).
- Streif, J. Fleischverbräunungen bei Elstar und Erklärungsmöglichkeiten mit Ergebnissen von Conference und Braeburn. Oberkirch 31 March 1999
- Veltman R.H., 1997. Core browning in Conference pear: relation vitamin C and storage conditions. Int. workshop on Antioxidants in higher plants. April 13-15, Ravensburg, Germany.
- Xuan, H. und J. Streif: Effects of pre- and postharvest treatments of 'Biofresh' on keepability of different apples varieties. 25. International Horticultural Congress, Brüssel, 2.-7.8.1998 (Poster).

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project		
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045 kECU
Participant no. 1 total cost:	608 kECU	*EC contribution to partner no.1:*	304 kECU (50 %)
Commencement date:	01-06-1997	*Duration:*	4 years
Completion date:	31-05-2001		
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3		

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Participant no.: 1 (ATO-DLO)

A. PARTNER INFORMATION

Name and address

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Scientific team (1998-1999)

- Dr. H.W. Peppelenbos	task 6, task 9, coordinator
- Dr. Ir. H.A.G.M. van den Boogaard	task 9
- Ing. E.C. Otma	task 6
- Ing. M.G. Sanders	task 8
- Ing. A.C.R. van Schaik	tasks 2, 3
- Drs. R.H. Veltman	task 7
- Ir. J.P.J. de Wild	task 6

Objectives

The main objective of the project is the optimisation of the quality of Conference pears, being the most important pear grown within Europe, and the reduction of losses during storage, by preventing the development of disorders resulting in Brown Heart in pears. The key element in the project is the development of technology, which enables a rapid measurement and decision about post-harvest treatments and optimal storage conditions. The main advantage of such a technology is that it leads to an accurate advice based on product characteristics of the same harvest. Three pathways are most promising towards such an approach:

1. development of a predictive model based on registrated variances in orchard and weather conditions of a broad group of European countries;
2. development of a predictive model based on a rapid assessment of pear physiology (respiration rate, fermentation rate, resistance to gas diffusion);
3. testing and implementation of post-harvest treatments, which improve the storability of the harvested pears, leading to pears with a better quality.

The models will be used for advice on optimal storage en pre-storage treatments, and for predictions on maximum storability. In addition to adapting storage conditions to changes in pear physiology, it might be possible to increase storability, or reduce the risk of Brown Heart, by specific post-harvest treatments. Physiological and biochemical measurements are carried out to elucidate the processes, which have to be influenced in order to develop the treatments.

Actions in the project

The ATO is involved several tasks as described in the technical annex (page 5):

- task 2: Harvest of pears (in cooperation with partner 4);
- task 3: Storage of pears;
- task 6: Gas exchange and diffusion measurements;
- task 7: Destructive measurements (quality evaluation, biochemical analysis);
- task 8: Non-destructive measurements;
- task 9: Modeling;
- task 10: Validation.

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1997 TO 31-05-1998

Task 2

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	2

Objectives

The objective of this task is to get fruits that were grown under known conditions. By harvesting the fruits ourselves, we can decide on the exact date(s) of harvest. Harvest date is strongly related to the risk of Brown Heart. In general; the later the harvest, the higher the damage. Therefore it is important to define strict rules for harvesting. First it is important that every year pears are harvested at different dates to introduce a variance of development stages and pear characteristics at every location. Secondly the dates themselves have to be based on maturity and determined by all partners by comparable methods. Maturity was determined by the Streif index and firmness.

Material and methods

To compare fruits with a different sensitivity for brown heart, pears were harvested from two locations, at five harvest dates. Pears were harvested in Ingen (grower 1) and Ommeren (grower 2), both in the centre of the Netherlands (province of Gelderland). From an historical perspective the pears from Ingen are relatively sensitive to the development brown of heart, whereas the pears from Ommeren are usually not. Harvest dates were selected based on advice from the national extension service (DLV), who base their advice on a combination of firmness readings and the Streif-index. Considering the storage results found, the advice was correct.

Results and Discussion

The pears from both locations were harvested at five different dates: August 27, September 3, 10, 17 and 24 of 1999. Harvest three, September 10, was comparable to the harvest date generally advised and used in the Netherlands at these locations. Most pears were harvested at that date, because they were necessary for several experiments. Pears harvested at the other dates were used only for a comparison between harvest dates (especially gas exchange rates).

Task 3

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	2

Objectives

To simulate standard storage conditions and to generate fruits for experiments throughout the year, they have to be stored at specific conditions.

Material and methods

The standard pre-storage procedure can be described as follows: the cooling down procedure will be started as soon as possible after harvest, and after 48 hours the minimum temperature should be reached. From then on the temperature is constantly monitored. One week after the start of the storage the storage rooms are flushed with N_2 until a concentration of 4% O_2 is reached. The rest of the O_2 in the storage room is removed by respiration of the pears, until 2% O_2 is reached. From then on the gas concentrations are continuously monitored and controlled. The pears were stored in boxes, placed in 600 litre containers with a water sealing, at -0.5 ^{0}C to -1 ^{0}C . Oxygen levels were kept at 2 kPa. Carbon dioxide was kept at two different conditions: low (<0.5 kPa), using a KOH scrubber, or high (5 kPa). The low CO2 conditions simulates storage conditions used in practice. The high carbon dioxide condition was selected to generate the disorder in every year.
In some experiments pears were kept in 60 liter tanks connected to a flow-through system (Peppelenbos et al., 1996). A duplicate range of gas-conditions was selected at 5 ^{0}C. Every quarter of an hour the temperature was measured with a Vaisala thermometer (HMP 31UT). Relative humidity during storage was kept 97-99%. The right gas mixture of N_2, O_2 and CO_2 was composed with the aid of Brooks 5850 TR series mass flow controllers. The total flow rate was established between 490

and 510 ml/min.. Every three hours gas compositions were measured with an ADC 7000 gas analyser (Analytical Development Company Limited, Haddesdon, England), and if necessary adjusted.

Results and Discussion

The cooling down procedure was carried out according to the guidelines given. Pears were stored for maximally 9 months (last pears removed in the beginning of June 1998). The storage conditions were maintained close to the conditions agreed. Sometimes temporarily the CO_2 concentrations in some of the containers were CO_2 should be below 0.5 kPa were a little bit higher (1.0 kPa).

Task 6

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	29
No. of man-month devoted already to the task:	19

Objectives

The work of the first year is mainly focussed on testing the methodology on gas exchange and diffusion resistance measurements. In addition measurements have been carried out on pears in order to quantify variation caused by picking date, ripening stage, storage period and delaying ULO storage. The measurements in relation to picking date and ripening will clarify minimum and maximum values for gas exchange rates and diffusion resistance. Delaying ULO was carried out because in previous experiments this appeared to be a good treatment to lower the risk of brown-heart, however, without understanding the mechanism behind this result.

Material and Methods

Harvest dates

Conference pears were harvested on various dates (see task 2). Pears used for task 6 were harvested at the grower located at Ingen. The pears were stored at 0°C, 2% O_2 and 0% CO_2. Pears from the optimal harvest date were stored at 2% O_2 and 5% CO_2 as well. Gas exchange characteristics were measured at 2°C.

Gas exchange rate and diffusion resistance

Fresh weight and underwater weight (Bauman and Henze, 1983) were measured. Samples from pears were taken to measure the specific weight of pear juice (used in the calculation of the porosity). Outer surface area of the pears was estimated using the length and the maximum circumference of the pear (see Annual report 1998)). Two pears were put in 1500 ml cuvettes. The cuvettes were connected to a flow through system. Gas conditions were all combinations of 0, 0.5, 1, 2.5, 6 and 21% O_2 with 0 and 5% CO_2. Relative humidity was high (>95%) since the gas was led through water flasks. After 4 and 5 days of storage O_2 uptake and CO_2 production was determined. This was done by disconnecting the cuvettes from the flow through system, and sampling the headspace directly and after a period of 6 hours. The GC used was a Chrompack CP 2002. The measured O2, CO2 and N2 was corrected to 100% to account for possible pressure variations inside the GC. Then the concentration values (in %) were multiplied with the actual pressure inside the cuvette (in kPa). Because the volume of the cuvette and the volume of the pears is known, the gas exchange rates can be calculated in nmoles/kg.s.
The method of measuring diffusion resistance as described by Peppelenbos and Jeksrud (in press) was slightly adjusted. The inert gas neon was used as well, but instead of measuring the diffusion of neon into the fruit the diffusion of neon out of the fruit was measured (see Annual report 1998).

Delaying CA

Pears from the optimal harvest date were put under CA conditions or stored at 0°C. Gas exchange characteristics were measured directly after harvest and after a storage period of 5 weeks (all at 2°C).

Storage period
Gas exchange characteristics of pears were measured after 5 different storage periods: 42, 86, 126, 177 and 233 days of storage. Pears from both storage conditions (0 and 5 kPa of CO_2) were used. Pears were subjected to a range of gas conditions described under 'gas exchange rates' at a temperature of 2°C. Gas exchange models were used to analyse the data. A special attention was paid to pears measured at 2kPa O_2 and 0 or 5 kPa of CO_2, representing the conditions during storage.

Statistical analysis
The gas exchange rates were analysed using the nonlinear regression analysis of the statistical package Genstat. The models used within the package were derived from Peppelenbos and van 't Leven (1996) and Peppelenbos et al. (1996), and are mentioned in the technical annex page 11 as well.

Results and discussion
The results of 1999 are mentioned first, and then a comparison with the results of 1998 is made for every type of measurement and every experiment.

Harvest date
Respiration rates were not much different between the 5 harvest dates. Instead of an increase in respiration with harvest date, a normal phenomenon, which accompanies ripening, a small decrease was observed. Also modelling resulted in the lowest VmO_2 for harvest 5 (table 1).
Fermentation rates of pears from different harvest dates are also quite similar. The lowest value was found for harvest 4 (table 1). No obvious explanation can be given for this observation.

Table 1. Results of the 'harvest date' experiment.

Analysis	Parameter	Harvest date				
		1	2	3	4	5
O2model	R2	80.6	88.8	83.6	88.8	87.0
	VmO2	18.0	19.3	17.0	17.4	15.0
	KmO2	0.312	0.430	0.329	0.792	0.613
	KmCO2	23.8	18.4	19.3	21.4	20.6
CO2model	R2	42.8	60.4	57.8	62.8	52.3
	VmfCO2	23.4	25.1	19.5	14.8	24.1
	KmfO2	0.075	0.082	0.068	0.116	0.032
	RQox	0.858	0.781	0.832	0.722	0.948
Resistance		616	437	507	509	519

*Tables 1 to 3 give an overview of the statistical results of fitting gas exchange models to the data. Statistical parameters: Fit = fitted value, se = standard error, R^2 = percentage of explained variance, ** = could not be estimated. Model parameters: Vm_{O2} = maximum O_2 uptake rate (nmol/kg.s), Km_{O2} = Michaelis-Menten constant for the influence of O2 on O2 uptake (kPa), KmCO2 = Michaelis-Menten constant for the influence of CO2 on O_2 uptake (kPa), Vmf_{CO2} = maximum fermentative CO_2 production rate (nmol/kg.s), Kmf_{O2} = Michaelis-Menten constant for the influence of O_2 on fermentative CO_2 production (kPa), RQox = RQ for oxidative processes, Resist = resistance for diffusion of neon (s/mm).*

Also differences in diffusion resistance between the 5 harvest dates were minimal, and not significant considering the large variation between individual pears.
Gas exchange date and diffusion resistance values were used to calculate internal CO_2 concentrations (figure 1). Although the difference between internal CO_2 and external CO_2 is increasing in ambient air, the difference is very constant at the standard CA condition. This leads to the conclusion that internal CO_2 is *not* the main factor influencing increased browning at later harvest dates.

Delaying CA

Because delaying CA largely prevents the occurrence of browning during subsequent storage, it was investigated whether metabolic rates contributed to this phenomenon. Pears directly subjected to CA conditions showed slightly lower respiration and fermentation rates then pears subjected to the delay treatment (table 2). Differences in 1997 were larger.

Table 2. Results of the 'delay' experiment.

Analysis	Parameter	Harvest date		
		Harvest	direct CA	delay CA
O2model	R2	83.6	91.1	90.6
	VmO2	17.0	19.0	22.2
	KmO2	0.329	1.92	1.41
	KmCO2	19.3	12.3	17.6
CO2model	R2	57.8	71.3	76.8
	VmfCO2	19.5	11.1	*51.0**
	KmfO2	0.068	0.201	0.033
	Rqox	0.832	0.866	0.824
Resistance		507	523	483

** VmfCO2 of delayed CA was overestimated by the statistical package. On average the actual value was 13.8*

When internal gas concentrations were calculated, no differences were found. Therefore it can be concluded that internal CO_2 is also *not* the main factor influencing browning after direct CA. Because metabolic rates increase during the delay treatment, increased energy production might be related to the prevention of browning. A relation with energy consuming processes must be made (vitamin C content). Also the role of fermentation should be investigated. In figure 4 it can be seen that after the delay treatment VmfCO2 is higher. It is interesting whether Vmf CO2 remains higher throughout the whole storage period.

Table 3. Results of the 'storage' experiment.

Analysis	Parameter	2 months		5 months		8 months	
		0 CO_2	5 CO_2	0 CO_2	5 CO_2	0 CO_2	5 CO_2
O2model	R2	82.8	90.7	87.6	76.8	84.7	87.2
	VmO2	19.9	25.4	18.2	21.5	18.3	23.2
	KmO2	1.00	1.63	1.00	1.53	0.894	1.82
	KmCO2	15.0	13.8	14.9	9.67	7.70	9.87
CO2model	R2	63.5	68.3	50.5	54.6	52.8	56.2
	VmfCO2	8.64	10.9	15.4	12.1	20.6	7.15
	KmfO2	0.320	0.335	0.155	0.349	0.153	1.19
	RQox	0.821	0.825	0.896	0.871	0.805	0.711
Resistance		483	539	343	369	421	378

Storage period

Respiration rates during standard CA storage conditions (2 kPa O_2 with 0 kPa CO_2) stay very stable. Diffusion resistance is decreased after 150 days of storage (figure 5). Fermentation rates increase to values close to values directly after harvest (compare table 1and 3, VmfCO2). Remarkable is the large reduction in maximum fermentation rate found after 2 months of storage (figure 4).

When modelling results are analysed, it can be seen that the inhibitory effect of CO_2 on respiration increases during storage (table 3, reduction in $KmCO_2$).

Once pears are returned to ambient air, the respiration rates of pears stored at 5kPa CO2 are higher than pears stored at 0 kPa CO2 (figure 3). Interestingly the maximum fermentation rates are strongly reduced, especially after prolonged storage (table 3, figure 4). Also another model parameter is analysed; KmfO2. When the value for KmfO2 is low, it means that even at low O2 concentrations fermentation is inhibited. In other words, fermentation starts to increase at very low O2 concentrations. From figure 4 it becomes clear that the delay treatment causes a very low value for

KmfO2, while the long storage under 5kPa CO2 causes a dramatic increase of this parameter. Pears are stored under 2 kPa O2, and the maximum fermentation rate is measured at o kPa O2. Therefore kmfO2 is more important to predict fermentation rates at actual CA conditions. From figure 4 it can be concluded that fermentation at 2 kPa O2 is reduced by the delay treatment, and increased by storage at 5 kPa O2. This should be measured by metabolites such as ethanol and acetaldehyde.

Comparison 1998-1999

With regard to respiration, no differences between 998 and 1999 are found. Fermentation rates, however, were higher in the 1999 season. KmfO2 increased in the 1999 season, especially after storage at 5 kPa CO_2. This, however, was not observed in 1998. Both in 1998 and 1999 VmfCO2 and KmfO2 were lower after the delay treatment.

Diffusion resistance values were lower in the 1999 season. Where in the 1998 season resistance values increased during storage, values decreased in the 1999 season during storage.

The regression analysis, were gas exchange models were fitted to gas exchange data, resulted in comparable R^2 values for respiration in both years (R^2 stands for the % of the total variance explained by the model, between 0 and 100). In 1998 the average R^2 values for respiration was 88.2, while in 1999 it was 86.0. For fermentation, however, R^2 values were remarkably lower in 1999. The average R^2 in 1998 was 72.1, while in 1999 it was 59.1. Functioning of the GC is very likely the main cause for this difference.

Conclusions Task 6

1. Internal CO_2 is *not* the main factor influencing increased browning at later harvest dates.
2. Internal CO_2 is *not* the main factor influencing browning after direct CA.
3. Fermentation is affected by the delay treatment.
4. Fermentation changes during storage.
5. Reduction in fermentation at actual CA (2 kPa O_2) seems related with low occurrence of brown heart.
6. The differences in physiological parameters between 1998 and 1999 must be integrated in the physiological model. The parameters do not give a clear indication when analysed separately.

Planning Task 6 1999-2000

1. Differences between harvest dates will no longer be measured
2. Instead, at a late harvest pears from two orchards will be measured; one with a history of brown, and one without brown
3. Pears will no longer be stored at 5 kPa CO_2, brown is always found at 0 kPa as well.
4. Instead, pears with and without a delay treatment will be stored and monitored throughout the storage period.
5. Fermentation rates at actual CA conditions will be measured by ethanol and acetaldehyde emissions. These measurements will be carried out throughout the storage season.

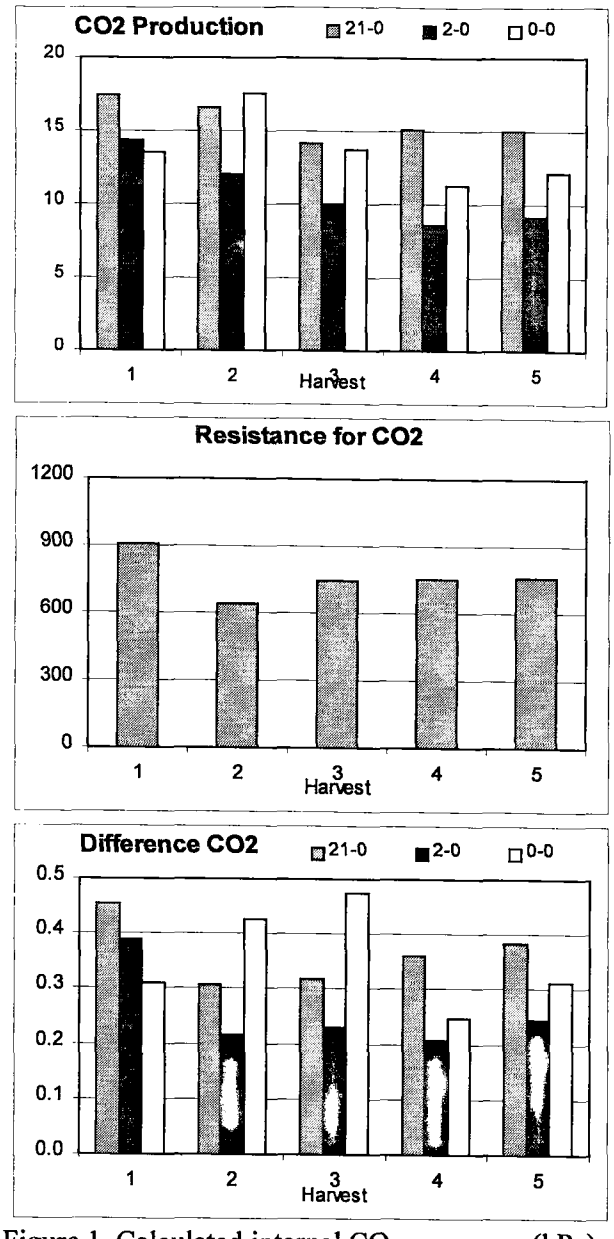


Figure 1. Calculated internal CO_2-pressures (kPa) per harvest date, based on CO2 production data (upper graph) and resistance for the diffusion of CO_2 (calculated from neon diffusion resistance).

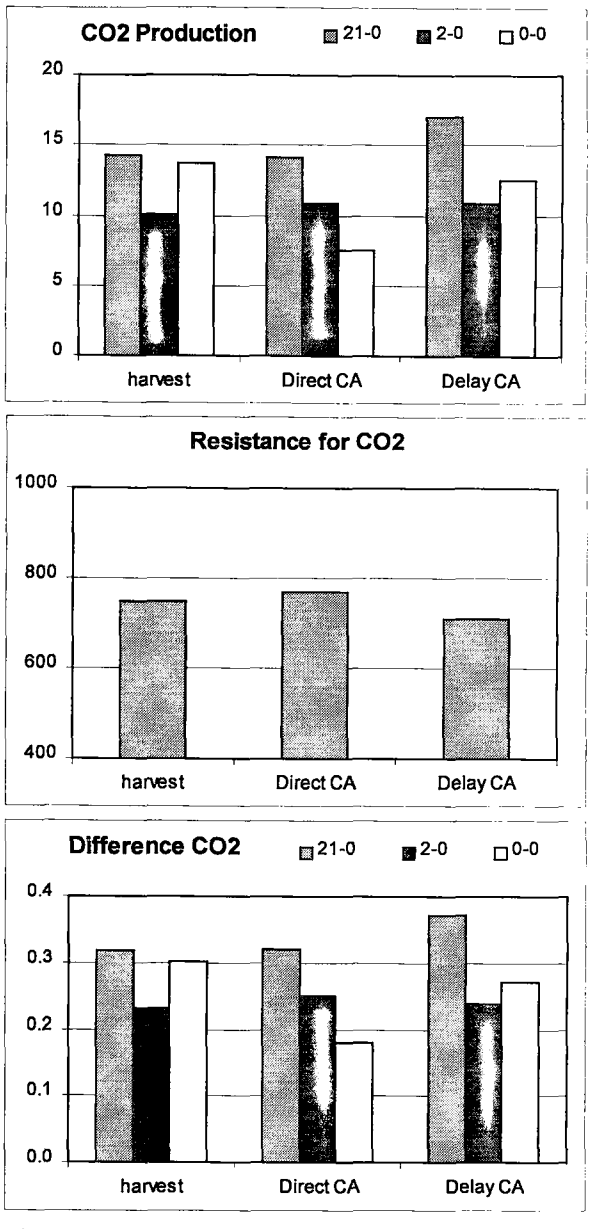
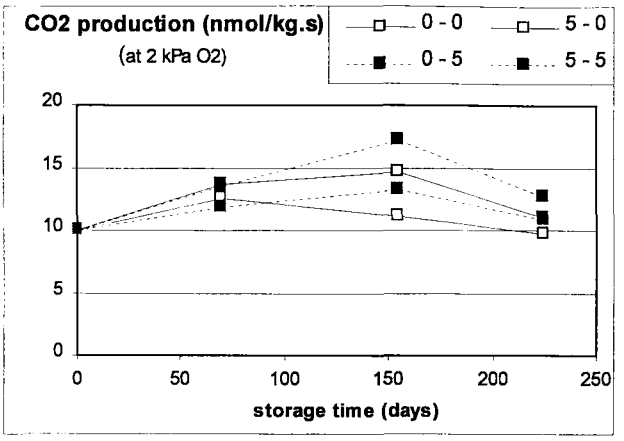
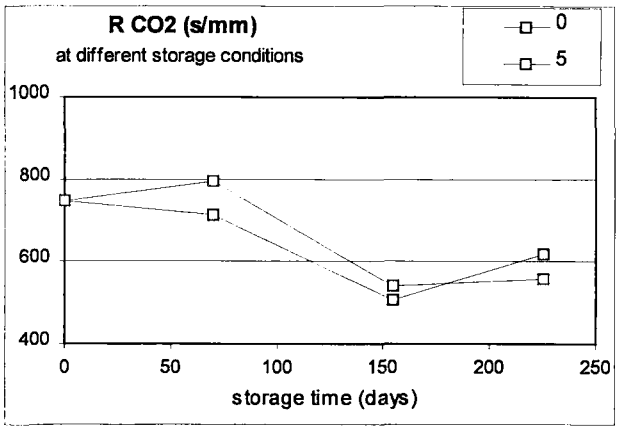


Figure 2. Calculated internal CO2-pressures (kPa) per treatment (direct CA or delayed CA). Calculations as in figure 1.





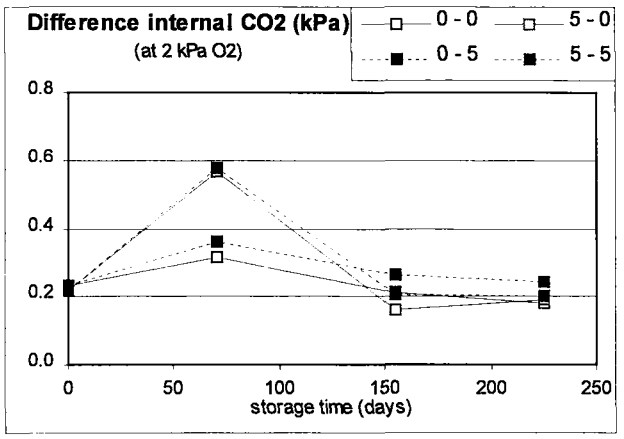
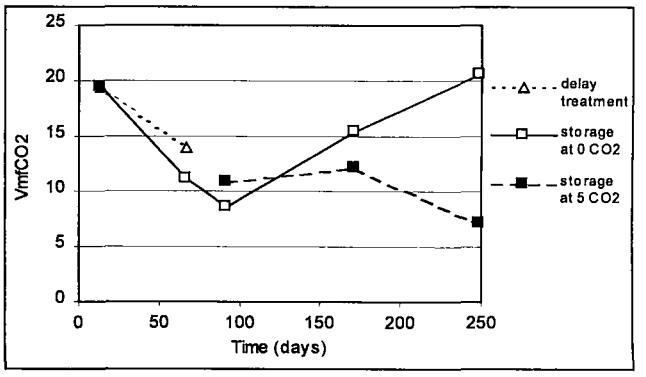


Figure 3. The CO_2- production (nmoles/kg.s), diffusion resistance and calculated internal CO_2 of pears throughout the storage season. Pears were stored at 0 or 5 kPa of CO_2, and then subjected to 0 or 5 kPa CO_2 for gas exchange measurements. 5-0 therefore means: stored at 5 kPa, measured at 0 kPa CO_2.



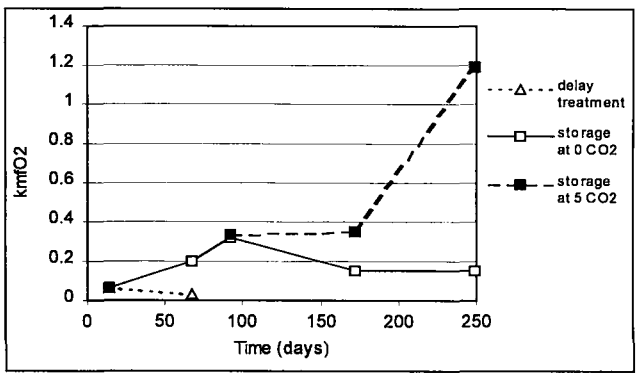


Figure 4. The maximum fermentation rate (VmfCO2) and the Michaelis-Menten value for fermentation (kmfO2) during storage

Task 7

Duration:	**48 months**
Current Status:	**24 months to completion**
Total estimated Man-month:	**29**
No. of man-month devoted already to the task:	**19**

Objectives

The objective of this task is to clarify the biochemical processes involved in the development of brown heart in pears. The research in the second year focused on the relation between vitamin C and browning, and how vitamin C levels could be influenced.

Materials and methods

Plant material and storage

For the experiment in which the change in ascorbic acid was followed, and conditions were adapted to avoid browning, Conference pears were obtained from a local auction (orchard 1). Fruits were stored at the auction under standard CA conditions for 6 months before the experiment started. During the experiment fruits were stored in a flow-through system at 2.0 ± 0.1% oxygen combined with 0.0 ± 0.2% (four-fold) or 10.0 ± 0.2% (eight-fold) carbon dioxide. After 40 days half of the containers with 10% carbon dioxide were switched to 0% carbon dioxide. Ascorbic acid was determined in two mixed samples of 10 pears in every container. The experiment lasted 83 days in total. Every week containers were opened and pears were judged on browning. Conference pears (orchard 2) were harvested by own personnel in The Netherlands (province Gelderland) at five dates, pick 1, 2, 3, 4 and 5 respectively: September 3, 10, 16, 24 and October 1. Pick 2 was the optimal harvest date for controlled atmosphere storage. For bulk storage Conference pears were stored in crates (about 100 per crate) in a static system. Eight crates were placed in 650 litre containers with a water sealing, at -1°C air temperature, 2% ± 0.1 O_2 and <0.7% (standard controlled atmosphere (CA) condition for Conference) or 3% CO_2. Relative humidity in the containers was 97-99%.

Ascorbic acid measurements

High-pressure liquid chromatography (HPLC) was performed using a Waters (Milford, USA) chromatograph model 510 with a Waters 486 UV-VIS detector (251 nm). A Symmetry C-18 column (3.9 x 150 mm, particle size 5 µm, Waters), with a Sentry Guard column C-18 (Waters), was employed. Measurements were performed at 25 □C. The mobile phase consisted of 2.5 g tetrabutylammoniumhydrogensulfate (z.s. 818858, Merck, Dorset, UK) and 55 ml methanol (p.a. Merck 6009) dissolved in 942.5 grams Milli-Q water (Millipore (Waters), Milford, USA) (Keijbets and Ebbenhorst-Seller, 1990). Before use the eluent was filtrated and degassed with a 0.45-µm Millipore filter (HVLP 04700). The flow rate during measurements was kept at 1 ml min^{-1}. Analyses were completed within 5.5 min including a post-column elution time of about 1 min. As a standard 62.5 mg ascorbic acid (Sigma, Perth, Australia) was dissolved in 100 ml Milli-Q water (stock I). One ml of stock I was diluted with 10-ml 9.5% (w/v) oxalic acid and 10-ml methanol (comparable with the extraction procedure)(stock II). A dilution series from stock II was stored on ice and kept in the dark before injection. Fresh stocks were prepared daily. For exp 1 fruits were divided in three samples: peel, cortex and core. In other experiments only the cortex tissue was examined. Samples were immediately frozen in liquid nitrogen. Then the samples were crushed in a Braun kitchen mixer. All following steps were carried out in the refrigerator or on ice in diffuse light. Ten grams of sample were diluted with 5 ml 9.5% (w/v) oxalic acid (Merck 100495), 5-ml methanol (p.a.) and 30 ml Milli-Q water. The mixture was homogenized with an ultra turrax mixer and filtered through fluted paper (Schleicher & Schüll 595½, Dassel, Germany). The filtrate was passed through a unit consisting of a 0.45-µm sterile filter and a Sep-Pak C18 cartridge (Waters), and was directly injected in a manual-injector system with a 20-µl sample-loop. HPLC measurements were done directly after the extraction procedure. Results were analyzed by means of the Millenium HPLC manager (Waters).

Brown index
To describe the extent of browning, pears were divided in 4 classes: no browning (class O), slight browning (class I), moderate browning (class II) and severe browning (class III). The following brown-index was used:

$$Brown\ index = \frac{I + 2II + 3III}{3(O + I + II + III)}$$

O: number of pears without browning; I: number of class I pears; II: number of class II pears; III: number of class III pears. Brown index value 0 means 'no browning'; brown index 1 means 'maximal browning'.

Ethane measurements
Pears were stored in a static system at conditions that induce browning (0.5 ± 0.1% oxygen and 3.0 ± 0.2% carbon dioxide). For ethane measurements with the laser equipment, 3 pears were enclosed in a 2-l cuvette for 4 h, while the cuvette was connected to the flow-through system of the laser-based detector at 2.5 ± 0.1% oxygen and 8.0 ± 0.2% carbon dioxide (flow rate 5-l h^{-1}). The set-up of the laser equipment for determining ethane has been described by Bijnen et al. (1996). The detection limit of ethane was around 1 ppb. Additional equipment, for example a cold trap to pre-clean the incoming trace gas flow and tubing has been used to lower the detection limit for ethane to 1 ppb (Oomens et al. 1998).

Statistical analysis
Ascorbic acid values were analysed for significant differences by analysis of variance (ANOVA) with the statistical package Genstat (release 5). When significant differences were found, comparisons between pairs of data were made using the least significant differences between means (LSD) at a significance level of 95%.

Results and discussion

Relation between ascorbic acid and internal browning
When pears were stored at 2% oxygen, 10% carbon dioxide and 5°C in a flow-through system, ascorbic acid levels slowly decreased (Fig. 1). After 40 days of storage, levels decreased from 1.5 mg $(100g\ fresh\ weight)^{-1}$ at t=0 to 1.3 mg $(100g\ fresh\ weight)^{-1}$. When pears are stored more than 40 days at these conditions ascorbic acid levels continued to drop. After 48 days the first clear incidences of browning appeared. After 71 days ascorbic acid levels had dropped to 0.75 mg $(100g\ fresh\ weight)^{-1}$, while the brown index of the pears exceeded 0.35. In brown tissue ascorbic acid levels are zero, but after 71 days storage, browning was not maximal (browning index 1.0). However, healthy tissue close to brown tissue can contain 'normal' ascorbic acid concentrations. In experiments presented here, ascorbic acid in the whole cortex of the fruit was determined, through which levels were never zero. In this experiment an ascorbic acid level of 1.3 mg $(100g\ fresh\ weight)^{-1}$ on average was practically regarded as a limit. Beneath this limit browning of the pears was expected to be unavoidable.

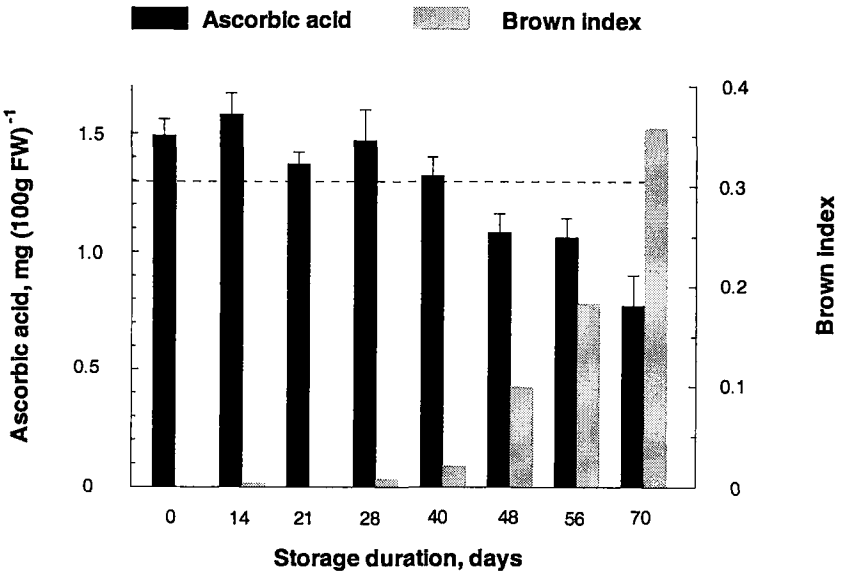


Fig 1. Ascorbic acid and browning in pears (orchard 1) during storage at 2% oxygen and 10% carbon dioxide in a flow-through system (5°C). The ascorbic acid content and the brown index of pears was monitored for 71 days. For the ascorbic acid determinations two mixed samples of 10 pears each were taken from 4 containers (n=4). Before ascorbic acid was determined, pears were judged on browning. When ascorbic acid levels drop below ca 1.3 mg (100g FW)$^{-1}$ (horizontal, dashed line in graph) browning seems to be initiated.

Switching storage conditions

In different experiments we examined the possibility to use decreased ascorbic acid levels of the fruit as a marker for the initiation of brown core. Under enhanced carbon dioxide concentrations ascorbic acid levels drop; beneath a certain threshold value pears seem to become brown. The question might be asked if browning can be avoided by changing gas conditions just before ascorbic acid values drop below this threshold value, and the formation of aberrations is initiated. In Figure 6 conditions were switched from storage under enhanced carbon dioxide to storage without carbon dioxide when ascorbic acid levels had dropped to 1.3 mg (100g fresh weight)$^{-1}$.

Ascorbic acid levels in pears stored at standard CA conditions increased slowly to ca 2.9-mg (100g fresh weight)$^{-1}$ after 30 days. Under enhanced carbon dioxide ascorbic acid levels dropped slowly, and after switching to CA-conditions at day 40 ascorbic acid concentrations in the pears increased again, paralleling concentrations of control fruits after about 2 weeks (Fig. 2). Concentrations in pears stored under enhanced carbon dioxide showed a continued decrease. Instron values, measured at day 56, were 33, 64 and 55 N (n=20) for pears stored under standard conditions, enhanced carbon dioxide and switched conditions, respectively. These measurements show that pears of the control are riper. However, ascorbic acid levels are probably not affected by ripening when compared to controls. Ethylene production in containers where conditions were switched increased to the level of control pears at day 56: ca 0.15 nmol kg^{-1} s^{-1}. In the enhanced carbon dioxide containers the ethylene production was about 5 times lower: 0.03 nmol kg^{-1} s^{-1}. These values can be compared to the ethylene production in ripening control experiments. As far as a comparison can be made ascorbic acid levels seem not to be affected by ripening at day 56. After 40 days the first incidences of browning could be seen in pears stored at enhanced carbon dioxide. After 56 days browning was also detected in the control pears. At switched conditions there were some incidences of browning. Table 4 gives an overview of brown induction after 83 days of storage.

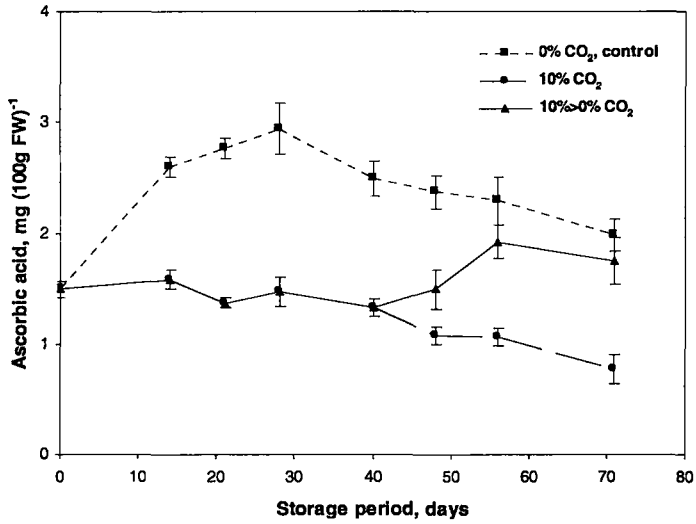


Fig 2. Ascorbic acid in pears as an indicator for browning before and after switching gas conditions. Fruits from orchard 1 were stored at 2% oxygen without carbon dioxide (control, n=4) or under 2% oxygen and 10% carbon dioxide (n=8). After 40 days of storage four of the enhanced carbon dioxide containers were switched to the control condition (10%>0% CO_2). During storage ascorbic acid was determined in two mixed samples of 10 pears from each of the 12 containers. Values are expressed as mg (100g FW)$^{-1}$ ± SE.

Stage of browning	Gas conditions (O_2-CO_2)		
	2-0	2-10	2-10 > 2-0
0 (no browning)	41.3	23.9	74.7
1 (slight browning)	36.7	14.7	6.1
2 (moderate browning)	16.6	13.0	11.6
3 (severe browning)	5.4	48.5	7.7
Brown index	0.29	0.62	0.17

Table 4. Brown core in pears after 83 days of storage. Pears from orchard 1 were stored at 2% oxygen, with or without 10% carbon dioxide (2/0 and 2/10, n=4). One series of containers was switched from high carbon dioxide (10%) to no carbon dioxide (2/10>0) after 40 days (n=4). Pears were classified as healthy (class 0), and slightly (class I), moderately (class II) or severely (class III) brown. For every condition (2/0, 2/10 and 2/10>0) circa 120 fruits were examined. Values are expressed as percentages. From the percentages the brown index is calculated.

The effect of switching conditions from enhanced carbon dioxide to control conditions is clear; nearly 75% of the pears did not show browning compared to only 24% of the pears continuously stored under carbon dioxide. About 50% of the pears stored at enhanced carbon dioxide showed severe browning, compared to only 8% of the pears stored in containers that had switched conditions. Ponting and Joslyn stated already in 1948 that there is an intimate relation between browning of apple tissue and ascorbic acid levels. It seems that browning does not occur until all ascorbic acid in the tissue is oxidized. Brown tissue does not contain ascorbic acid. Furthermore, in vitro the PPO reaction is overall inhibited until ascorbic acid is depleted, probably because ascorbic acid reduces formed *o*-quinones back to precursor polyphenols (own observations).

Ethane

Due to lipid peroxidation in cell membranes ethane can be formed which diffuses out of the fruit. In a preliminary test ethane emission has been monitored during storage at unfavourable, brown-inducing conditions (0.5% oxygen and 3.0% carbon dioxide). Three fruits were placed in 2 l cuvettes and connected to the flow-through system of a photoacoustic detector. The ethane emission was monitored for 4 h (Fig. 3). In one cuvette the average ethane concentration in the flow was 30 ppb, while one

pear showed small cavities, the second showed moderate-sized cavities, and the third pear did not show aberrations. In a second cuvette the average ethane emission was 60 ppb, while two pears showed moderate browning and cavities, and the third pear did not show browning or cavities. Pears without browning or cavities did not show a detectable ethane emission.

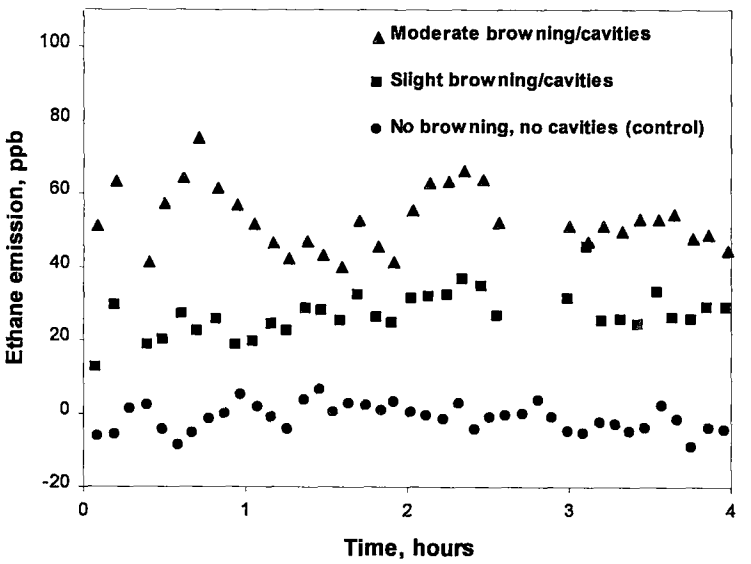


Fig 3. Photoacoustic laser measurements on the ethane emission of brown pears. Before the experiment fruits (from orchard 1) were stored at 0.5% oxygen and 3.0% carbon dioxide for 5 months, to initiate aberrations. Three cuvettes were connected to the flow-through system of a photoacoustic CO laser, all with three pears, at 2.5% oxygen and 8% carbon dioxide. The total flow through the cuvette was 5 l h^{-1}. Ethane emission is expressed as ppb. Pears were judged afterwards. (▲): Moderate browning/cavities; (■): slight browning/cavities; (●) no browning or cavities.

Veltman et al. (1999) hypothesized that brown core in Conference pears is a consequence of cellular decompartmentation, caused by membrane disintegration. During senescence, internal membrane damage results in leakage of phenolic compounds from the vacuole. A higher susceptibility for browning is neither caused by an enhanced activity of PPO, nor by a higher concentration of phenolics. Destructive rearrangements of the mitochondria and other organelles give grounds to suggest that these phenomena are caused by an energy deficiency. Probably maintenance energy (ME) requirements are not constant during controlled atmosphere storage of fruits (Peppelenbos and Rabbinge 1996). Such changes were found in potato tissue, where increased lipid peroxidation, progressive loss of membrane integrity and substantially higher rates of Cyt-mediated respiration are characteristics of older potato tubers during sprouting (Kumar and Knowles 1993). As tubers age, membrane permeability to electrolytes and age induced loss of membrane integrity increase. Potential ATP sinks during aging are ATPases in the plasmalemma that become leaky with advanced age. Older tubers respire with a faster rate to achieve the same AEC (Kumar and Knowles 1996a, b). In the case of pears stored in CA, respiration cannot be accelerated due to the limiting oxygen concentration in the storage room. A second parameter affecting respiration is the diffusion characteristics of the fruit. When ME requirements increase during aging, this parameter could become of in increasing importance. An increase in diffusion resistance, for example, could be an explanation for browning in ripe pears at CA conditions, which is seen in Table 4 Browning can be seen at control conditions (2/0) after 83 days storage. Although ascorbic acid levels slowly decreased in the control after 30 days storage, levels were not determined after 70 days, so that no statements can be made about the course of this decrease after 70 days.

While initiation of browning can probably be explained by energy deficiency, it is not clear why the appearance of browning takes so long. When pears are stored at 2% oxygen and 10% carbon dioxide

(5 °C) the first incidences of browning become clear after more than 5 weeks.
Results presented in figure 7 indicate that brown pears and pears with cavities produce ethane. Konze and Elstner (1978) reported that ethane is formed from linolenic acid in potato mitochondria. In their paper they stated that oxygen radicals mainly drive ethane (and ethylene) production by potato mitochondria. Ethane and pentane, end-products of a process called β-scission, can be produced followed by hydrogen abstraction from ω-3 and ω-6 unsaturated fatty acids respectively (Aust 1985).

Ascorbic acid during static storage
Ascorbic acid levels in the cortex tissue of Conference pears strongly decline after harvest (Table5). Most of the ascorbic acid is lost when fruits were brought under standard controlled atmosphere (CA) conditions. After 100 days of CA storage pears of the first and the second pick lost 60.6% and 62.7% of their ascorbic acid content respectively. At enhanced carbon dioxide (3.0%) losses are 81.6% and 75.6% respectively. These values are only slightly increased after 200 days. After 100 days storage at standard CA, for example, pears from the second, optimal harvest date contained 2.80 mg/100g FW ascorbic acid (Table 5). After 200 days storage the same pears contained 2.54 mg/100g FW on average, a decline of less than 3% per month. When pears are stored under cooled conditions (no CA) after harvest the higher ascorbic acid levels at harvest are retained for more than a month (data not shown).

Harvest	Storage duration (days)	Ascorbic acid content (mg / 100 g FW)		
		at harvest	after storage	
			0.5 kPa CO_2	3 kPa CO_2
1	0	7.57		
	100		2.99	1.39
	200		2.42	1.40
2	0	7.51		
	100		2.80	1.83
	200		2.54	1.42
3	0	7.62		
	100		2.24	1.16
	200		2.44	1.34
4	0	7.21		
	100		1.77	0.83
	200		2.52	0.98
5	0	6.16		
	100		1.64	0.13
	200		1.75	0.64

Table 5. Pears from orchard 2 were monitored during static storage, and ascorbic acid values were determined. Every value is the average of two measurements. Every measurement is done on a mix of the cortex tissue of 5 fruits. Values are expressed as mg $(100g\ FW)^{-1}$ ± SE.

After 100 days CA-storage of Conference pears the effect of picking date and elevated CO_2 concentrations on the ascorbic acid content became visible (Table 5 and Fig. 4). Browning was only found during storage at standard CA conditions, when fruits were picked after the optimal CA picking date (pick 2) (Fig. 4). Low ascorbic acid levels were found in these fruits. In brown tissue ascorbic acid is nearly or completely absent, therefore average ascorbic acid values were lower in the mixed samples. Because of browning, it was difficult to draw conclusions on ascorbic acid declines from later picked fruits. No pears from pick 1 and 2 with ascorbic acid levels below 1.2 mg $(100g\ FW)^{-1}$ were found. The correlation between harvest date and ascorbic acid levels is significant in figure 8. The model in this figure shows that an ascorbic acid limit can be seen for pears from pick 3, 4 and 5 at circa 2.5-3.0 mg $(100g\ FW)^{-1}$. Below this limit internal browning is developed. The harvest date seems to be the main factor in the relationship between ascorbic acid and browning. A putative threshold, below which browning is developed, strongly depends on the harvest date. Other significant factors influencing ascorbic acid levels are carbon dioxide during storage and the storage duration.

The difference in ascorbic acid levels between 100 and 200 days storage is not significant, but the difference between levels at harvest and after 100 or 200 days storage is. From figure 5 and 6 we concluded that ascorbic acid levels in Conference pears of the optimal harvest date for CA storage decreased under brown inducing conditions. When these levels dropped beneath a certain threshold (1.3 mg (100g FW)$^{-1}$), browning seemed to be initiated. These experiments were performed under extreme conditions. Increased storage temperature (5°C) and enhanced carbon dioxide concentrations (10%) were used to initiate brown core quickly. From figure 8 it can be seen that results do not contradict with experiments under extreme, experimental conditions. Although no brown pears were found in pick 1 and 2, ascorbic acid levels were above the putative, suggested threshold (1.3 mg (100g FW)$^{-1}$).

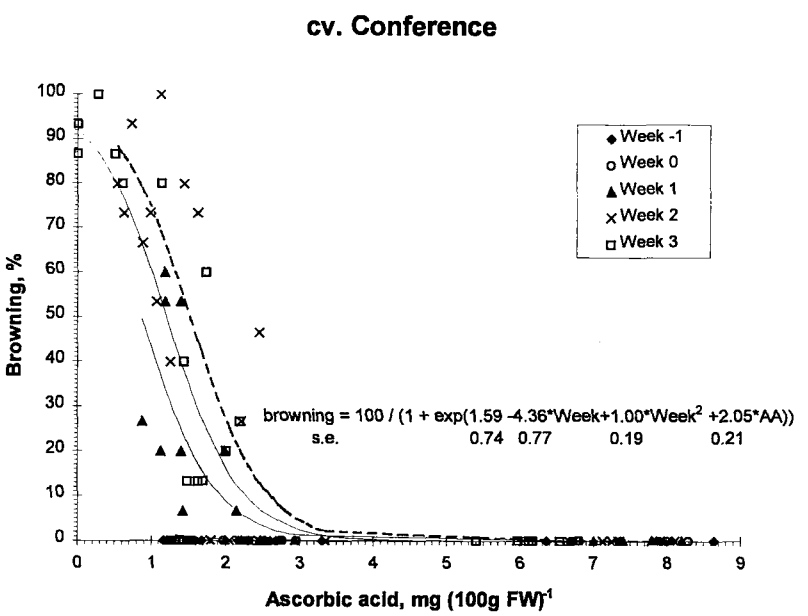


Fig. 4. Model of ascorbic acid levels of pears from 5 harvest dates, and data on internal browning. 'Week 0' is the optimal harvest date. For every point in the graph 5 pears were examined. Ascorbic acid was determined in a mixed sample of the cortex tissue of 5 fruits, and befor pears were cut the brown index was determined. Ascorbic acid values are expressed as mg (100g FW)$^{-1}$ ± SE. (Acknowledgements to R. Kho).

ascorbic acid losses during storage of fruits and vegetables have been often reported. In potatoes, for example, such losses vary from 21% to 60% when tubers are stored for 8 months at 5-6 °C, depending on the cultivar (Keijbets and Ebbenhorst-Seller, 1990).
Data on vitamin C levels during storage at MAP or CA is scanty. Vitamin C was lost more quickly in air than in low O_2 concentrations in asparagus, pea, snap bean, spinach, broccoli and Brussels sprout, and losses were dependent on storage temperature (Platenius and Brown-Jones, 1944). Contradictory, ascorbic acid levels in Conference pears dropped significantly when fruits were brought at CA conditions (Table 5), compared to immediately after harvest. The same drop was seen in apples of 5 different apple cultivars by Haffner et al. (1997). In both pear cultivars enhanced carbon dioxide and lowered O_2 concentrations induce browning as well. Comparable results were found by Agar et al. (1997) in several berry fruits. A rapid decrease of ascorbic acid was monitored in strawberries, when stored at 15% CO_2. The highest ascorbic acid concentrations were found in strawberries stored under air. Comparable results were found in raspberries, red and black currants and black berries. In juice from pomegranate ascorbic acid decreased with increasing CO_2 concentrations (Küpper et al., 1995). Under experimental conditions ascorbic acid could be used as an indicator. It became clear that carbon dioxide lowers ascorbic acid levels before brown core is developed. From the results in figure 8 it seems that the harvest date is a complicating factor. Conference pears picked after the optimal date for CA storage show browning at higher ascorbic acid levels. From these data it seems very likely that, at a physiological level, there is no direct relation between ascorbic acid and browning, but, that a third factor is involved. The effect of this third factor is strongly dependant on the harvest date. One

idea is that this third factor is the energy status of the tissue. Notwithstanding this observation ascorbic acid levels are indicative when only the optimal harvest date is observed.

Conclusions

Optimalization of gas conditions is applied to reduce maturation of the fruit during storage. However, during storage energy requirements could increase, by which an energy shortage is created and vital processes, like the regeneration of antioxidants are abolished. Depending on parameters like diffusion characteristics of the fruit, browning of the tissue is initiated.

It can be concluded that storage conditions, storage duration and picking date have an evident influence on ascorbic acid levels in Conference pears. AA levels can probably act as a quality parameter, being an indicator for the development of brown core. These levels can give information about the quality of fruit during storage. Secondly, ascorbic acid is an essential substance in the human diet, and, therefore, one of the reasons fruit is consumed. Suboptimal storage conditions, like enhanced CO_2 or lowered O_2 concentrations, can result in a diminished food value, and AA is a tool to monitor quality and prevent quality decline. Probably developing an interactive storage system, in which energy needs play a crucial role, can prevent disorders in pears and other fruits.

Future research

In the two years the Europear project is running we examined the different aspects of the development of internal browning in Conference pears. PPO activity and polyphenol concentrations are no limiting factors in the browning process. There are strong indications that energy levels in pears during storage are crucial. A shortage of energy causes very likely decreased ascorbic acid concentrations in the fruit, and damage to internal cell compartments can probably not be repaired.

In the near future we will focus on the relation between the Adenylate Energy Charge (AEC), respiration and concentrations of ascorbic acid in the fruit tissue. On the basis of these data we will examine if it is possible to predict browning in Conference pears.

Secondly, we will (already are) investigating the kinetics of the PPO reaction. PPO is not only able to oxidize polyphenols. The enzyme breaks down ascorbic acid as well. Experiments will be done on purified pear PPO, and ascorbate oxidase kinetics (of PPO) will be described.

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Task 8

Duration:	**48 months**
Current Status:	**24 months to completion**
Total estimated Man-month:	**29**
No. of man-month devoted already to the task:	**19**

Introduction

The aim of this task is to develop a non-destructive measurement by which brown-core can be detected. We tested a non-destructive technique that is currently used for measuring fruit firmness (the Peleg firmness tester or PFT). The technique is based on the frequency response of fruits after oscillating it. The PFT contains a small electrodynamic vibrator, which contacts the fruit and can apply series of minute input oscillations of a predetermined frequency. The frequency can be altered at a constant rate while the input acceleration is kept constant. The absolute output acceleration is measured by a transducer attached to a device which keeps the fruit in a specific position.

Material and methods

Calibration

In an initial experiment the characteristic frequency and acceleration patterns of pears with or without brown core were measured. Conference pears were marked near the core and measured at this position with 8 different calibration methods (which differ in frequency range and acceleration). Afterwards the pears were cut and the brown index value was determined. Based on these experiments the optimal measuring method was selected.

Validation

Conference pears (n=229) were stored at 1 ° C under normal and high CO2 levels. Directly after storage pears were put on ice and were measured with the PFT. After the measurement the pears were cut and the brown index value was determined. A method to distinguish brown and not-brown pears was developed by combining PFT signals at different frequencies.

Results

An average signal for brown and not brown pears were calculated for each calibration method. From these results the optimal measuring method (results not shown) was chosen.

In figure 9 the average PFT signals of pears with a different a brown index are shown. Large differences between pears with no or moderate browning and pears with sever browning can be found in 2 frequency areas, around 700 Hz and 1000 Hz. These differences in PFT signal could not be related to differences in firmness. In table 6 the results of 3 different prediction methods are shown. All three methods are based on a combination of two frequencies.

Prediction method 1 showed the highest percentage of correctly classified pears (83.8%). When only the prediction of actual brown pears are compared, method 2 was the best, with only 1.7% brown pears that were not detected. This second method, however, resulted in a large number of pears (28.8%) that were classified as brown while they did not show any brown. To prevent brown pears

reaching the consumer method two is to be preferred. The disadvantage is a large number of rejected pears that are not brown.
It is neccesary to validate whether pears during storage show the same PFT response, and wheter a prediction method can be used throughout the storage period.

Conclusions

- Measuring brown core non destructively seems possible with a PFT.
- Other combinations of frequencies will be analyzed. The measurements will be validated next season.

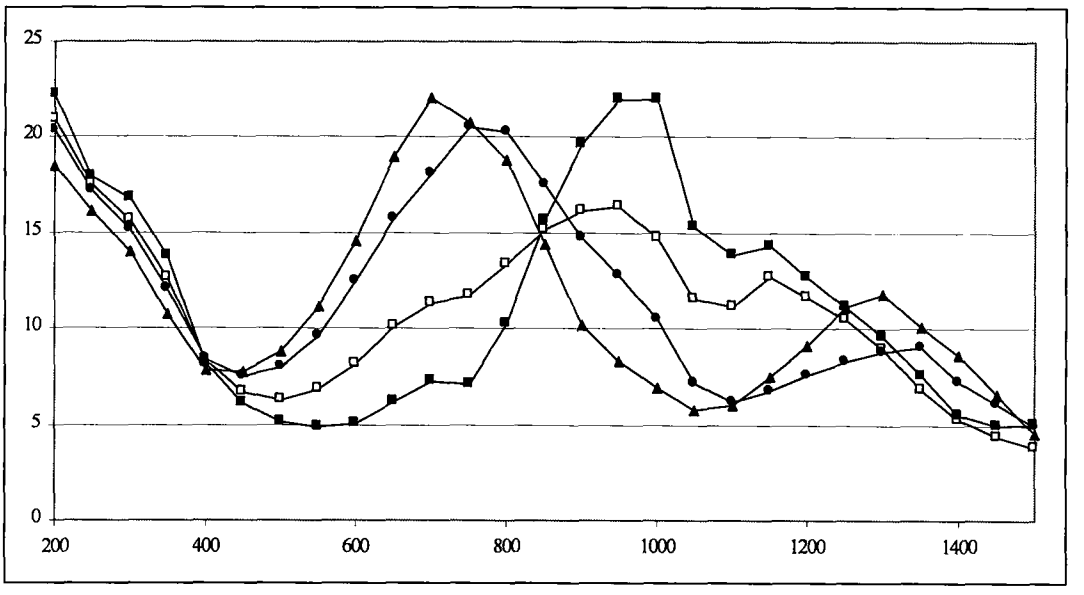

Figure 9. Average PFT signal of Conference pears with different brown index value. Symbols: □ brown index 0; ■ brown index 1; ● brown index 2; ▲ brown index 3.

Table 6. Non-destructive measurements of Conference pears (prediction and actual values in % of total pears).

Judgement	Prediction	Actual	Prediction method		
			1	2	3
Correct	Not Brown	Not Brown	65.9	48.5	56.8
	Brown	Brown	17.9	21.0	18.3
	Total		83.8	69.5	75.1
Not Correct	Not Brown	Brown	4.8	1.7	4.4
	Brown	Not Brown	11.4	28.8	20.5
	Total		16.2	30.5	24.9

Task 9

Duration:	**48 months**
Current Status:	**24 months to completion**
Total estimated Man-month:	**29**
No. of man-month devoted already to the task:	**19**

Objectives

The aim of the modelling task of the Europear project is to twofold. The first aim is to develop a model that can predict the occurrence of Brown-Heart based on orchard and weather conditions in the

different European countries. The second aim is to develop a model that can predict the occurrence of Brown-Heart based on the physiology of the pear fruits. For these two models different approaches are used. To find the relationships between orchard and climate factors and the occurrence of Brown-Heart, multivariate statistical techniques such as principal components analysis and multivariate regression analysis can be used. This model requires data-sets with climate and orchard factors in the different European countries over different years, together with data on the occurrence and severeness of Brown-Heart at he different locations and in different growing seasons. The second model is a mechanistic model that quantitatively describes the physiological processes related to the occurrence of Brown-Heart such as metabolic rates, energy fluxes and antioxidant levels. This model requires experimental data on the various physiological processes. In this report we describe the work carried out so far, in which we concentrated on the first, predictive model for the occurrence of Brown-Heart.

Data-set

Orchards

For the analysis measurements of the first two growing seasons were available. In these years, 1997 and 1998, data were collected by all partners. In most cases pears were grown in orchards at different locations, and the climate at these different locations was recorded throughout the growing season. At FPO in the Netherlands, at some locations data of several orchards were collected. The data from VCBT of 1997 were not included in the data-set because the storage conditions deviated from those in the other countries. Table 7 gives an overview of the origin of the data collected thusfar.

Table 7. Overview of the origin of the data-sets of climate factors and occurrence of Brown-Heart collected by different partners during 1997 and 1998.

Partner	1997				1998			
	Orchard	Nr of locations	Nr of harvests	Nr of subsets	Orchard	Nr of locations	Nr of harvests	Nr of subsets
FPO	Numansdorp	4	5	1	Numansdorp	3	5	1
	Wilhelminadorp	3	5	1	Wilhelminadorp	3	5	1
	Kapelle	1	5	1	Kapelle	1	5	1
	Dodewaard	2	5	1	Dodewaard	1	5	1
VCBT					Velm	1	3	1
					Zellik	1	3	1
UHOH	Bavensdorf	1	3	3	Bavensdorf	1	3	3
	Salem-Mittelsweil.	1	3	3	Salem-Mittelsweil.	1	3	3
IVTPA	Malaguti/Rinaldi	2	3	1	Malaguti	1	3	2
IRTA	Giminels	1	3	3	Giminels	1	2	3
	Albatarrech	1	3	3	Albatarrech	1	2	3
					Mollerusa	1	2	3

Data recorded

The data-set consisted of the different types of variables. Firstly, orchard factors such as type and amount of fertilisation, soil type and the age of the trees was recorded. During the growing season the climate at every location was monitored by standard weather stations. The registered variables were: minimum (Tmin), maximum (Tmax) and average (Tavg) daily temperature, relative humidity, total global daily radiation and daily precipitation. Then, in every season the development of the fruits was described by the time of flowering and the different harvest times. Fruits were always harvested at the optimal harvest date but also once or twice before and after the optimal harvest time. Using the date of flowering and the date of optimal harvest a relative developmental time scale was defined. The time of flowering was defined as stage 0 and the optimal harvest date as stage 1. This allows the comparison at similar developmental stage of fruits from different countries that vary in absolute development measured in days. This is necessary as e.g. in 1997 the duration of the growing season was 160 days in the Netherlands, 140 days in Germany, 140 days in Italy and 141 days in Spain. An illustration of the development on an absolute time-scale and a relative developmental time scale is given in Figure 10. At harvest a number of quality parameters was measured, though not all measurements were carried out by all partners. The following quality parameters were assessed: firmness (N 0.5 cm^{-2}), sugars, starch, mineral content (Ca, K, Mg), vitamin C, diffusion resistance and porosity. Finally, after

storage the occurrence of Brown-Heart was assessed. Individual fruits were scored for the degree of browning (class 0,1,2 and 3) and for the severity of cavities (class 0,1,2, or 3). Pears can either be healthy, have only browning, have only cavities or be affected by both browning and cavities.

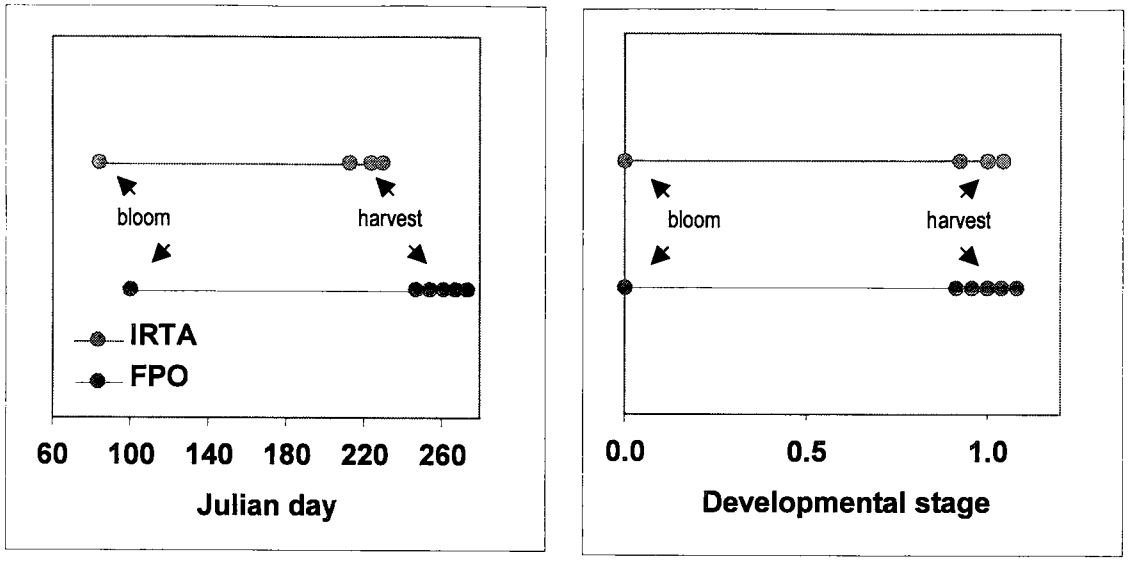

Figure 10. Development of pear fruits at FPO (The Netherlands) and IRTA (Spain) on an absolute time-scale (left) and on a relative time scale.

Transformation of the climate data

The original climate data consisted of daily values of minimum, maximum and average temperature, relative humidity, total global radiation and precipitation. To correlate such data with the occurrence of Brown-Heart at a given time after storage, it is necessary to transform these data into integrated values that describe the climate not per day, but over a certain period of time. We choose to calculate integrated values of the climate parameters over different periods of time. Firstly over the entire growing season, and next over 5 periods each representing a period of a subsequent 20% of the total development, i.e. period 1 is from bloom to developmental stage 0.2, period 2 is from developmental stage 0.2 tot 0.4, etc. Besides the average values of the climate parameters over the defined periods, we also calculated the temperature sum, radiation sum, precipitation sum over the complete growing season, and for every period we calculated the average difference between maximum and minimum temperature (Tdiff), the highest maximum temperature, the lowest minimum temperature and the largest difference between the maximum and minimum temperature that occurred in a period. In this way, every season the climate was described by 63 climate parameters.

Principal Components Analysis of climate variables

The purpose of the principal component analysis (PCA) was to see which climate parameters contributed most to the variation in climate between the different orchards. We already know that the levels of occurrence of Brown-Heart varies between the different countries in Europe, i.e. higher occurrence of Brown-Heart in northern European countries and a lower occurrence in southern counties. Therefore, a principal component analysis describing the main contributors to variation in climate between the locations in different countries gives a first indication about which climate parameters could be related to the occurrence of Brown-Heart. As a result of a PCA factors are formed that describe a data-set. Every factor can consist of a number of the original variables. In this way the data-structure is simplified as the multivariate data-set (63 climate parameters in our case) is described by a new set of factors (3 new factors in the current example).

Procedure followed

Radiation was excluded from the data-set as these data were not complete and the units were not comparable. Thereafter a number of consecutive PCA's was performed. Every following PCA we

only included those variables with factor loading higher than 0.7, in other words that had a correlation coefficient higher than 0.7 with the one of the factors. A first PCA was performed including all climate parameters over all periods. Thereafter a second PCA was performed including all climate parameters over all periods except precipitation. In the third PCA only climate parameters from the first period (0 - 0.2 development) and those over the complete growing season were included. In the last PCA we including all climate parameters except precipitation, from the first period (0 - 0.2 development; P1) and from the complete growing season. Table 8 gives the explained variance by the factors. Table 9 and Figure 11 give the factor loadings that resulted from this last PCA.

Table 8. Eigenvalue, percentage of explained variance and cumulative explained variance of PCA including average values over the growing season of Tavg, Tmax, Tmin and Tdiff and average values over the period of 0 – 0.2 development of Tavg, Tmax and Tmin.

	Eigenvalue	% Explained variance	% Cumulative variance
Factor 1	3.49	49.8	49.8
Factor 2	2.27	32.4	82.3
Factor 3	1.13	16.1	98.5

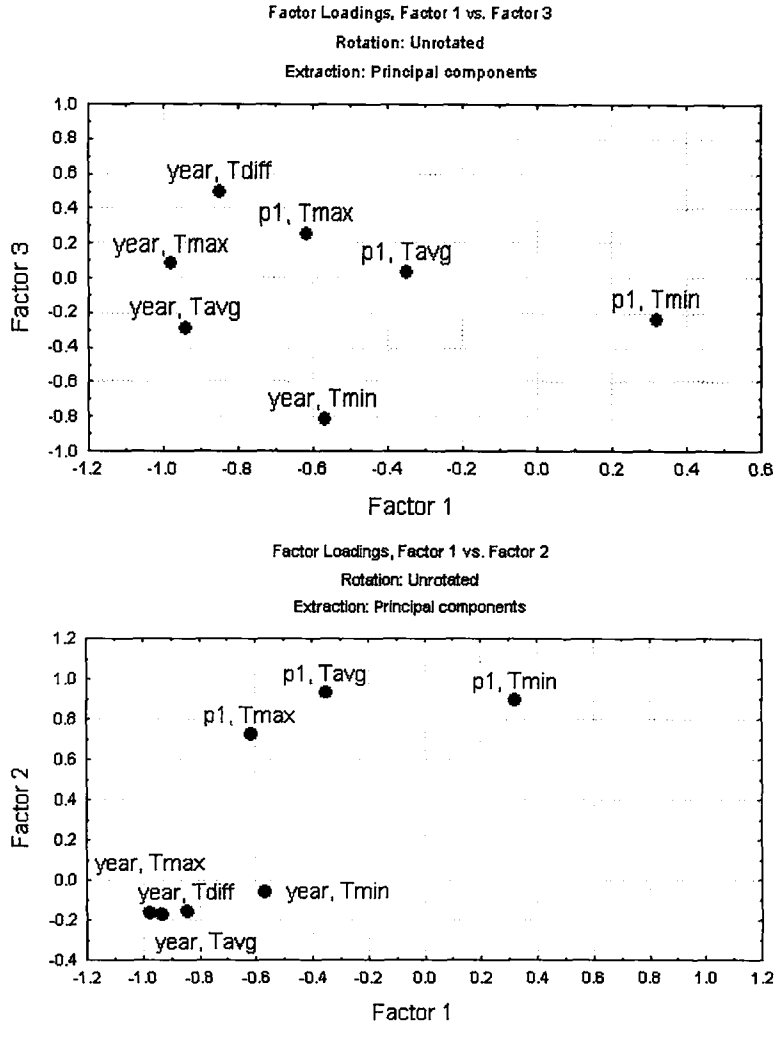

Figure 11. Factor loadings f PCA including average values over the growing season of Tavg, Tmax, Tmin and Tdiff and average values over the period of 0 – 0.2 development of Tavg, Tmax and Tmin. From these graphs it can be seen that the first factor 1 describes the temperature over the entire growing season, factor 2 describes the temperature during the first part of the growing season and factor 3 describes the difference between minimum and maximum temperature.

Table 9. Factor loadings of PCA including average values over the growing season of Tavg, Tmax, Tmin and Tdiff and average values over the period of 0 – 0.2 development of Tavg, Tmax and Tmin.			
	Factor 1	**Factor 2**	**Factor 3**
Year-Tavg	-0.938	-0.170	-0.292
Year-Tavg	-0.980	-0.165	0.077
Year-Tmin	-0.571	-0.061	-0.816
Year-Tdiff	-0.847	-0.154	0.497
P1-Tavg	-0.350	0.932	0.027
P1-Tmax	-0.618	0.724	0.253
P1-Tmin	0.321	0.892	-0.244

Thus, 3 main factors emerged from the principal component analysis. The first factor describes the temperature over the entire growing season , the second factor the temperature during the first period (0 - 0.2 development), and the third factor describes the difference between minimum and maximum temperature. After plotting the factor scores of the individual data-points (the different locations with a different climate), these different locations can be grouped using cluster analysis. The results of this cluster analysis are shown in Figure 12. It can be seen that 3 different climate groups could be separated: a group of climates including mainly the northern European countries in the first growing season, a group containing these locations in the second growing season, and a group mainly consisting of the southern countries. The preliminary conclusion from these results is that the occurrence of Brown-Heart is higher when there is a low temperature over the whole growing season, there is a low temperature early in the season and when there is a small difference between the minimum and maximum daily temperature. From the analysis it resulted that especially the first part of the growing period contributed to variation among the countries. Interestingly, this period overlaps with the period of cell division. This may indicate that problems with the occurrence of Brown-Heart during storage may originate from the period of cell division. It should be noted that the current analysis was performed using only a limited data-set including climate parameters over 2 growing seasons. The analysis will improve if more data are included later in the project.

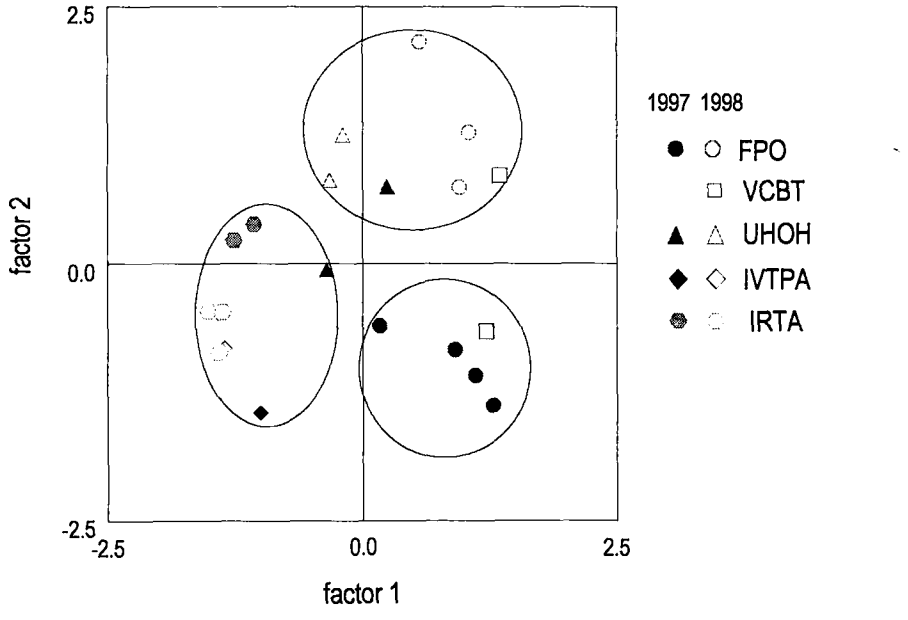

Figure 12. Factor scores of the individual locations in the growing seasons 1997 and 1998. Grouping is according to cluster analysis.

Occurrence of Brown-Heart

The data of the occurrence of Brown-Heart under the different growing conditions and storage conditions in the first two years were analysed. There was a very strong increase of Brown-Heart with increasing harvest time. Figure 13 gives an example of this using data from FPO, the Netherlands. From this figure it can also be seen that at high CO_2 the effects of harvest time were less clear, as the overall levels of Brown-Heart were increased.

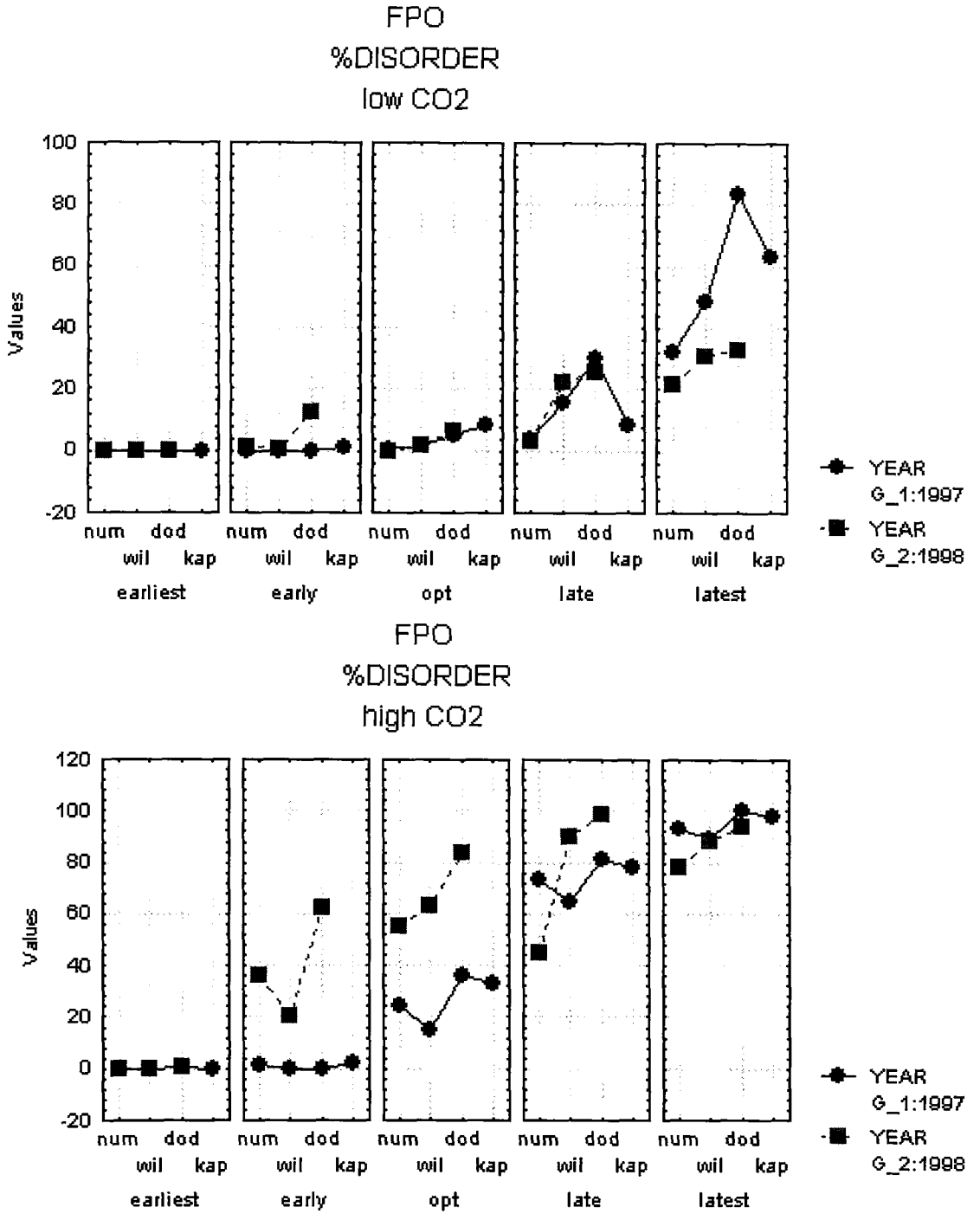

Figure 13. Percentage Brown-Heart in pears from earliest, early, optimal, late and latest harvest at different location in the Netherlands in 1997 and 1998, from pears stored at low or high CO_2.

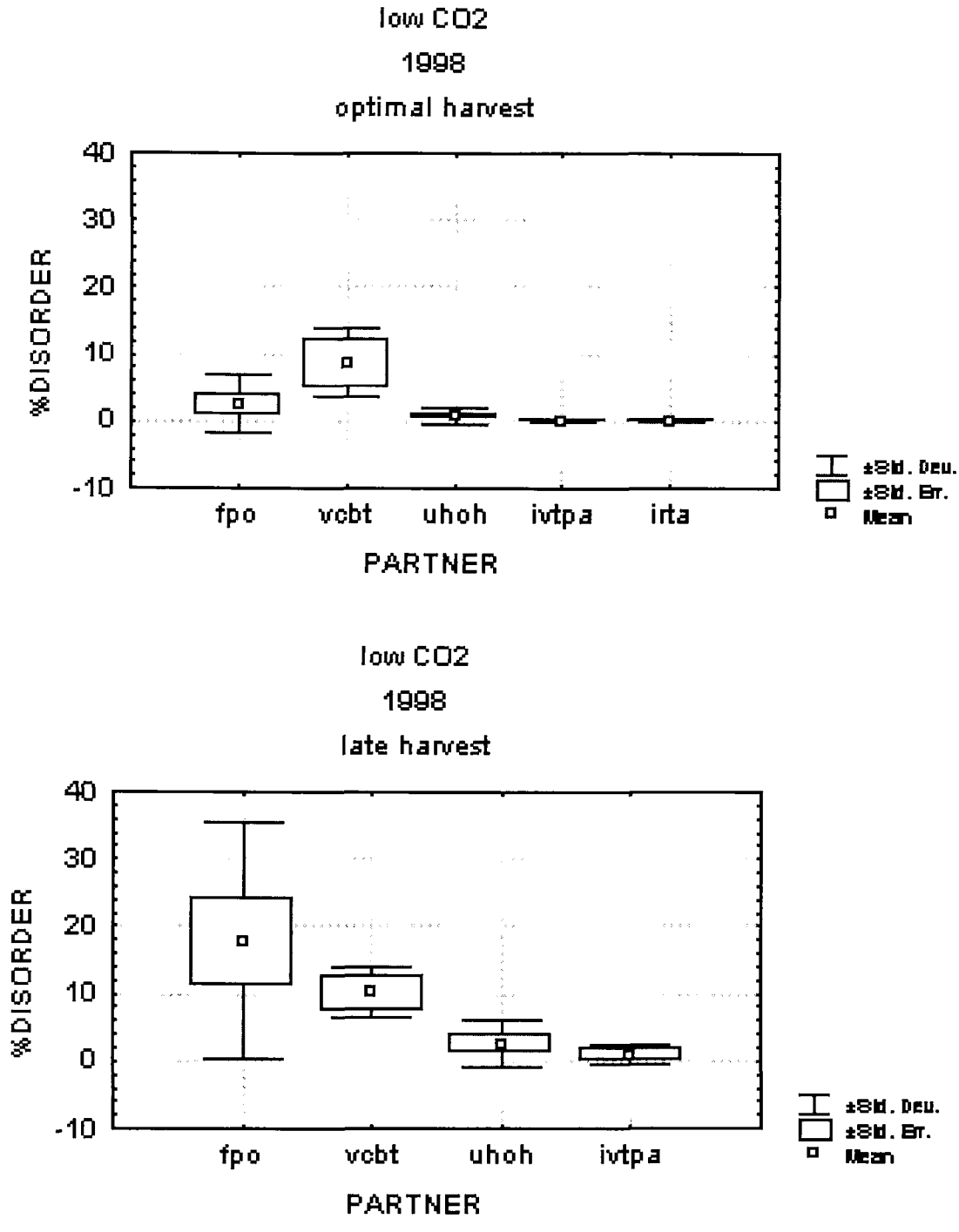

Figure14. Occurrence of Brown-heart in pears from the optimal harvest date and from the late harvest, stored at low CO_2, from locations in the Netherlands (FPO), Belgium (VCBT), Germany (UHOH), Spain (IRTA) and Italy (IVTPA) in 1998.

As can be seen from Figure 14, the variation in occurrence of Brown-Heart between the different countries were not very large when fruits of the optimal harvest date were considered. However, when fruits were harvested later that the optimal harvest date, large differences between the countries became apparent.

Conclusions

- The analyses carried out so far was done using data of only two growing seasons. More data on climate factors and occurrence of Brown-Heart will improve model.
- Late harvest of pears can be used to assess the potential for Brown-Heart.
- Proper assessment of optimal harvest date is very important as the largest variation in the occurrence of Brown-Heart was due to variation in harvest date
- The preliminary prediction can be drawn that low temperatures in spring and low temperatures throughout the growing season lead to high occurrence of Brown-Heart

Improvements model 1

- More locations with consequently more variation in climate will improve the ability of the modelling techniques to find the causes for the occurrence of Brown-Heart.
- Similar measurements of radiation among all partners would allow including radiation into the model.
- Including harvests later than the optimal harvest will give a better impression on the potential for development of Brown-Heart
- Low CO2 and 2% O2 can be used as standard storage procedure, while storage at 5% can be skipped.
- In the present data-set storage time varies between 176 and 259 days. An equal storage time among partners will lead to a more fair comparison of the occurrence of Brown-heart.

Model 2: physiological parameters

Currently the relation between metabolic rates, reduction in vitamin C levels, energy metabolism and the onset of the brown-heart disorder are quantified. Based on this analysis the measurements directly after harvest will be re-evaluated. Predictions on the risk of brown heart based on such measurements will be generated for the next harvest period (September 2000).

Planning 1999-2000

- The correlative model on the relationship between the occurrence of Brown-Heart and climate factors can be improved by including data from the 1999 season and by performing multivariate regression analysis.
- A start will be made with the physiological model using metabolic network modelling to describe ascorbate levels in pears.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1998 TO 31-05-1999

None

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1999 TO 31-05-2000

The plans as described in the technical annex will largely be followed. Changes are mainly expected for task 7, biochemical measurements, where some very interesting findings on vitamin C are worth to be further analysed.

Task 7 will focus on two aspects. First monitoring vitamin C during storage at different gas conditions, and the consequences of changing conditions during the storage period. Important questions are: can adaptation of gas conditions avoid browning, and what happens to ascorbic acid levels during adaptation? More fundamentally, we are interested in the effect of ascorbic acid on PPO, and, reciprocally, the effect of PPO on ascorbic acid. For example, is PPO able to break down ascorbic acid? Secondly, we will focus our attention on energy metabolism. Especially fermentation and the effect of carbon dioxide at ULO conditions. Enhanced carbon dioxide concentrations during storage suppresses energy production. For measurements on fermentation products we will cooperate with the Department of Molecular and Laser Physics of the Catholic UniversityNijmegen. This group

is able to detect fermentative metabolites in the gas atmosphere at very low concentrations (ppt-ppb range).

Work on the first version of the physiological model, focussed on relating internal gas concentrations to energy metabolism and the onset of disorders, will start in the next period. Results of this analysis will be used for advice on measurements in the coming growing season.

E. DISSEMINATION

Journals

(1=international refereed journal, 2=proceedings, 3=national journal)

1 De Wild H.P.J., Woltering E.J. and Peppelenbos H.W., 1999. Carbon dioxide and 1-MCP inhibit ethylene production and respiration of pear fruit by different mechanisms. J. Exp. Botany, 50 (335): 837-844.

2 Peppelenbos H.W., Jeksrud W.K., 1998. A method for the simultaneous measurement of gas exchange and diffusion resistance of fruits and vegetables under various gas conditions. Acta Horticulturae, 464: 333-338.

2 Peppelenbos H.W., Oosterhaven J., 1998. A theorethical approach on the role of fermentation in fruits and vegetables. Acta Horticulturae, 464: 381-386.

2 Peppelenbos, H.W., 1998. Gas exchange models and the prediction of disorders in fruits. Proc. COST915 - Copernicus CIPA-CT94-0120 workshop on Food Quality Modelling. Leuven, 3-6 June 1997, pp 69-74.

3 Peppelenbos H.W., 1999. European research on causes and solutions for brown heart (in Dutch). Fruitteelt, 31: 14-15.

3 Veltman R.H., Peppelenbos H.W., 1998. Healthy Conference pears in CA storage thanks to vitamin C (in Dutch). Fruitteelt, 22: 14-15.

1 Veltman, R.H., C. Larrigaudiere, H.J. Wichers, A.C.R. van Schaik, L.H.W. van der Plas and J. Oosterhaven, 1998. PPO activity and polyphenol content are no limiting factors during brown core development in pears (Pyrus communis L. cv. Conference). Journal of Plant Physiology, 154: 697-702.

1 Veltman, R.H., M.G. Sanders, S.T. Persijn, H.W. Peppelenbos, and J. Oosterhaven. 1999. Decreased ascorbic acid levels and brown core development in pears (Pyrus communis L. cv. Conference). Physiologia Plantarum, 107: 39-45.

Lectures and posters

- Peppelenbos, H.W., 1997. Gas exchange models and the prediction of disorders in fruits. COST915 - Copernicus CIPA-CT94-0120 workshop on Food Quality Modelling. Leuven, 3-6 June 1997.
- Peppelenbos, H.W. and Schouten, S.P., 1998. Control of fermentation in harvested plant products. XXV Int. Hort. Congress, Brussels, 2-7 August 1998.
- Veltman R.H., 1997. Core browning in Conference pear: relation vitamin C and storage conditions. Int. workshop on Antioxidants in higher plants. April 13-15, Ravensburg, Germany.

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 2 total cost: (100%)	165.4 kECU	*EC contribution to partner no. 2:*	165.4	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

Coordinator: Dr. H.W. Peppelenbos
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Participant no.: 2

A. PARTNER INFORMATION

Name and address
Name of the institute: IRTA
Address: Alcalde Rovira Roure, 177. 25198 LLEIDA, SPAIN

Scientific team
Names of the scientists participating in the project:

- C. Larrigaudiere - Group leader
- Herrero - Orchard factors
- M. Vendrell - Research scientist
- Recasens - Profesor Physiology
- T. Casero - Mineral analisis
- Lentheric - PhD student

Objectives
As in the technical annex

Actions in the project
IRTA (partner 2) is particularly involved in the following tasks:

- task 1: cultivation of pears
- task 2: harvest of pears
- task 3: storage of pears
- task 4: variation in climate and orchard characteristics
- task 5: post-harvest treatments
- task 7: a- quality evaluation, b- biochemical studies, c- mineral analysis
- task 9: model development
- task 10: validation of the model

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1998 TO 31-05-1999

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	6
No. of man-month devoted already to the task:	4

Objectives
The objective was to select different orchards presenting various sensitivity to brown heart.

Material and methods
We used the same orchards than the previous year:
- orchard 1: "Gimenels", low susceptibility
- orchard 2: "Albatarrech", high susceptibility
A third orchard placed in Mollerussa, was added to complement the model.
Pears were grown according to the local recommendations.

In Gimenels, a complementary study which concerns the effects of the fertilization (N_2 fertilization) was carried out. Fruits were grown with 90, 180 and 270 U / ha and the effects of this strategy on quality and brown heart (BH) incidence was studied. In an other experiment, trees were sprayed with

different Ca^{++} solutions (Seniphos: 310 g/l P_2O_5 + 56 g/l CaO, Stopit: 224 g/l CaO, Metalosate: 12 % P_2O_5+ 6.7 % CaO) to investigate the effect of calcium pretreatment on quality and brown heart incidence.

Results and Discussion

✣ Effect of the fertilization dosis:
The study was carried out trying to answer the following questions:
- is there any significant differences in the mineral composition at harvest ?
- can the fertilization dosis affect the Vit.C content of the fruit ?
- what happen to the quality parameters during storage ?
- is there any significant differences in disorder incidence between different N_2 dosis.

As regard the first question and as shown in table 1, we didn't observed significant differences in the mineral composition in the fruit between the different N_2 doses. Furthermore, no significant differences in the content in ascorbate were observed for the different treatments immediately after harvest (fig.1). After storage no differences in the various quality parameters such as the firmness (fig.2), SSC, acidity were observed.
No physiological disorders were observed this year after storage. In consequence, the relationship between N_2 doses and BH incidence could not be established.

✣ Effect of preharvest calcium treatments:
In the same way, the fruits treated with calcium didn't present significant difference in their mineral composition at harvest (table 2). Calcium treatment was not effective to preserve the quality of the fruit during storage and even cause a slight increase in maturity in the treated fruits. For the same reason than before, the relationship between Ca^{++} treatment and BH incidence could not be established.

Conclusions
Although we could not established the direct relationship between the treatments previously described (N_2 doses and Ca^{++} treatments) and browning incidence the results obtained on quality parameters (similar loss in firmness and maturity in the treated-fruits) are an indication that these parameters are not directly involved in BH incidence in Conference pears.

Task 2: Harvest of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	3

Objectives
Experiment 1: To study the influence of picking date on the development of brown heart.
Experiment 2: During year 1997/98, we showed (cf progress report), that the more mature fruits showed a significant decrease in their enzymatic capability of defense against oxidative damage and peroxidation. Significant decrease in Superoxide Dismutase (SOD), Catalase (CAT) and increase in Ascorbate Peroxidase activity were found.
This year we repeat this experiment and complement it determining the levels of antioxidants such as Ascorbate and Glutathione.

Material and Methods:
Experiment 1: To compare orchards, pears were harvested from 3 locations (Gimenels, Mollerussa and Albatarrech), at two harvest date. Fruits were picked at 1 week-intervals, the 17 and 24 August

for H_1 (harvest 1) and H_2 respectively. Maturity was determined according to standard procedures analysing the ground color, titratable acidity, firmness and sugars content (SSC value).
Experiment 2: Fruits were picked at one week interval, the 17, 24/08 and 01/09. Immediately after harvest, we determined the following parameters: content in ascorbate and glutathione, activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX).

Results and Discussion
Experiment 1: Quality data of the pears directly after harvest are listed in table 3
Experiment 2:. As regards antioxidants, we showed that:
- delayed harvest leads to a significant decrease in ascorbate and glutathione content
- we noted a change in the reduction state of ascorbate
- results on enzyme activities were similar to those of the previous year.

Conclusions:
Experiment 1: Harvesting pears with one week interval resulted in satisfactory differences in maturity as indicated by the different quality parameters (table 3). However, and for a reason which remain unknown, no physiological disorders were observed this year for the different harvest dates.
Experiment 2: Delayed harvest leads to reduction in the antioxidative defense system in Conference pears and likely explains the harvest-maturity related susceptibility of this fruit to physiological disorders such as B.H.

Task 3: Storage of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	2

Objectives
Pears from the different orchards were stored under various AC storage conditions to study mainly the influence of CO_2 on the occurrence of core browning during storage.

Material and methods
As for the first year two different storage procedures were established:
- a standart storage, - 0.5 °C, 95 % RH, 2 % O_2 and 0.7 % CO_2, representative of conventional commercial conditions
- a stress storage, - 0.5 °C, 95 % RH, 2 % O_2 and 5 % CO_2, in order to induce B.H
Fruits were removed from storage at time 90 d and 180 d.

Results Discussion and Conclusions
1- Effect of storage conditions on quality and biochemical parameters: see the other tasks
2- Effect of storage conditions on brown heart incidence:
No storage disorders were observed this year for the different storage conditions..

Task 4: Variation in climate and orchard characteristics

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	1

Objectives
One main objective was established for partner 2: to collect meteorological data in order to feed the predictive model

Material and methods
Meteorological data for orchard 1 were directly checked on the site, for orchard 2, data were collected from a meteorological station near the orchard. Parameters checked were the following ones: T°C / day (min., max. and average), humidity in %, radiation in J s^{-1} m^{-1} and rainfall in mm / day.

Results, discussion and conclusions:
Data on meteorological parameters have been sent to partner 1 for the validation of the predictive model

Task 5: Post-harvest treatments

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	8
No. of man-month devoted already to the task:	4

Objectives
Partner 2 was involved in this task in determining the effect of a stress scenario (high CO_2 treatment prior to conservation) on the physiology of pear and its incidence on B.H.

Material and methods
After harvest, the fruits picked at commercial harvest maturity (H_2, orchard Gimenels) were immediately treated with CO_2 as follows:
Te. Control no treated
3d – 10 %: fruits treated 3 days with 10 % CO_2 + 2 days at 1°C
5d – 10 %: fruits treated 5 days with 10 % CO_2
3d – 15 %: fruits treated 3 days with 15 % CO_2 + 2 days at 1°C
Immediately after this treatment the fruits were analysed or put in storage as previously described.
The parameters analysed both after treatment and after storage were the following:
- quality parameters: color, firmness, SSC, acidity
- biochemical parameters: fermentative metabolism (etanol, acetaldehyde, ADH and PDC), oxidative metabolism (Lipoxygenase, antioxidant scavenging enzymes: SOD, CAT, APX), levels in antioxidants (ascorbate and glutathione) and peroxidation state of the membranes.
- effects on the storage-related disorders.

Results and Discussion

1- Effects on quality parameters:
Fruits treated with CO_2 shows higher firmness values during all the storage period (table 4). On an another hand, SSC and acidity remains lower in the CO_2-treated fruits. The CO_2 treatment delays ripening and may be of interest for a prevention of the senescence-induced physiological disorders such as BH.

2- Effects on fermentative metabolism:
After treatment we noted a slight decrease in acetaldehyde levels and also a slight increase in ethanol particularly for the treatment at 15 % of CO_2. On an another hand, no significant changes in the activity of ADH and PDC were found.

3- Effects on oxidative metabolism:
✣ Immediately after the treatment we noted some significant changes in the oxidative metabolism. These changes are the following:
- a slight decrease in LOX activity (fig.3)
- a significant increase in SOD activity in the CO_2-treated fruits associated with a slight decrease in CAT activity (fig.4)
- a slight increase in APX activity
✣ After storage the CO_2-treated fruits generally presented less capability to produce peroxides (lower activity in SOD, lower activity in LOX) and this result likely explain why the membranes of these fruits presented less peroxidative damage and lower electrolyte leakage (fig.5).

4- Effects on brown heart incidence:
Any physiological disorders were observed this year during storage. In consequence, the relationship between the CO_2 treatments and BH incidence could not be established.

Conclusions
Although we could not established the direct relationship between these CO_2 treatments and browning incidence the results obtained on quality parameters (delay in ripening, higher firmness values) and on the biochemical parameters (less electrolyte leakage, short-term activation of SOD, without prejudicial effect on LOX and fermentative metabolism) are an indication that these treatments could be useful to prevent senescence disorders in pears..

Task 7: Destructive measurements

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	44
No. of man-month devoted already to the task:	22

Objectives

7-a: Quality evaluation
Quality changes during storage were studied for the different orchards, storage conditions and harvest date.

7-b: Biochemical studies
The aim of this work was to determine the underlying mechanisms involved in B.H incidence. Two different studies were carried out:
✣ study of the biochemical changes on a short term basis:
The aim of this work was to investigate the biochemical changes which occur on a short-term basis (from 0 to 21 days) when the fruits are kept in AC and to determine a specific effect of the storage at high CO_2 level.
The final objective was also to determine the relationship between these biochemical changes and the disorders.
✣ study of the biochemical changes on a long term basis:
The same plan was follow for fruits kept in long-term storage.

7-c : Mineral content
Study of the mineral content and of its influence on B.H occurrence was already described in task 1 : effect of fertilization doses and effect of preharvest calcium treatments.

Material and methods

✣ short-term, basis:
Immediately after harvest, fruits were put in microchambers at 2 % O_2 and 0.7 or 5 % CO_2. Determinations of biochemical parameters (antioxidants, polyamines and antioxidant-scavenging enzyme activities) were carried out after 2, 4, 8 and 21 days of storage.

✣ long term, basis:
Immediately after harvest, fruits were put in AC storage at 2 % O_2 and 0.7 or 5 % CO_2. Determinations were carried out after 3 and 6 months of storage.
Methods for the determination of antioxidants and antioxidant-scavenging enzyme activities are similar to those described in references.

Results and Discussion

✣ Quality evaluation:
In general there is no significant difference in the quality parameters between fruits stored at 5% and 0,7% CO_2. Quality parameters are not available to predict the sensibility to B.H. Due to the abnormal incidence in physiological disorders observed this year, the relationship between changes in quality parameters and B.H incidence could not be established.

✣ Biochemical changes in stored fruits, short-term analysis:
- *Effects on polyamines*:
Putrescine accumulated differently as a function of the CO_2 concentration. The concentration in this compound remained higher at 5 % CO_2 than at 0.7 % during days 8 (fig.6). After 10 days this tendency is reversed.
As regards the others polyamines spermine and spermidine, there was not significant differences between the two storage atmospheres.
- *Effect on antioxidants*:
Immediately after storage we noted a sharp decrease in Vit.C (fig.7). This decrease was similar for the fruit kept in air or at 0.7 % CO_2 but higher when the fruits were kept at 5 % CO_2.
In the same way, the amounts in glutathione sharply decrease with time. No differences between the different storage conditions were found.
- *Effect on antioxidant-scavenging enzymes*:
After a significant decrease still 2d, superoxide dismutase (SOD) activity increase with time in the fruits stored at 5 % CO_2 (fig.8). This increase is lower for the fruits stored in air. Concomitantly, the catalase (CAT) activity remains stable in the CO_2-treated fruits still 4d but decrease later (fig.9).
As regards the AsA-GSH regenerating system, the effects of the storage at high levels of CO_2 (5 %) were the following:
- a sharp increase (we noted a peak in activity at time 2 d) in the ascorbate peroxidase (APX) activity specific of the storage at high CO_2 level (fig.10)
- a general increase of the glutathione reductase (GR) activity in the CA stored fruits (fig.11).
Both these results are consistent with an idea of a regulation system involving the rapid oxidation of ascorbate and it regeneration through the AsA-GSH cycle. Further studies are needed to confirm this hypothesis and to determine the role of the peroxides and mainly of hydrogen peroxide (H_2O_2) in this system.
On an another way we also noted a rapid and significant increase of the lipoxygenase (LOX) activity in the CA stored fruits (fig.12). This increase paralleled those of the antioxidative metabolism previously described and can likely acts in synergy to cause peroxidative damage.

✣ Biochemical changes in stored fruits, long-term analysis:

- *Effects on polyamines*:

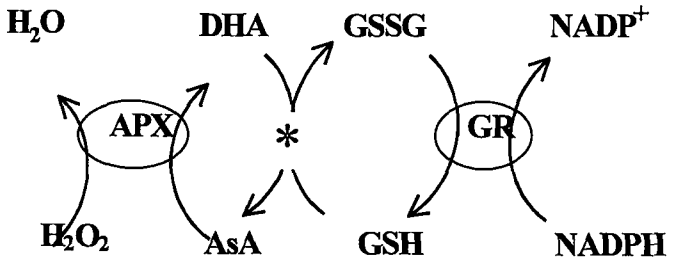
Data on polyamines are still in progress and will be presented later.

- *Effects on antioxidants and enzymes*:

Changes in ascorbate content followed 2 phases:

- a first phase for which the levels of ascorbate decrease sharply independently of the storage conditions (cf. short-term behaviour)
- a second phase for which the levels of ascorbate can be regenerated (fig.13). The regeneration depends directly of the storage atmosphere, the higher the CO_2 in the atmosphere, the lower the regenerative capability. In this way, **the fruits stored at 5 % CO_2 were unable to regenerate ascorbate**. This result is of great interest to explain the CO_2-related physiological disorders such as B.H

In order to understand this specific biochemical behaviour and to know why ascorbate could not be regenerated in the 5 % stored fruits, the research was focussed later on the AsA-GSH regenerating system which is the main way of regeneration of this compound (see schema).



Different parameters of this cycle were studied this year and the results were the following:

1- the 5 % stored fruits shown the same amount in glutathione after 6 months of storage than the controls (figure 14). In consequence, the difference in the regenerating capability cannot be explain by a difference in the amount of this antioxidant.

2- logically and as supposed at the view of this previous result, there were no differences between the different storage regimes in the GR activity (fig.15).

3- furthermore, there were no differences between the different storage regimes in the activity APX (fig.16) and the loss in the capability to regenerate ascorbate is not due to an increase in the activity of this enzyme.

Conclusions:

Where is the factor of regulation ? The more probable candidate will likely be found in the missing element marked with a * in the previous scheme: the deshydroascorbate reductase or DHAR. Further studies are needed and planned for the next year to confirm this new hypothesis.

Task 8: Non-destructive Measurements
Duration: 48 months
Current Status: 36 months to completion
Total estimated Man-month:
No. of man-month devoted already to the task:

Objectives

A complementary study has been carried out with damaged fruits. The objective of this study was to propose a non destructive tool which will permit detect BH disorder in Conference pear.

Material and methods

Quality parameters like weight, colour, firmness, soluble solids concentration and titrable acidity were measured individually from 45 fruits picked on the 24th of august 1998 and stored during 6 months at 2 % O_2 and 5 % CO_2. Simultaneously, the level of brown heart disorder was evaluated (from 0 to 60 % of damaged pulp) and fruits were classified into eatable (low level of damage, < 10 %) and non eatable fruits (high level of damage, > 10 %). Data on quality parameters and BH incidence were compared using a multivariate analysis procedure.

Results and Discussion

As we noted this year a very slight number of physiological disorders, multivariable analysis was carried out with a very limited sample. However, results were promising and were the following:

- a very poor linear correlation has been found between each quality parameter and the percentage of alteration
- as shown in figure 17, the model pointed out the importance of the non destructive parameters L*, a* and weight in the explanation of the percentage of damaged pulp
- relaxing the quantitative prediction, the information was used to built up a classification of the fruits into 2 classes (fig.18): low level of damage (<10%) and high level of damage (>10%)
- the distance between the two models was high enough to enable a classification of the samples to each group with only a 9 % of error (fig.19).

Conclusions

Though this method is less precise than for instance nuclear magnetic resonance detection, it gives an indication of the damage in the fruit with the advantage of being cheaper and easier to fit out. More experiments are needed with a larger sample size to improve the procedure and to assess precisely its accuracy.

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C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1998 TO 31-05-1999

In general:
A very slight incidence in BH disorder has been observed this year for all the experiments. As a consequence, the relationship between the different treatments and BH incidence could not be established.

Task 1,2:
- first year, season 97/98: experiments were carried out with 2 orchards and 3 harvest dates
- second year, season 98/99: experiments were carried out with 3 orchards and 2 harvest dates. This change which allows us to harmonize the cooling period prior to AC, did not affect the number of data for the validation of the model.

Task 5:
The experiment which concern the application of high CO_2 pretreatments has been realised this year. Results are encouraging and will be completed next year.

Task 7:
Results on the relationship between polyamines content and BH incidence remains unclear. Complementary studies are planned for the next year.

Task 9:
A new model using a multivariate analysis procedure has been carried out this year. This model, not planned in the technical annex, aimed to detect BH disorder in Conference pears. It differs from the general model whose objective is a prediction and not a detection of BH and will complement it.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1999 TO 31-05-2000

1- Some works and tasks will be stopped:
Task 1: the studies on N_2 fertilisation and calcium treatments will be stopped
Task 7: the relationship between BH disorder and the fermentative metabolism and between BH incidence and the activity of some enzymes such as the SOD, CAT and LOX will be stopped.

2- Others works will be completed or initiated:
- as previously shown in results, the fruits kept at 5 % CO_2 are unable to regenerate ascorbate. This specific behaviour is likely one of the more important result already obtained to explain this CO_2-related disorder. In consequence, emphasis will be given on this aspect and more precisely on the metabolic pathway regenerating the ascorbate: the AsA-GSH cycle. New methodology will be developed to analyse the levels of H_2O_2 and the activity of the deshydroascorbate reductase (DHAR) enzyme.
- others studies will be carried out to characterize the BH-damaged fruits. These studies will include both a biochemical analysis and a study on the relationship between the incidence in BH and the quality parameters able to detect BH.
- study of the relationship between BH and polyamine content will be continued. In complement, fruits will be treated with a potential activator of the synthesis of polyamines (algae cream,GA14) and the effect of this treatment on quality and BH incidence will be evaluated.
- high CO_2 treatments will be repeated in order to complement the results obtained this year.
- in collaboration with partner 1, experiments on energy metabolism and oxidative stress will be carried out in Wageningen.

E. DISSEMINATION

Papers

- Larrigaudiere C., I. Lentheric and M. Vendrell. 1998. Relationship between enzymatic browning and internal disorders in controlled-atmosphere stored pears. Journal of the Science of Food and Agriculture, 78(2): 232-237.
- Lentheric I., E. Pinto, M. Vendrell and C. Larrigaudiere. 1999. Harvest date affects the antioxidative systems in pear fruits. Journal of Horticultural Science and Biotechnology, 74 (6) – in press.

Presentations

- Larrigaudiere C., I. Lentheric and M. Vendrell. 1998. Late harvest induced changes in antioxidant defense mechanisms in Conference pears. 11th FESPP (Federation of European Societies of Plant Physiology) congress, Varna (Bulgaria), 7-11 September 1998. (Poster).
- Lentheric I., E. Pinto, C. Larrigaudiere., and I. Recasens. 1998. Effects of N_2 fertilization on storage, quality and brown heart incidence in Conference pears. Madrid98–COST915 conference "Physiological and technological aspects of gaseous and thermal treatments of fresh fruits and vegetables". 15-16 October 1998. (Poster).
- Lentheric I., E. Pinto and C. Larrigaudiere.. 1999. Multivariate analysis as a mean to detect brown heart in Conference pears. Workshop on optimal harvest date, St Remy de provence (France). Oral presentation .
- Lentheric I., E. Pinto and C. Larrigaudiere. 1999. Efecto a corto plazo de las concentraciones de CO_2 sobre el metabolismo antioxidativo de la pera Conference. Will be presented to the XIII reunión de la sociedad española de fisiología vegetal. 19-22 September 1999. Presentation of a poster.

F. ANNEX

	90 U	180 U	270 U
Ca	4,59	3,5	3,84
Mg	5,08	4,62	4,57
K	106,8	101,2	101,6
P	10,3	9,8	9,8
K/Ca	23,3	28,9	26,5

Table 1: Changes in the mineral composition of the pulp (in mg/100 g F.W) as a function of the N_2 fertilization strategy.

	Ca	Mg	K	K/Ca
Control	3,98	4,76	103,2	25,9
Seniphos	4,41	4,58	107,1	24,3
Stopit	4,29	4,66	96,6	22,5
Metasolate	4,19	4,67	102,2	24,4

Table 2: Changes in the mineral composition of the pulp of fruits treated with different calcium solutions.

	GIM	ALB	MOLL
	Harvest 1		
Firmness	14,9	14,5	14,9
SSC	11,9	11,1	12,1
Acidity	0,97	1,44	1,29
	Harvest 2		
Firmness	14,3	15,2	15,8
SSC	13,3	11,4	12,5
Acidity	1	1,25	1,05

Table 4: Changes in quality parameters in Conference pears treated with CO_2. Firmness is expressed in lb, SSC en % and acidity in g malic acid / l

	COLOR	FIRMNESS	SSC	ACIDITY
Control	-17,6	12,8	12,9	0,85
3d/10%	-12,4	13,8	14,1	0,66
5d/10%	-13,4	14,2	14	0,61
3d/15%	-12,5	15,2	14,2	0,62

Table 3: Changes in quality parameters in Conference pears harvested at 3 different orchards. Firmness is expressed in lb, SSC en % and acidity in g malic acid / l

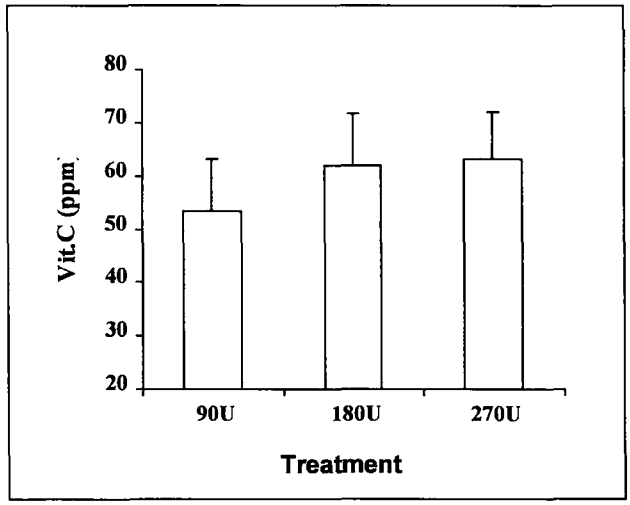


Figure 1: Change in the content in Vit.C as a function of the fertilization strategy.

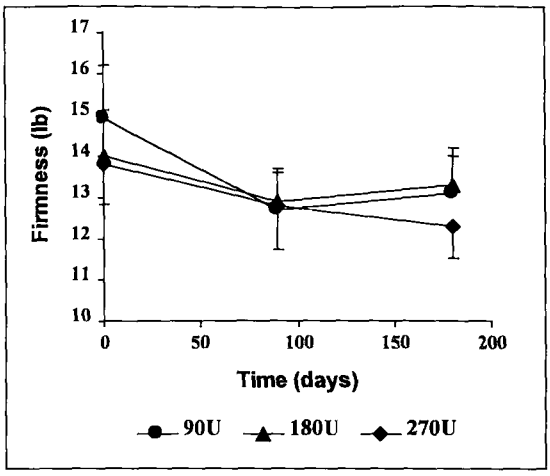


Figure 2: Changes in firmness during storage at 5 % CO_2 as a function of the fertilization strategy.

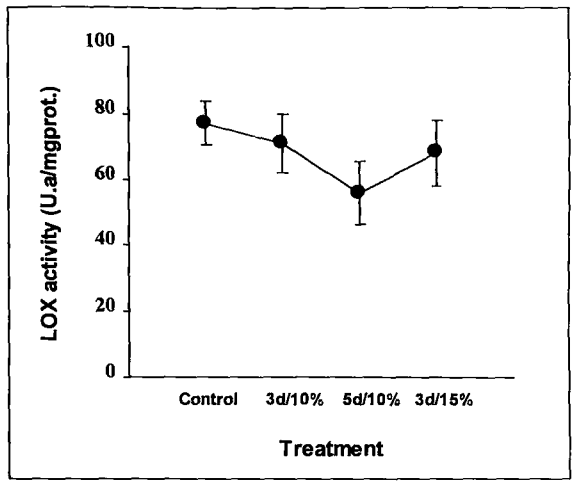


Figure 3: Changes in LOX activity in the CO_2-treated fruits immediately after treatment.

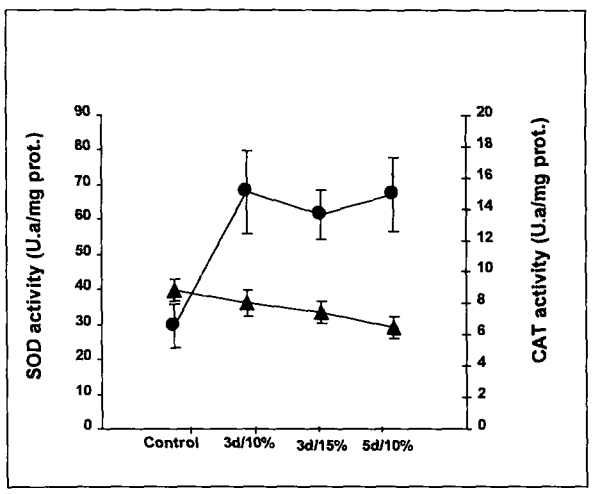


Figure 4: Changes in SOD and CAT activity in the CO_2-treated fruits immediately after treatment.

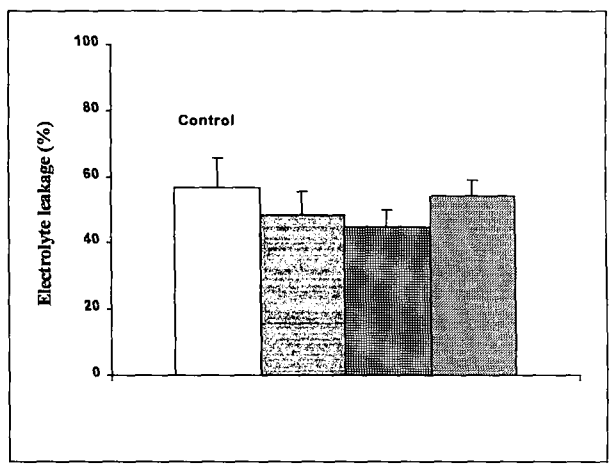


Figure 5: Effect of CO_2 pretreatment on electrolyte leakage after 6 months of storage at 5 % CO_2. Values are expressed as a percentage of total leakage.

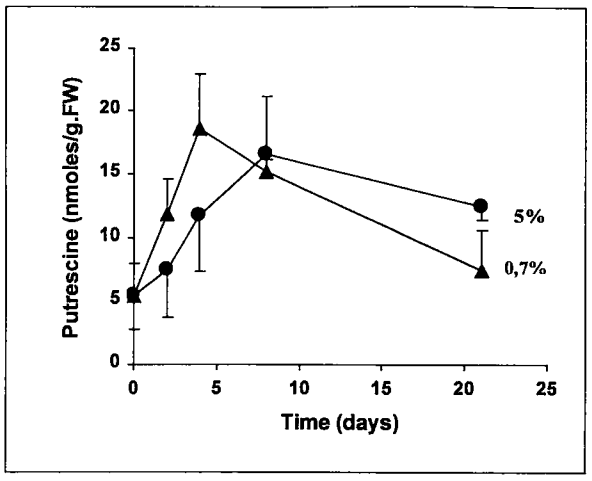


Figure 6: Short-term changes during storage at various CO_2 concentration in putrescine levels.

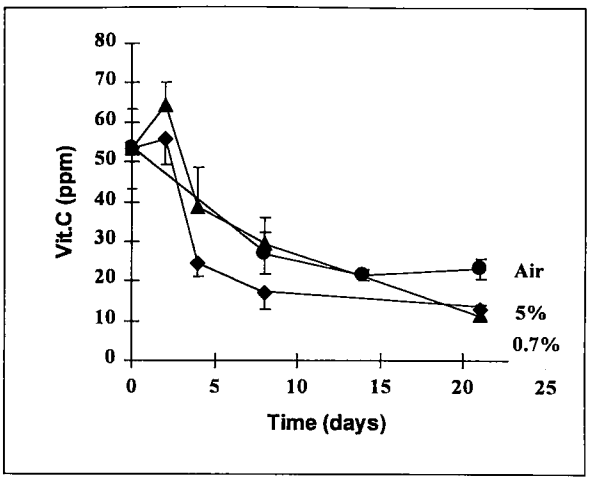


Figure 7: Changes in ascorbate content during the short-term storage of pears under various atmophere conditions.

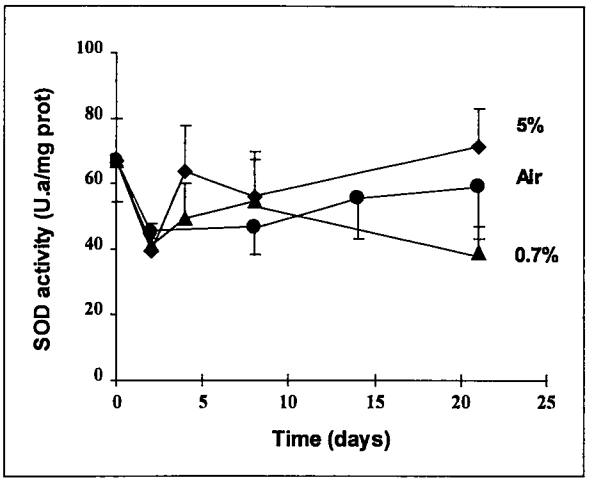


Figure 8: Effect of a short-term storage under various conditions of atmosphere on the activity of the superoxide dismutase (SOD) enzyme.

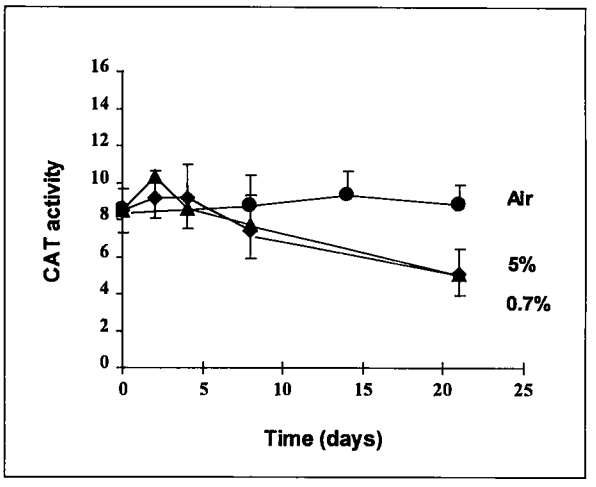


Figure 9: Effect of a short-term storage under various conditions of atmosphere on the activity of the catalase (CAT) enzyme.

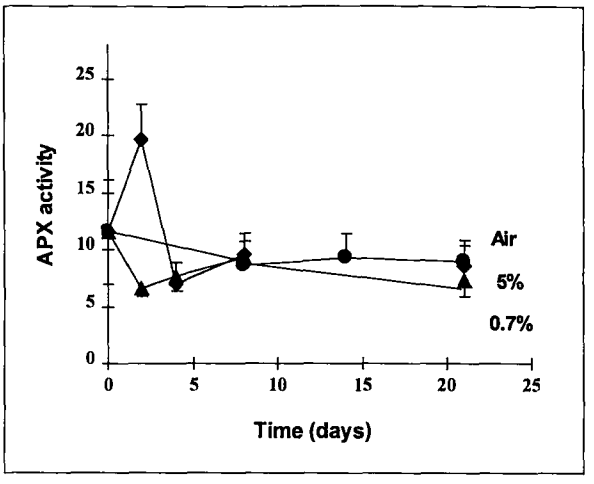


Figure 10: Effect of a short-term storage under various conditions of atmosphere on the activity of the ascorbate peroxidase (APX) enzyme.

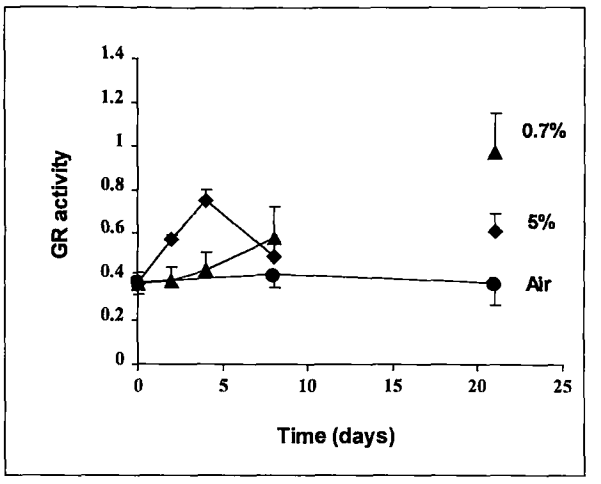


Figure 11: Effect of a short-term storage under various conditions of atmosphere on the activity of the glutathione reductase (GR) enzyme.

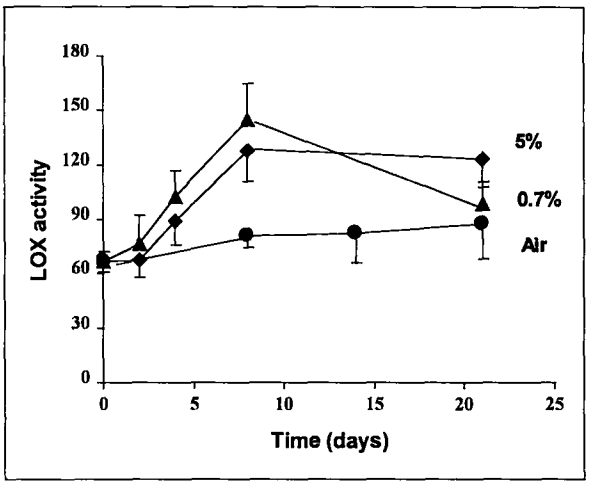


Figure 12: Effect of a short-term storage under various conditions of atmosphere on the activity of the lipoxygenase (LOX) enzyme.

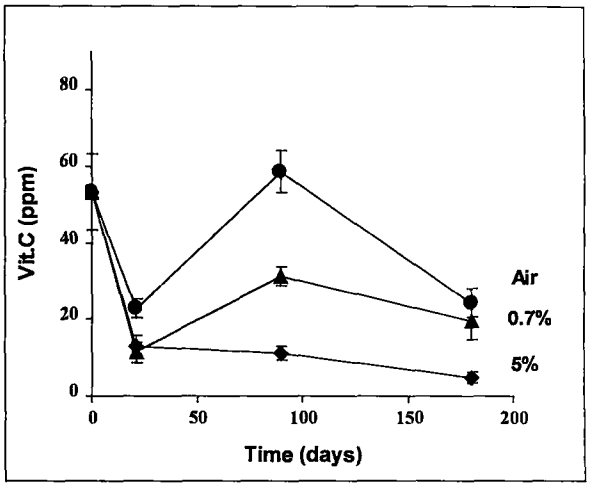


Figure 13: Changes in ascorbate content during storage in pears kept under various atmophere conditions.

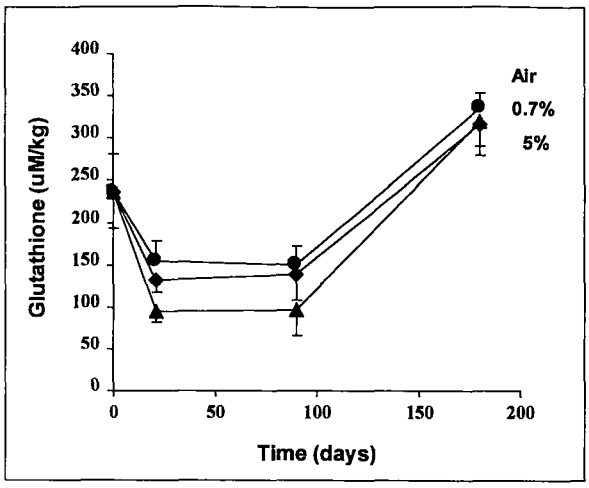


Figure 14: Changes in glutathione content during storage in pears kept under various atmophere conditions.

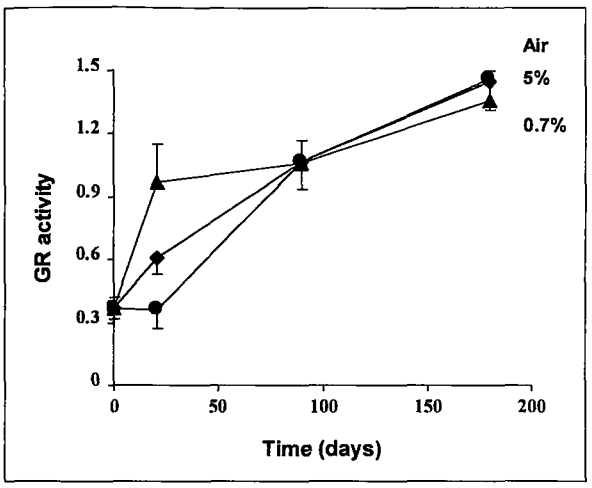


Figure 15: Effect of storage under various conditions of atmosphere on the activity of the glutathione reductase (GR) enzyme.

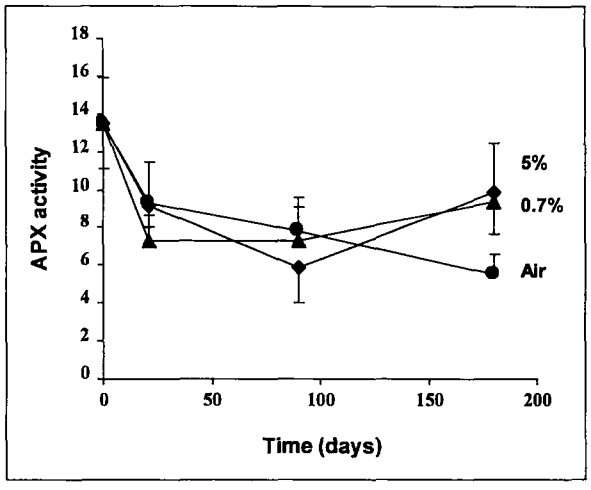


Figure 16: Effect of storage under various conditions of atmosphere on the activity of the ascorbate peroxidase (APX) enzyme.

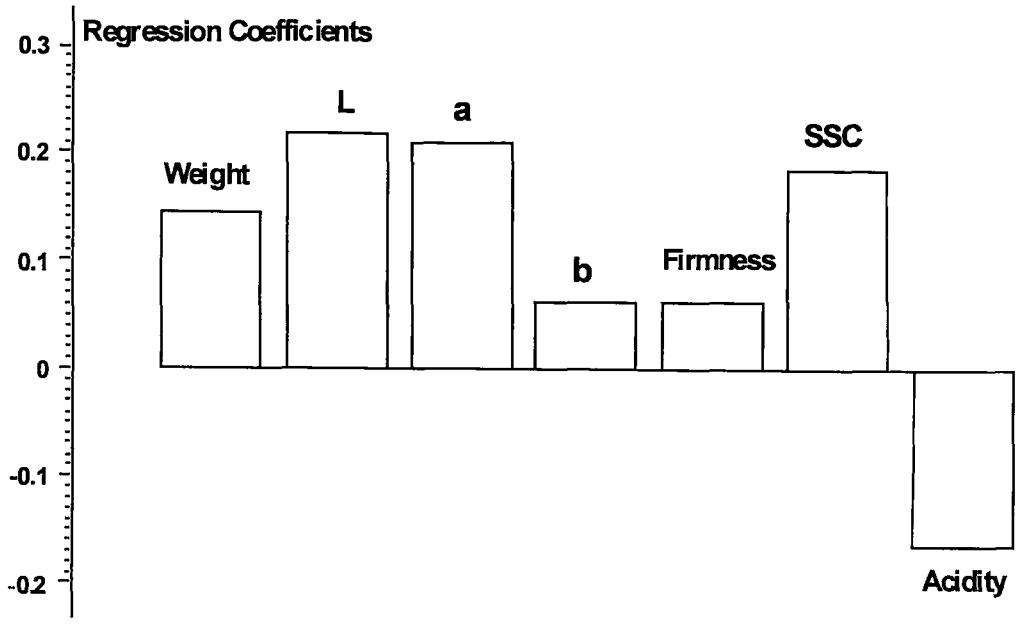


Figure 17: Multivariate analysis: regression coefficients between quality parameters and disorder percentage.

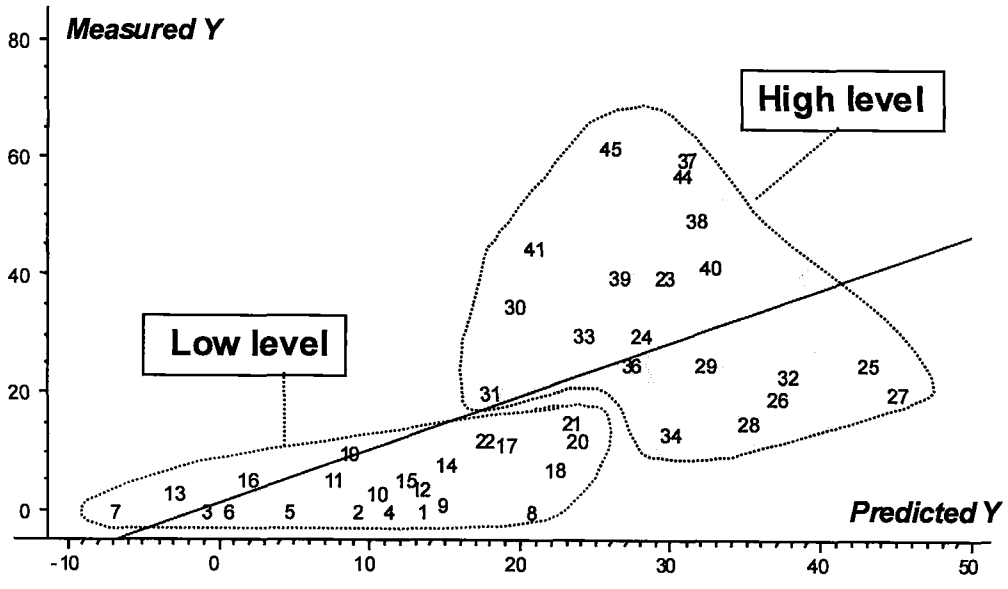
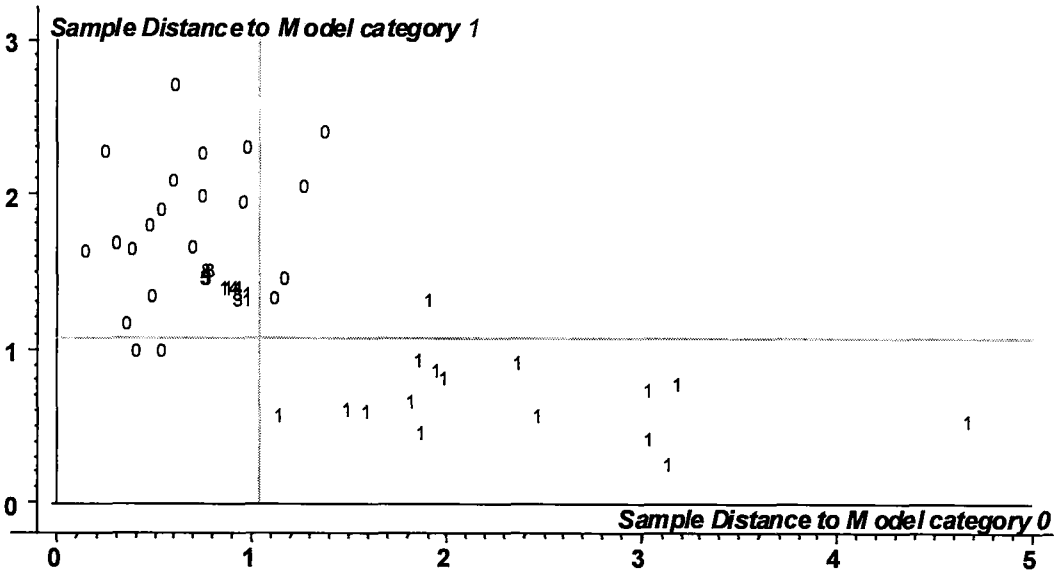


Figure 18: Multivariate analysis: plot of the y predicted versus the y measured



Significance = 5.0%, Model 1: category 0, Model 2: category 1

Wrongly classified: low level: 1 / 22
high level: 3 / 23
no classified: 7 / 45

Figure 19: Multivariate analysis: sample distances to the model.

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 3 total cost: (100 %)	127,2 kECU	*EC contribution to partner no. 3:*	127,2	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

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Contractor

A. PARTNER INFORMATION

Name and address
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Scientific team
Dr. Paola Eccher Zerbini, Dr. Alberto Pianezzola, Dr. Anna Rizzolo, Dr.Ada Brambilla, Maurizio Grassi, Gianni De Colellis.

Objectives
The main objective of the project is the optimization of the quality of Conference pears and the reduction of losses during storage by preventing the development of disorders resulting in Brown Heart. Brown Heart is a disorder related to controlled atmosphere storage, which causes problems throughout Europe, but more severely and more often in North-Werstern countries than in Southern countries. Comparison of pears grown in both regions, in different climates, should give information about the causes of the disorder. Prevention of the disorder should be achieved by development of technology which enables a rapid measurement and decision about optimal storage conditions.
The main objectives of the project are:
1. development of a predictive model based on registrated variances in orchard and weather conditions ;
2. development of a predictive model based on a rapid assessment of pear physiology (respiration rate, fermentation rate, resistance to gas diffusion)
3. testing and implementation of post-harvest treatments, which improve the storeability of pears, leading to pears of a better quality.

Actions in the project
Research work at IVTPA is focused on the relationships between orchard factors, pear physiology and storage disorders. The work programme includes:
Task 1 - selecting orchards;
Task 2 - picking fruit at different dates;
Task 3 - storing in experimental CA containers;
Task 4 - recording meteorological variables and agronomical features in selected orchards;
Task 6 - measuring gas exchange in pears at harvest and during storage.
Task 7 - analysis of fruit at harvest to evaluate fruit maturity;
- analysis of fruit after storage to evaluate quality by common physical and chemical analysis;
- control of the occurrence of storage disorders;
- biochemical analysis of antioxidants in the pear tissue.

Task 10- the predictive models built with the data of all partners will be validated by comparison with storage results in commercial packinghouses.

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1998 TO 31-05-1999

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	0.3
No. of man-month devoted already to the task:	0.3

Objectives
The development of a predictive model requires fruits of known history and growing conditions.

Material and methods

Orchard. The orchard selected (Malaguti) was one of the two orchards used in the previous year. The orchard is located in a pear growing region (Campogalliano, in Modena province).
Planting year: 1985. Rootstock: MA. Training system: palmetta. The trees were planted originally at a distance of 3.3 x 1.5 m, but in 1987 new trees were planted in the interspace on the row, obtaining distances of 3.3 x 0.75 m (4040 trees/Ha). Only the older trees were used for the research. Full bloom occurred on 2 April 1998. No spring frost occurred, but the flowering was scattered over a long period. The orchard was irrigated one time during the season. Pesticide treatments followed the instructions of the local "Phytosanitary defense plan". No treatment was made with growth regulators.

Trees. In the orchard two rows of 35 trees were selected and considered as blocks.
On each tree the number of fruits was counted.

Results and Discussion

Yield capacity. On the average each tree produced 101 fruits (15.2 standard dev.) with an average yield of 21.97 kg (4.6 std. dev.).

Task 2: Harvest of the pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	1.6
No. of man-month devoted already to the task:	0.8

Objectives
The development of a predictive model requires fruits of known maturity; from previous experience fruits of different maturity stages have different susceptibility to brown heart.

Material and methods
In the orchard two rows were selected and considered as blocks (subsets). In each block 30 average vigour, 13-years-old trees were selected for harvesting pears at different times. Pears were harvested on 10, 17 and 24 August 1997. Commercial harvest of Conference pears began on the 17th August. About 60 fruits per tree were picked from 10 trees at each harvest time for Tasks 3, 6 and 7, and were randomized between different controlled atmosphere treatments and different times of analysis.
The day after harvest a sample of 20 fruits per treatment was analysed for quality and maturity parameters. Fruit weight, colour (Minolta CR-200), firmness (plunger 8 mm diameter, Instron crosshead speed 200 mm/min), starch hydrolysis as % white section of the fruit, soluble solids by refractometric index (R.I) and titratable acidity of juice were measured, and Streif and deJager maturity indexes were computed (deJager, 1996).

Results and Discussion

Harvest date. With delaying harvest time significant differences were found in fruit weight (about +20% from first to third harvest), in hue of fruit colour (decrease of green hue) and in soluble solids (Table 1). As regards firmness, starch hydrolysis and Streif and deJager indexes of maturity, only the fruits of the first harvest were significantly different from the fruits harvested later, and no difference

was significant between fruits from the 2nd and 3rd harvest. The values of the parameters found in the second year are very similar to those found in the first year of experiment, indicating that the pears were picked at a comparable stage of maturity.

Task 3: Storage of the pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	3.6
No. of man-month devoted already to the task:	1.6

Objectives

The development of a predictive model requires to store fruits in different known controlled armosphere conditions, which are likely to favour (high CO_2) or to prevent (low CO_2, delayed CA) the Brown Heart disorder.

Material and methods

Harvest experiment. After harvest 4 boxes per harvest were stored at IVTPA in controlled atmosphere containers with 2 % oxygen and 0.7 or 5 % CO_2, (pull-down=2.5 % O_2) at 0/-1 C after cooling for one week. These fruits were examined in January and in March.
Four more boxes per harvest were also stored in two commercial stores. In one of them (Campofrigo) 2 boxes per harvest were stored in CA with 4.5 % O_2 + 1.5 % CO_2 until 1 February 1999, then in air for 4 weeks. In the other store (Eurofrutta) 2 boxes per harvest were stored in CA with 1.4% O_2 + 2.4% CO_2 until 10 December, then in 3% O_2 + 2% CO_2 until 18 February, then in air for 2 weeks. In both stores pears were cooled at -1 C for 1 week before storing in CA.

Position experiment. Fruits picked at the optimal harvest date (second harvest) from the high and low branches of the trees (see Task 4) were stored at IVTPA in controlled atmosphere containers with 2 % oxygen and 0.7 or 5 % CO_2, (pull-down=2.5 % O_2) at 0/-1 C (2 boxes per position).

Delayed CA experiment. In the Eurofrutta store, 2 boxes per harvest were cooled for 5 weeks before storage in CA as above.
A small delayed CA experiment was performed also at IVTPA on third harvest fruits. One box of fruits from the 3rd harvest was stored in 2 % oxygen and 0.7 or 5 % CO_2 after cooling for 5 weeks.

Results and Discussion

Gas exchange rates (Task 6) were measured and samples for vitamin C analysis (Task 7) were prepared in October, in December and in April with fruits stored at IVTPA. Quality parameters and disorders of stored fruits were examined in January and in March (Task 7).

Task 4: Variation in climate and orchard characteristics

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	0.5
No. of man-month devoted already to the task:	0.3

Objectives

The development of a predictive model requires to know the variations in climate during the growing season among the different orchards and countries, and the effect of orchard factors. Fruits in different positions on the tree seem to be differently susceptible to the disorder, so fruits at the optimum harvest date were picked separately from top and bottom of the tree.

Material and methods

Meteorological data. Minimum, maximum and average temperature and relative humidity at a height of 2 m and global radiation (daily integral of energy flux in the 0.3-3000 nm range with a solarimeter Kipp & Zonen) as MJ/m^2 were recorded from March to August 1998 by the Osservatorio Geofisico del Dipartimento di Scienze dell'Ingegneria, Università di Modena (Geophysical Observatory of the Engineering Sciences Dept. of the University of Modena) in a meteorological station located at Marzaglia, at 3 km distance from the orchard. The geographical coordinates of the orchard are: 44° 42' N 10°50'E.
The meteorological data were obtained free of charge for scientific use, provided that the institution is mentioned when publishing the results.

Orchard effects: position on the tree. At the second harvest (17/8/98), considered as optimum, fruits were picked separately from the high and the low part of 5 trees per block, and randomized between the two controlled atmosphere treatments at IVTPA (8 boxes in total). High position was from 2 m to about 3.2 m (top of trees), and low position was below 1.4 m from the ground.

Results and Discussion

Position. The weight of fruit, the colour hue and L, s.s. content, Streif and deJager indexes were all affected by position, with the fruits in top position showing values corresponding to more advanced maturity (Table 2). The advance in maturity was of about one week, if compared to the values of the harvest date experiment (Table 1).

Meteorological data. In July and until mid August the temperature was very high. Average daily data are reported in Table 3.

Task 6: Measuring gas exchange and diffusion rates

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	24
No. of man-month devoted already to the task:	20

Objectives
The development of a predictive model requires the assessment of pear physiology related to gas exchange and diffusion rates in fruits with different susceptibility to brown heart.

Material and methods

Fruits. Groups of fruits of the same size and colour were picked from the same tree for gas exchange measurements in different oxygen and CO_2 concentrations.
Gas exchange and diffusion rates were measured on fruits from the three harvest times, immediately after harvest and after 2 and 4 months in storage at 2 % O_2 and 0.7 or 5 % CO_2. Two months in storage correspond to 8 weeks for 1st harvest fruits, and 6 weeks for 3rd harvest fruits.
Gas exchange and diffusion rates were also measured after 5 week storage on fruits of the 3rd harvest cooled for 1 or 5 weeks at -1/0 C (delay experiment) and in April after 8 month storage in high or low CO_2.
At harvest and after 2 and 4 months of storage internal gas concentration was measured in pears immediately after the gas exchange measurement, then the weight, length, diameter and volume of the fruit and the juice density were measured, and the internal space volume was computed according to the method of Baumann and Henze (1983). The pears were cut and examined for brown heart.

In April a different method for internal gas analysis was used, which was destructive, so the measure of density and of internal space volume of the same fruits was no more possible.

Diffusion resistance measurement. The method used is the linear one described by Banks (1985) as modified by Peppelenbos in the first Europear report. All the experiment, except GC analysis which were made at room temperature, was carried out at -1 C. Fruits were put in 1700 ml glass jars (one fruit per jar) and flushed with a known mixture of nitrogen, oxygen and carbon dioxide, then the jar was closed and 7 ml Neon were added in the jar. The concentration of Neon was measured in the jar after overnight equilibration (16-20 hours). Then the measurement of diffusion was carried out: the fruit was taken out of the jar, a stopwatch was triggered on, the fruit was left for 3-4 seconds in front of a fan then it was quickly put in a new jar with air, and the latter was taken out of the cold room to the GC for the analysis of headspace Ne diffusing out of the fruit. The GC analysis was carried out 4 times within 7-8 minutes from the opening of the jar with Ne. Number of replications was 2. Diffusion resistance for neon was computed according to Banks (1985).

Gas exchange measurements.
Pears were put in 1700 ml glass jars (one pear per jar) and the jar was flushed at 110 l/h for 10 min with a known gas mixture. Each day the atmosphere was renewed. The fruit equilibrated in the gas mixture for four days, except for the time (about 10 minutes) necessary for the diffusion measurement on the second day. On the third day the jar was flushed for the last time with the gas mixture, and after 24 hours the gas in the headspace was analyzed with GC for O_2 consumed and CO_2 produced. All the experiment, except GC analysis (10 min for diffusion, 5 min for gas exchange), was carried out at -1 C. The fruits measured at harvest were cooled to -1 C overnight before flushing with gas mixtures.
Two jars (replications) were used for each measurement.
O_2 consumption and CO_2 production rates were expressed as nmol/kg/s in standard conditions, considering the volume of the jar and the volume and weight of the fruit, correcting for actual values of temperature in the cold room and of pressure in the jar.
Gas mixtures used for respiration measurements were: 100% N_2 , oxygen at 0.5, 2 and 21% with low (0 or 0.7%) or high (5%) CO_2 concentration. The mixtures with 0.5% O_2 were used only after 4 month storage. The mixtures had been prepared in cylinders, which were kept in the storage room, i.e. at -1 C.

Internal gas concentration measurement.
At harvest and after 2 and 4 months of storage, the pear was taken out of the jar immediately after gas exchange measurement, and put underwater in order to limit gas exchange with air. Internal atmosphere was sampled by inserting the Luer probe with a side hole fitted to the gas-tight syringe near the calyx end of the fruit and extracting the internal atmosphere from the locular space while keeping the fruit underwater. After having locked the gas sample in the syringe using the valve, the sample was injected in the GC by removing the probe from the syringe.
The expected internal gas concentration was also computed for each pear from diffusion resistance found for Ne, corrected for O_2 and CO_2 according to Graham's law, and from gas exchange results, knowing the dimensions of the fruit and of the jar.
In April a different method was used to extract internal atmosphere from the fruit, following the suggestion of Streif. The internal atmosphere was extracted with vacuum, while the pear was under water. Normal air pressure was restored before sampling and analysing the internal gas extracted.
Internal gas concentration was also computed, based on Fick's law and on diffusion resistance measured for Ne and corrected for O_2 and CO_2 according to Graham's law.

GC analysis.
Neon, O_2 and CO_2 concentrations in the headspace were analyzed with a MicroGC MTI model P-200 (Hewlett-Packard) equipped with a molecular sieve column for permanent gases and an OV-1 column for CO_2.. In the molecular sieve column, O_2 and Ar have the same retention time. Argon was only present in the air mixture (21% O_2 + 0% CO_2), and it was added to O_2 in the calibration.
The Micro GC was new and some time was spent to set up the methods for gas analysis.

Gas samples of internal fruit atmosphere were analyzed by a GC Dani, model 86.10, equipped with a 100 µl loop injection valve and a TCD; CO_2, O_2 and N_2 were analyzed in a single run within 5 min using He as carrier gas with a 6 ft CTR-I column (Alltech) consisting of two concentric columns; the outer column (i.d. 1/4") is packed with activated molecular sieve, and the inner column (i.d. 1/8") is packed with porous polymer mixture.

Statistical analysis. The data were processed by analysis of variance using the GLM procedure of SAS System. Non linear models (Hertog et al., 1998) were fitted to gas exchange data (O_2 consumed and CO_2 produced) in function of O_2 and CO_2 partial pressure. The software used for non linear modelling was the NLIN procedure of the SAS/STAT package.

Results

Gas exchange measurements.
Modelling gas exchange rates (Table 4) was quite successful for O_2 uptake: a model with noncompetitive inhibition by CO_2 was best fitted in all cases except for fruits measured in October, after 2 month storage, where no inhibition by CO_2 was found by non linear modelling. For CO_2 production, modelling was not very successful: in many cases the model with inhibition of fermentation by O_2 could not be fitted to the data.
Parameters estimated by modelling are reported in Tables 5-8. Where maximum O_2 uptake (VmO_2) or maximum fermentative CO_2 production ($VmfCO_2$) could not be estimated by fitting the model, the original data respectively of O_2 uptake in air or of CO_2 production in N_2 atmosphere are reported.
Oxygen uptake at harvest decreased with later harvests, while RQox increased, indicating a slight increase in CO_2 production by respiration with later harvesting, but almost no difference in fermentative CO_2 production (Table 5). Delaying CA by 5 weeks instead of the normal 1 week increased both O_2 uptake (Figure 1) and CO_2 production; this effect was less evident but still visible after 8 month storage, especially on fermentative CO_2 production (Table 6). Gas exchange rates during storage were lower than at harvest, but there was not a clear trend with time (Table 7). At every time O_2 uptake and fermentative CO_2 production of fruits kept in low CO_2 atmosphere were higher than those of fruits kept in 5% CO_2 (Table 8). However RQox in fruits kept in low CO_2 and examined after 4 months is very low, indicating perhaps some problems in the analysis.

Diffusion resistance.
Diffusion resistance was very variable between fruits.
No difference was found in 3rd harvest fruits cooled for 5 weeks as compared to those measured at harvest. In December a significant difference was found between fruits of different harvests: the later the harvest, the higher the resistance to diffusion (Figure 2).
Diffusion resistance increased linearly during storage.
If these results are confirmed and, according to Graham's law, diffusion resistance of O_2 and CO_2 is proportional to that found for neon, it means that in late harvested, mature fruits after a certain storage time, diffusion resistances to O_2 and CO_2 can reach very high values so that the entry of oxygen in the fruit from external atmosphere and the diffusion of CO_2 out of the fruit could be hindered. These results are consistent with the fact that 3rd harvest fruits are more susceptible to brown heart, and that brown heart develops after some time in storage.

Internal gas concentration.
At harvest the difference between the computed internal and the external gas concentrations increased with harvest time both for O_2 and for CO_2. The same difference was higher in fruits of the 3rd harvest kept in air (delayed CA) than in those kept in 2% O_2 after 5 week storage (Figure 3), and also after 8 month storage (data not shown).
In the analyses made with the first method, the average sampled volume of internal gas was 0.91 ml, with a range from 0.2 to 3.5 ml. Often the volume was insufficient to flush the GC. Almost all the analysis of internal atmosphere resulted in high O_2 and low CO_2 concentration, indicating that air had entered between the GC and the syringe so diluting the sample atmosphere.

By extracting the internal atmosphere with the second method (under vacuum), both internal measured CO_2 and O_2 were higher than that computed acording to Fick's law and diffusion resistance measured for each fruit (Figure 4). To explain this difference, solubility of gases in the fruit cells was taken into account, with the hypothesis that, given a normal fruit (Table 9), a part of the gas solubilized in the cells could volatilize and be added to the internal gas atmosphere extracted under vacuum. By simulating different amounts of solubilized gas coming out of solution, with the hypothesis of 3% of total gas solubilized in the cells coming out with extraction, the simulated internal concentration of CO_2 and N_2 was similar to that measured, but the simulation could not explain the O_2 concentration higher than expected, and even higher than normal concentration in air, also taking into account the presence of Argon. Phosynthesis was also considered, measuring fruits in the dark, and internal atmosphere of sponge balls was also measured and compared to that of pears (Table 10).
Further research is needed on this point, and a better sampling method for internal gas analysis should be found.

Task 7: Destructive measurements (7a Quality evaluation, 7b Biochemical studies)

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	22
No. of man-month devoted already to the task:	12.6

Objectives

The development of a predictive model requires the assessment of pear physiology and the evaluation of the quality of fruit after storage. Vitamin C seems to be related to the appearance of brown heart, so it has been included in the biochemical analysis.

Material and methods

Quality analysis of fruit.

On 20 fruits per treatment (harvest, position, storage conditions and storage time) the following measurements were made on the day after the end of storage: background colour of the skin (Minolta CR-200) on the largest and greenest part of the fruit; largest diameter; height; weight; firmness with 8 mm plunger (Instron UTM); soluble solids and titratable acidity.

Brown heart and storage disorders.

Fruits from different harvest time, storage type and storage time were cut and examined for brown heart and other storage disorders in January and March. Fruits used for gas exchange measurements, stored in 2% O_2 and high or low CO_2 were examined for brown heart presence in October, December and April.

Biochemical studies: ascorbic acid content.

For ascorbic acid determination fruits were sampled at the same time of gas exchange measurements. So fruits of the three harvest times were sampled at harvest and after 2 , 4 and 8 months in storage at 2 % O_2 + 0.7 or 5 % CO_2. Fruits of the 3rd harvest cooled for 5 weeks in air at -1/0 C (delay experiment) were sampled after 5 week storage and again in April after 8 month storage. Fruits of the position experiment (high or low on the tree) from the second harvest were sampled only in April. Each sample (one replication) was made up of five fruits; two 5-pear replications were prepared per treatment. The inner and the outer part of the pulp (without peel and without core) were separated.
Using a routine sampling and extraction procedure (slicing the fruit in thin slices before freezing in liquid nitrogen, storing the samples at -20 C, thawing the sample at the beginning of the extraction procedure, and not protecting the tubes from light) no detectable amount of ascorbic acid was found in the extracts of samples collected at harvest, in October and December. The extraction was carried out at the beginning of January.

Both sampling and extraction procedures underwent a series of successive improvements. In April a final methodology was set up for AA determination in pears.
Taking into consideration the results obtained with a number of comparison tests between different procedures, with recovery tests and with stability tests, the following protocol was adopted for samples collected in April, and each extraction was coupled with a recovery test. Recovery of AA is 70-95%.
AA in MPA extracts is quite stable over a 72 hour period at 2 C.

a. Sampling
Four cylinders per pear were sampled at room temperature in the equatorial zone of the fruit using a brass cork borer with a 17 mm inner diameter. Then each cylinder was cut into two parts: the outer part was the 1-cm layer just under the peel and the inner part the remaining part of the cylinder. Each sample, made up of five cylinders of pulp, each one taken from a different fruit, was frozen in liquid nitrogen into a tulle little bag and then stored at -80°C until the analysis. In this way, four replicated ready-to-use samples were available for each five-pear pool.

b Extraction:
All the operations were performed on ice and under reduced light. 3-5 g of crushed frozen sample was weighted into a 90-ml centrifuge tube wrapped with black polyethylene film in the presence of 15 ml of 6% MPA; then the sample was homogenized using an Ultra-Turrax and the centrifuge tube sealed with a black cap; the suspension was centrifuged at 6000 rpm for 15 minutes and the extract was transferred into a 50-ml volumetric flask wrapped with alluminium foil. The procedure was followed through once more and the second extract was combined with the first and the volume was brought to the mark with 6% MPA. The extracts were kept at 2°C until HPLC analyses, which were performed overnight (within 3-4 hours from the end of the extraction procedure) using an automatic sampler. Before HPLC analysis the extracts were filtered through 0.45 μm Nylon 66 membrane with a 1 μm glass wool pre-filter unit.

c. HPLC separation:
A BIP-I liquid chromatographic pump (Jasco) fitted with a UVIDEC - 100V UV spectrophotometer (Jasco), a AS-1555 intelligent autosampler (Jasco) and a CR6A Chromatopac data processor (Shimadzu) were used.
The separation was achieved at room temperature according to the following conditions:
column: Inertsil ODS-3 (4.6 x 250 mm) GL Science; mobile phase: H_3PO_4 0.02 M; flow: 0.7 ml min^{-1}: ; detection: 254 nm at 0.02 AUFS; sample: 5-20 μl using the auto-sampler.

Results and Discussion

Brown heart and storage disorders.
According to the results of the last year, the early symptoms of brown heart were shown already in December. Brown heart was almost absent in pears picked in 1998. At the end of storage, even in the most susceptible treatments (late harvest, high CO2 storage), the incidence of BH was very low and not significant. Only the position of fruit on the tree had a significant effect on BH: fruits picked from high branches were significantly more affected by BH than those picked from low branches (Table 11).

Quality analysis of fruit.
After 5 and 7 month storage the results of quality analysis were similar. The effect of harvest time was significant on the colour of fruit (second harvest fruits were the greenest), on firmness and acidity (decreasing with harvest time) and on soluble solids content (highest in 3rd harvest fruits) (Figure 5).
The effect of the position on the tree, after 7 months storage, repeated that recorded at harvest: fruits from high branches were more yellow and had more soluble solids, as at harvest, but they kept more acid, although at harvest they were less acid (Figure 6).
As regards storage atmosphere, high CO2 compared to low CO2 (fruits stored at IVTPA), and low O2 compared to high O2 (fruits stored in commercial stores) kept the pears less yellow (colour coordinate

b) (Figure 7). The effect on firmness was not clear. Acidity was higher in low O2 as compared to high O2.
Fruits from the 3rd harvest subjected to a delay of 5 weeks in CA were more yellow and had more soluble solids and less acidity after storage at IVTPA (Figure 8) but the effect was less evident in fruits stored in the commercial store Eurofrutta (Figure 7).

Biochemical studies: ascorbic acid content.
After 8 month storage, ascorbic acid content increased with harvest especially in the outer part of fruit, but in fruits of the third harvest the amount of AA found in the inner part of the pulp was equal to that of the outer part (Table 12).
A strong influence of the storage atmosphere was found (Table 13). The higher the CO_2 percentage, the lower the ascorbic acid content, whatever the harvest date. This fact is also confirmed by the results obtained in the trial concerning the influence of the position on the tree, carried out at the second harvest time, and by those obtained in the delay experiment, carried out on fruit at the third harvest (Table 14).
As for the position experiment, fruit in the "high" position had significantly higher amount of ascorbic acid compared to the corresponding fruit in the "low" position only if stored in 0.7% CO_2. The delay treatment had no effect on ascorbic acid content after eight months of storage (Table 14).
The storage atmosphere influenced also the distribution of AA in the fruit (Table 15). On the average the 0.7% CO_2 stored fruit had higher AA in the outer part, while no significant difference was found between the outer and inner portions of the pulp in the 5% CO_2 stored fruit.
Taking into consideration the differences of AA content between the outer and inner part of the pulp in the different treatments (Figure 9) the difference was positive (i.e. higher AA content in outer than in inner part) in fruits stored in 0.7% CO_2, and negative (i.e. higher AA content in inner than in outer part) in fruits stored in 5% CO_2, with the only exception of fruits from low position on the tree. This result could be due perhaps to a different gradient in CO_2 concentration within the fruit, depending on CO_2 diffusion.

Task 10: Validation of the models

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	3
No. of man-month devoted already to the task:	0

Objectives
In the last two years of the project the models will be used to generate predictions about storage behaviour. Storage results will be compared with the predictions, which will be used for validating the models.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1998 TO 31-05-1999

Task 6.
The GC was changed so some time was necessary to learn how to use it, and much time was spent in checking the integration of the peaks.

Task 7.
The method used routinely at IVTPA for ascorbic acid determination had to be changed and adapted for pears.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1999 TO 31-05-2000

The planned activity for the next season will follow more or less the activity of the last year, with some important differences regarding Tasks 3, 6, and 7.

Task 3
Pears will be picked later in order to enhance susceptibility to brown Heart.

Task 6 and 7
More attention will be paid to the first 2 months after harvest.

Task 7
ATP and ADP analysis will be added if possible, in order to explain energy metabolism in connection with gas exchange measurements.

E. DISSEMINATION

It is planned to write a paper on "The influence of orchard management and storage conditions on fermentative metabolites and storage disorders of Conference pears".

F. ANNEX

Tables and figures are reported in the Annex.

Table 1. Quality and maturity parameters of Conference pears at harvest (1998).

harvest	weight (g)	L	hue (rad)	chroma	firmness (N)	Starch hydrolisys (%)	soluble solids (Brix)	titratable acidity (meq/100g juice)	n. of seeds	% fruits with seeds	Streif index	deJager index
1	192.5 b	59.62 a	2.010 a	38.08 a	70.8 a	5.5 b	11.8 b	2.75 a	1.00	65	0.612 a	6.122 a
2	219.2 ab	57.32 b	1.992 ab	36.92 a	64.7 b	13.5 a	12.3 ab	2.76 a	1.15	65	0.392 b	5.103 b
3	236.5 a	58.06 ab	1.963 b	36.99 a	66.7 b	14.3 a	12.8 a	2.53 a	1.30	80	0.446 b	5.057 b
average	216.1	58.33	1.989	37.33	67.4	11.1	12.3	2.68	1.15	70	0.483	5.427

Means followed by different letters are significantly different with P>0.05 (Tukey's test)

Table 2. Quality and maturity parameters of Conference pears picked in high or low position from the tree at optimum harvest date (1998).

position	weight (g)	L	hue (rad)	chroma	firmness (N)	starch hydrolisys (%)	soluble solids (Brix)	titratable acidity (meq/100g juice)	n. of seeds	% fruits with seeds	Streif index	deJager index
high	223.1 a	58.61 a	1.987 b	37.73 a	66.0 a	12.0 a	12.8 a	2.329 a	0.8	50	0.454 b	5.023 b
low	171.3 b	57.05 b	2.016 a	36.82 a	65.1 a	7.5 a	11.1 b	2.558 a	1.1	55	0.561 a	5.882 a
average	197.2	57.83	2.001	37.28	65.6	9.8	12.0	2.443	0.9	52.5	0.507	5.452

Means followed by different letters are significantly different with P>0.05 (Tukey's test)

Table 3. Mean values of daily average, maximum and minimum temperature, relative humidity, radiation, precipitation and sunshine hours in the orchard during the growing season.

	Tavg	Tmax	Tmin	RHavg	radiation	precipi-tation	sunshine
	°C	°C	°C	%	MJ/m2	mm	h
March 1998	6,9	14,8	-0,4	72,3	14,3	0,8	7,4
April	11,4	17,9	4,8	79,3	14,8	1,5	5,4
May	16,8	23,6	9,5	74,0	21,0	2,4	8,0
June	21,7	29,0	14,4	72,5	25,2	2,3	10,6
July	24,5	31,5	16,9	64,6	24,6	0,5	11,1
August	24,3	31,8	16,8	59,4	20,5	0,1	9,7

Table 4. Models for gas exchange (Hertog et al., 1998)

$VO_2 = \frac{Vm_{O2}.[O_2]}{Km_{O2} + [O_2]}$	O_2 uptake with no inhibition
$VO_2 = \frac{Vm_{O2}.[O_2]}{(Km_{O2} + [O_2])\,(1 + \frac{[CO_2]}{Kmn_{CO2}})}$	O_2 uptake with non-competitive inhibition
$VCO_2 = RQ_{ox} \cdot VO_2 + \frac{Vm_{CO2\,f}}{1 + \frac{[O_2]}{Kmc_{CO2\,f}}}$	CO_2 production

Table 5- Parameters estimated by nonlinear modelling of gas exchange data at harvest. For 1st and 2nd harvest a non-competitive model was fitted to the data.

	1st harvest		**2nd harvest**		**3st harvest**	
	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error
VmO_2	27.290	0.919	17.127	2.609	13.570*	
KmO_2	0.903	0.127	0.289	0.420		
$KmnCO_2$	36.305	12.703	18.235	16.58		
R^2adj	0.985		0.750			
$VmfCO_2$	16.4*		15.5*		17.3*	
RQox	0.63		1.11		1.35	

* original data - see text

Figure 1. Model of O_2 uptake in fruits stored without or with delayed CA (2% O_2 + 0.7% CO_2).

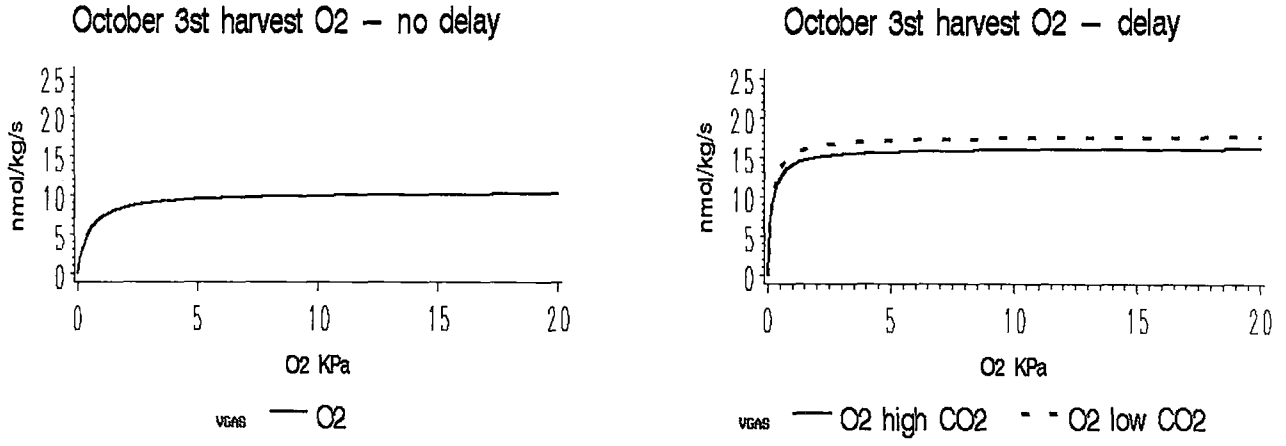


Table 6- Effect of delaying CA of 3rd harvest fruit on parameters estimated from gas exchange data after 5 weeks of air or CA storage, and after 8 months of CA storage (2% O_2 + 0.7% CO_2).

	after 5 weeks				after 8 months			
	no delay		**delay**		**no delay**		**delay**	
	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error
VmO_2	10.28	0.94	17.95	0.86	11.79	1.13	12.85	1.05
KmO_2	0.46	0.41	0.15	0.11	0.31	0.13	0.43	0.13
$KmnCO_2$	.		42.93	23.73	9.20	3.85	23.48	14.77
R^2adj	0.801		0.97		0.67		0.77	
$VmfCO_2$	14.9*		19.27	3.11	10.88	1.75	15.70	2.10
$KmfO_2$	.		0.03	0.07	0.06	0.04	0.08	0.04
R^2adj	.		0.38		0.58		0.37	
RQox	0.87		0.81		0.81		0.86	

* original data

Table 7- Effect of storage time on parameters estimated from gas exchange data of Conference pears.

	At harvest		**After 2 months storage**		**After 4 months storage**		**After 8 months storage** (only 3rd harvest fruits)	
	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error
VmO_2	18.84	1.84	9.18	0.53	18.94	1.07	12.33	0.80
KmO_2	0.43	0.28	0.57	0.27	0.89	0.20	0.38	0.10
$KmnCO_2$	38.09	38.84	.	.	35.12	21.01	13.90	4.90
R^2adj	0.71		0.74		0.78		0.70	
$VmfCO_2$	16.4*		12.2*		11.7*		13.05	1.41
$KmfCO_2$	.		.				0.07	0.03
R^2adj	.		.				0.42	
RQox	1.03		1.08		0.71		0.83	

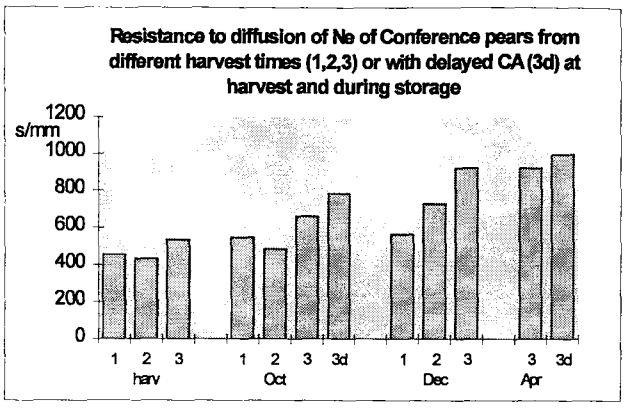
* original data

Table 8- Effect of storage conditions on parameters estimated from gas exchange data of Conference pears during storage.

	High CO_2 storage		**Low CO_2 storage**	
After 2 months	Estimate	Std. error	Estimate	Std. Error
VmO_2	8.52	0.38	9.97	1.05
KmO_2	0.55	0.22	0.64	0.47
R^2adj	0.91		0.65	
$VmfCO_2$	11.5*		12.8*	
Rqox	1.23		0.94	
After 4 months				
VmO_2	14.72	1.28	23.38	1.37
KmO_2	0.31	0.13	1.46	0.29
$KmnCO_2$	22.76	15.08	72.72	79.49
R^2adj	0.75		0.914	
$VmfCO_2$	10.83*		12.52*	
Rqox	0.71		0.38	
After 8 months				
VmO_2	11.56	1.05	12.99	1.03
KmO_2	0.47	0.16	0.29	0.10
$KmnCO_2$	11.44	4.97	18.00	9.26
R^2adj	0.71		0.77	
$VmfCO_2$	10.79	1.98	18.97	4.48
$KmfO_2$	0.12	0.07	0.02	0.01
R^2adj	0.32		0.50	
Rqox	0.84		0.82	

* original data

Figure 2. Resistance to diffusion of Ne out of Conference pears.



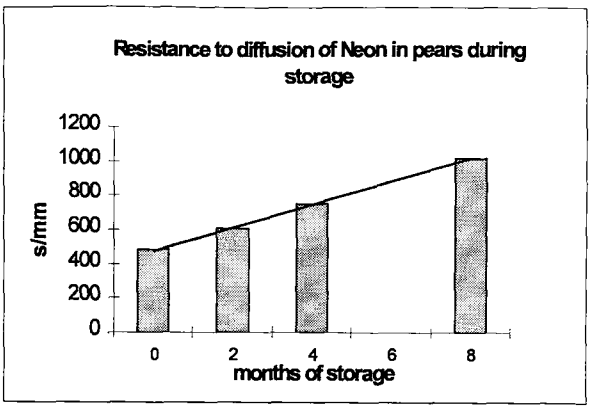
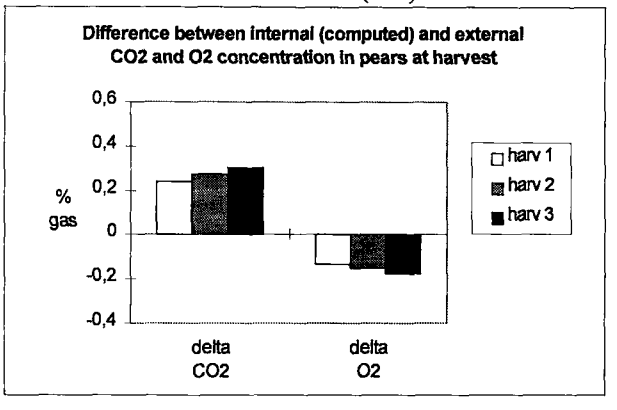


Figure 3. Differences between internal computed and external gas concentrations in Conference pears at harvest (left) and after 5 week storage (delay experiment, right).



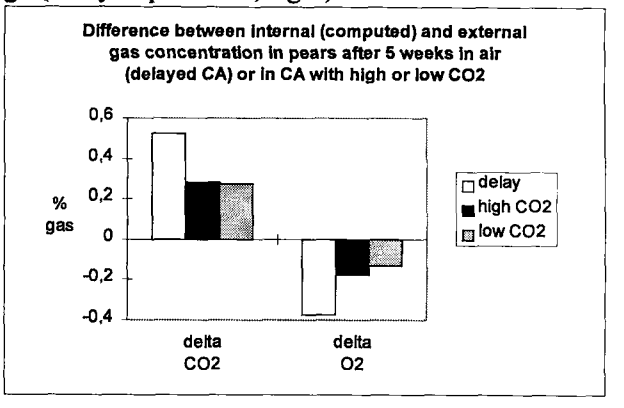
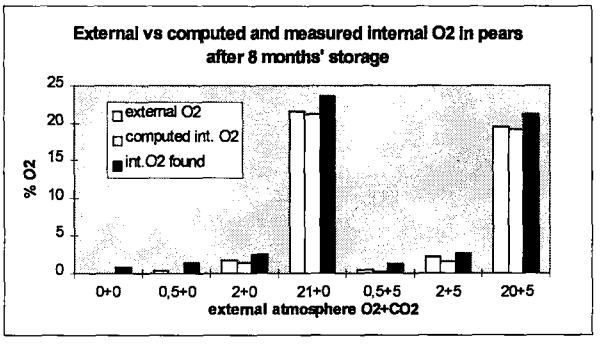


Figure 4. External, computed internal and measured internal O_2 (left) and CO_2 (right) concentrations in Conference pears.



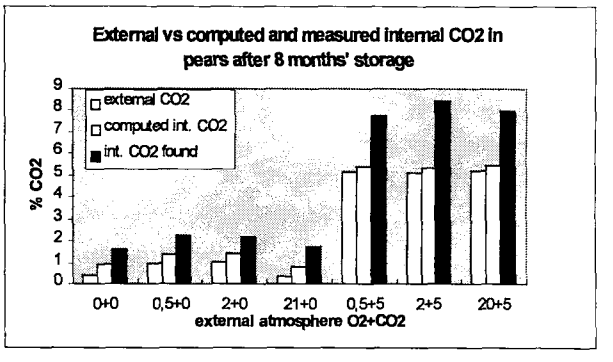


Table 9. Porosity of Conference pears

time of measurement	Relative Intercellular Space Volume %	Intercellular space volume ml	Fruit volume ml
	mean ± s.e.	mean ± s.e.	mean ± s.e.
October	5.1 ± 0.087	11.27 ± 0.32	219 ± 4.1
December	5.33 ± 0.094	10.77 ± 0.33	200 ± 3.6

Table 10. Measured external and internal atmosphere composition.

	$\%CO_2$	$\%O_2$	$\%N_2$
	mean ± s.e.	mean ± s.e.	mean ± s.e.
air	0.01 ± 0.01	21.38 ± 0.14	78.60 ±0.14
sponge balls	0.21 ± 0.08	21.34 ± 0.07	78.44 ±0.14
fruit in dark	1.39 ± 0.07	22.97 ± 0.08	75.64± 0.02
fruit in light	1.70 ± 0.15	23.61 ± 0.17	74.69 ± 0.30

Figure 5 - Effect of harvest time on maturity indexes at harvest and quality parameters after storage of Conference pears

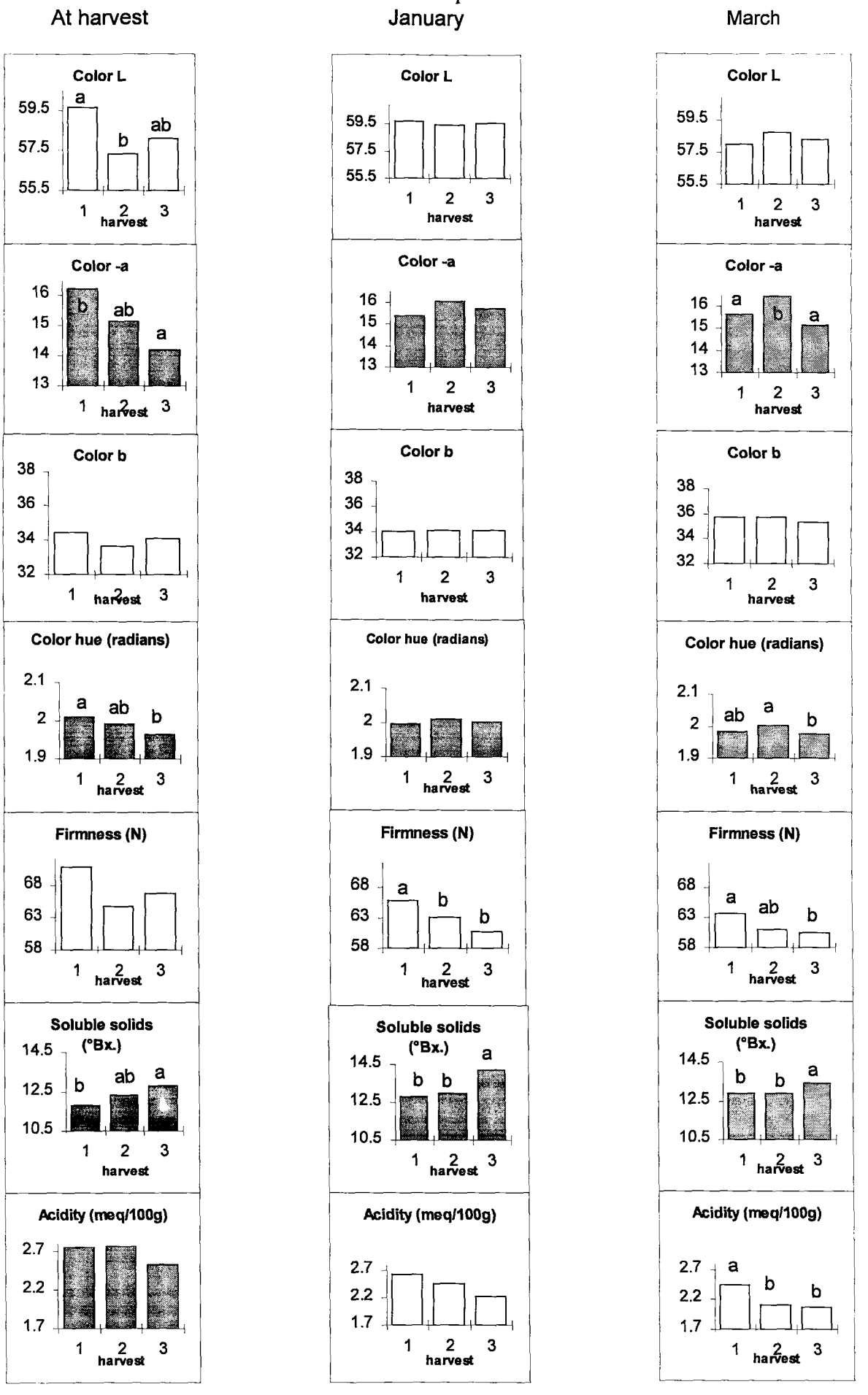


Figure 6 - Effect of fruit position on the tree on maturity indexes at harvest and on quality parameters after storage of Conference pears (L=low, H=high)

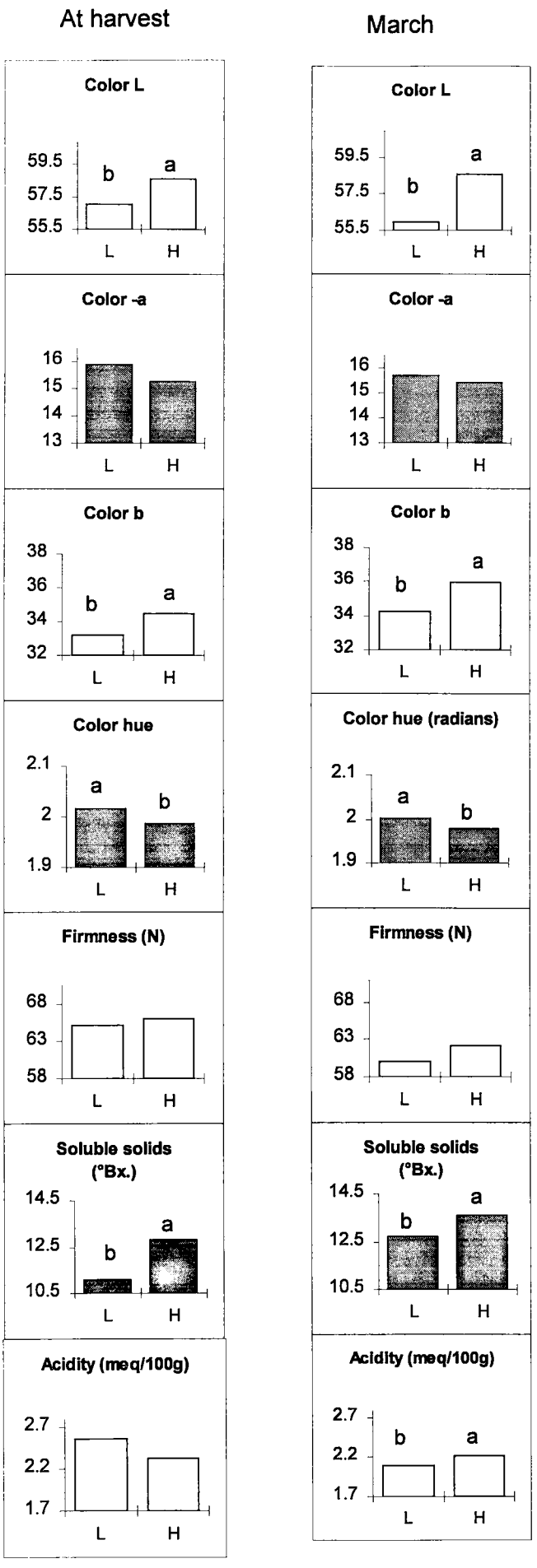


Figure 7 - Effect of storage atmosphere on quality parameters of Conference pears after storage.
IVTPA: 0.7 = 2% O2 + 0.7% CO2; 5 = 2% O2 + 5% CO2;
Commercial stores: C = Campofrigo, 4.5% O2 +1.5% CO2; E = Eurofrutta, 1.4% O2 + 2.2%CO2, Ed = Eurofrutta with CA delayed after 5 weeks in air at 0°C.

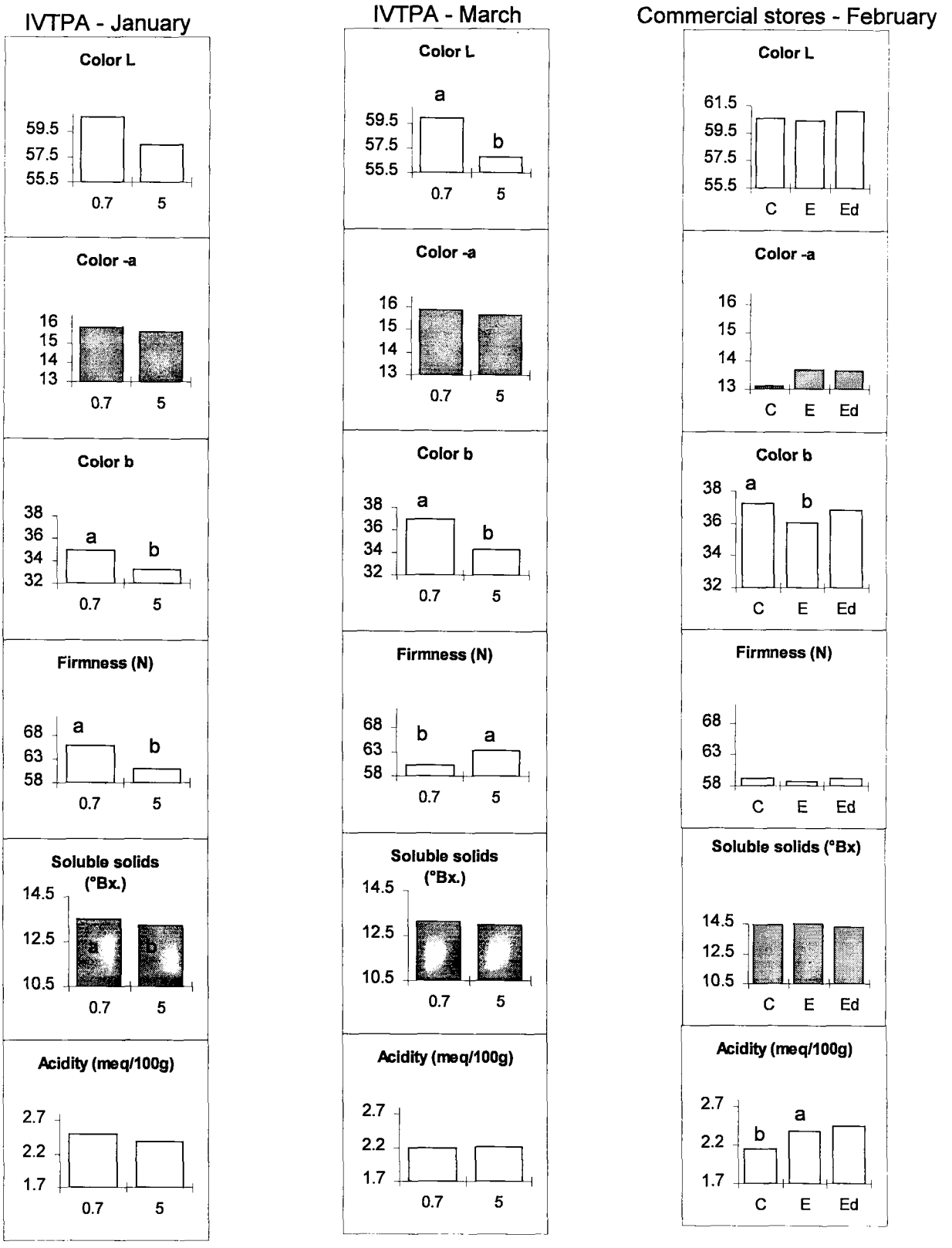


Figure 8 - Effect of delaying CA storage by 1 week (normal) or 5 weeks (delayed) in air storage on quality parameters of Conference pears (2nd harvest) after storage

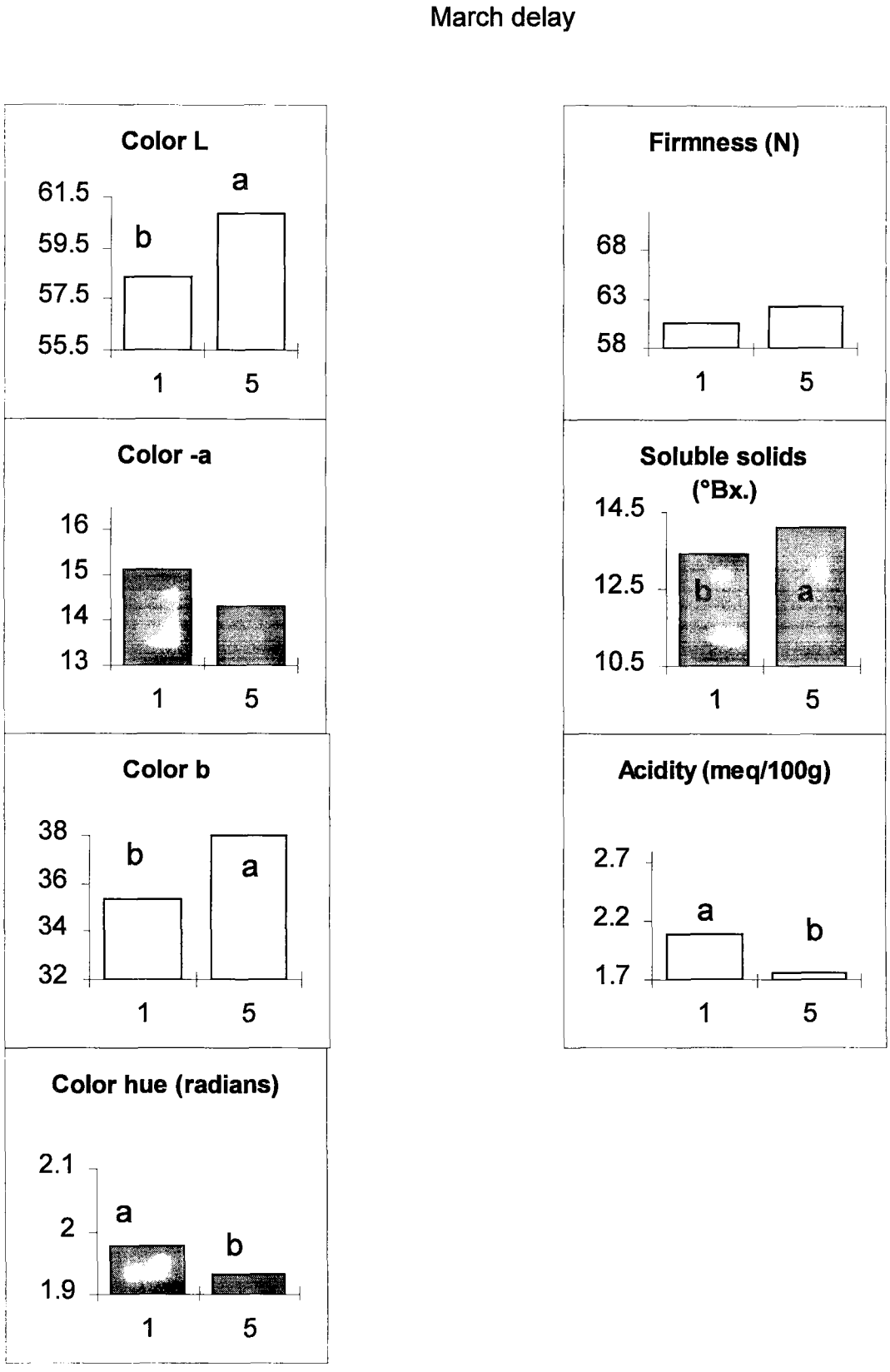


Table 11. Number and per cent Conference pears affected by Brown Heart after 7 month storage. Effect of position on the tree.

Position on the tree	2% O_2 + 5% CO_2		2% O_2 + 0.7% CO_2		mean
	n	%	n	%	%
High	8	4.8	1	0.6	2.7 a
Low	0	0	0	0	0 b

Table 12. AA content (mg/100 g F.W.) in stored pears after eight months in 2% O_2+0.7% CO_2 and 2% O_2+5% CO_2 storage: influence of harvest

harvest	outer pulp	inner pulp	mean
1	0.221	0.185	0.202 a
2	0.406	0.184	0.295 ab
3	0.464	0.466	0.465 b
stnd error	0.097		

Table 13. AA content (mg/100 g F.W.) in stored pears after 8 months in 2% O_2+0.7% CO_2 and 2% O_2+5% CO_2 storage: influence of storage atmosphere

	storage atm.	
harvest	2% O_2 +0.7% CO_2	2% O_2 +5% CO_2
1	0.335	0.069
2	0.459	0.131
3	0.671	0.259
mean	0.488 b	0.153 a

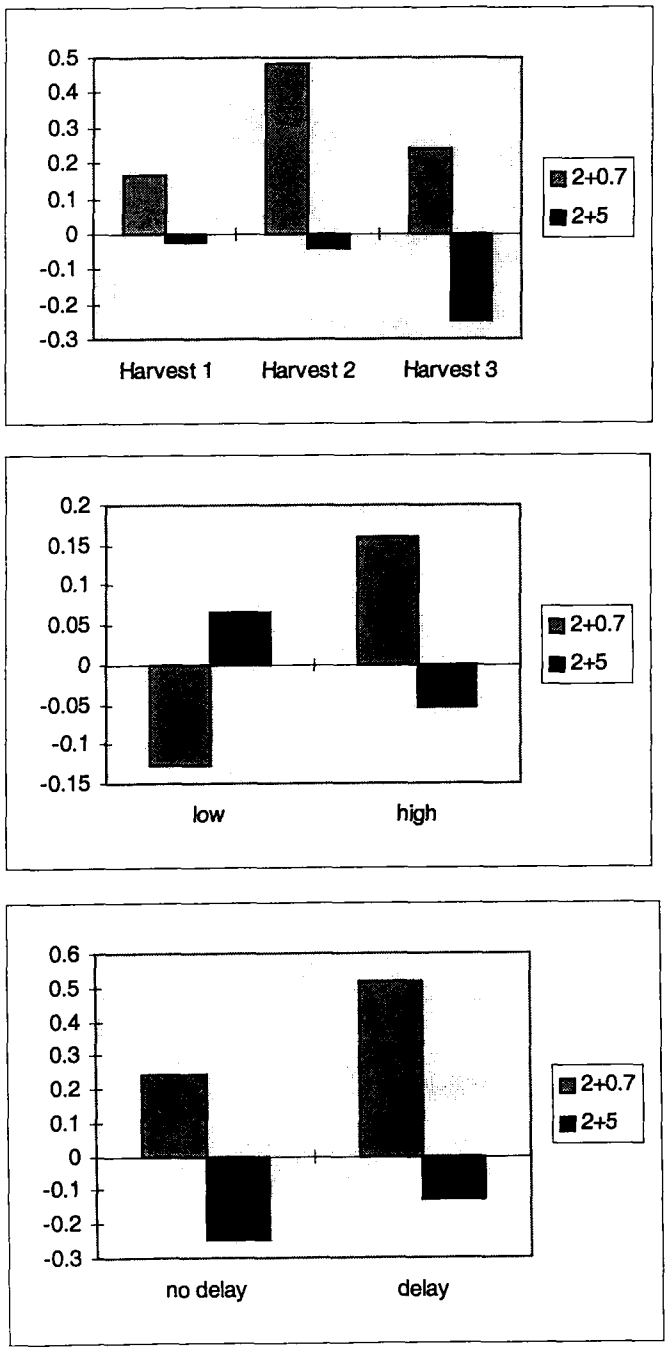
Table 14. AA content (mg/100 g F.W.) in stored pears after 8 months in 2% O_2+0.7% CO_2 and 2% O_2+5% CO_2 storage: influence of the position of pear on the tree (only 2ns harvest fruits), and of delayed CA (only 3rd harvest fruits).

	Position		Delayed CA	
	low	high	no delay	delay
2% O_2+0.7% CO_2	0.365	0.950	0.671	0.533
2% O_2+5% CO_2	0.188	0.236	0.259	0.290
stnd error	0.181		0.140	

Table 15. AA content (mg/100 g F.W.) in stored pears after 8 months in 2% O_2+0.7% CO_2 and 2% O_2+5% CO_2 storage: influence of pulp position

	outer part	inner part
2% O_2+0.7% CO_2	0.625	0.351
2% O_2+5% CO_2:	0.102	0.205
stnd error	0.079	

Figure 9. Differences of AA content between outer and inner pulp (mg/100 g F.W.) in stored pears after 8 months in 2% O_2+0.7% CO_2 and 2% O_2+5% CO_2 storage: effects of harvest time, of the position of fruit on the tree and of delayed CA.



FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project		
Total cost:	1600,3 kECU	*EC contribution:*	1045 kECU (65,3%)
Participant no. 4 total cost:	337,4 kECU	*EC contribution to partner no. 4:*	168,7 kECU (50%)
Commencement date:	01-06-1997	*Duration:*	4 years
Completion date:	31-05-2001		
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3		

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A. PARTNER INFORMATION

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Scientific team
Dr. A. de Jager
B.Sc. P. Willeboer

Objectives
See Technical Annex

Actions in the project
The following tasks are included in our work:
1. Cultivation of pears
2. Harvest of Pears
3. Storage of pears
4. Climate and Orchard factors
5. Post harvest treatments

7a. Quality evaluation
7b. Vitamin C determination
7c. Mineral analysis
11. Dissemination of results

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1998 TO 31-05-1999

Task 1. Cultivation of pears

Objectives
The objective was to select a range of orchards with sensitivity to brown heart **varying** from zero to high.

Material and Methods
Experiments were conducted in existing orchards. At first, seven orchards were selected representing cases from insensitive to very sensitive to brown heart. These orchard were located in the following three areas: Zeeland (south western part), Hoekse waard (south of Rotterdam) and Betuwe (central part between rivers Rhine, Waal and Maas). In these orchards treatments were carried out according to the special purpose. In all experiments the basic unit consisted of a field of at least three **adjacent** trees. The growers were asked to follow their normal cultivation program unless this interfered with a special treatment.

Results and Discussion
See the other Tasks

Conclusions
See the other tasks

Task 2. Harvest of pears

Objectives

The objective of this task is to harvest pears for subsequent analysis brown heart according to different stages of maturity, different orchard origin and different treatments at harvest and post harvest.

Material and Methods

Pears were picked weekly at five dates, from two weeks before expected optimum harvest date to two weeks after. Optimum harvest date was estimated according to firmness. A firmness of 6.3 kg with 7 mm plunger (penetrometer) is considered to represent the end of the 'picking window' for optimum harvest period.

Picking dates of fruits for different tasks is presented in table 1.

Table 1. Picking dates in 1998 of fruit for the different tasks

Pick	Task/Factor/Treatment	Date
1	Task 4/nutrition, fruit load, position	August 24
	Task 3/storage conditions	August 26
2	Task 4/nutrition, fruit load, position	August 31
	Task 3/storage conditions	September 2
3	Task 4/nutrition, fruit load, position	September 7
	Task 3/storage conditions	September 9
4	Task 4/nutrition, fruit load, position	September 14
	Task 3/storage conditions	September 16
5	Task 4/nutrition, fruit load, position	September 21
	Task 3/storage conditions	September 23

Immediately after harvest samples of 25 fruits were used for quality and maturity measurements for each object. We measured fruit weight (Mettler PM 600 in gram), ground colour (Minolta CR 300 in L*a*b), firmness (Instron, 7mm plunger in 2 sec to 8 mm depth), starch (visual, stages 1-10), total soluble solids concentration (TSS, with Atago DBX 55 in °Brix) and acidity (% malic acid) in samples from each harvest date. Mineral analysis (task 7a) was done on the samples of September 7^{h} (estimated end of safe picking period). Mineral analysis, total soluble solids and acidity were measured in a mixed sample, the other measurements were done on individual fruits.

In general, fruits were stored in containers of about 1m^3. Each container represented one treatment and was filled with fruits of one picking date of several orchards. Arrangement of the boxes was at random with respect to orchard. Storage conditions were normally reached within 24 hours after setting the conditions and were monitored by measurements each two hours.

From each harvest date all fruits from three trees were randomised and then stored for 7 days at -0.5 °C. Then storage was continued at -0.5 °C, 2% O_2 and 0.5 % CO_2.

Results and Discussion

See the other tasks

Conclusions

See the other tasks

Task 3. Storage of pears

Objectives
The objectives of this task were
(1) to study the influence CO_2 on brown heart.
(2) to study the influence O_2 of on brown heart

Material and Methods
Influence of CO_2
For fruits from all harvest dates, after 7 days at -0.5 °C, storage was continued at -0.5 °C, 2% O_2 and 0.5 or 5.0% CO_2.
Influence of O_2
For fruits from all harvest dates, after 7 days at -0.5 °C, storage was continued at -0.5 °C, 0.5% CO_2 and 2, 3 or 4% O_2

Evaluation of incidence of brown heart and of quality after storage were carried out from 6 to 9 April (after 7 month of storage) in the following way in order to guarantee good comparison between the treatments: on day one 25 fruits for quality analysis, on day two brown heart analysis on 25 fruits for each object and on day three and four the same. In this way inspection of fruits of all treatments was spread over the same period (too much work for one day).
Degree of brown heart was scored in three classes of brown and three classes of cavities relating to the stages shown in figure 1 and figure 2. From this an index was calculated, both for cavities and browning, as follows: index = class 1 + (3 * class 2) + (6 * class 3).

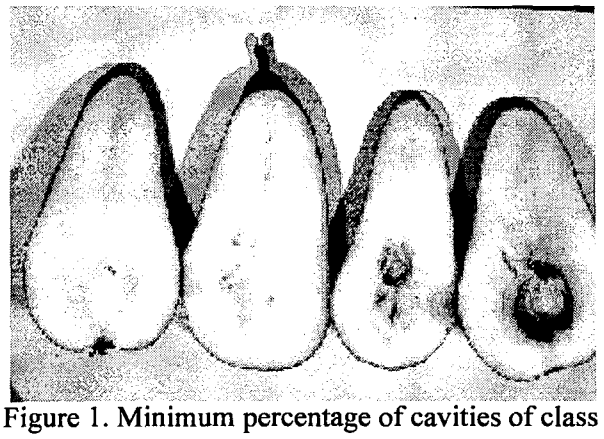

Figure 1. Minimum percentage of cavities of class 1, class 2, class 3 and an example of maximum percentage cavities.

Figure 2. Minimum percentage of internal browning class 1, class 2, class 3 and an example of maximum percentage browning.

Results and discussion

Comparison between CA-storage at 0.5 and 5% of CO_2

Cavities and browning

Table 2 shows that the % of pears showing cavities and/or browning is increasing from pick 4 at normal CO_2 and already from pick two at high CO_2 level. Compared to 1997, the incidence of disorders seems to be higher.

Table 2. The effect of increasing CO_2 level from 0.5 to 5% on the total incidence of disorders (% of pears affected).

	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5
0.5% CO_2	1.2 a	5.8 ab	4.2 ab	19.7 bc	31.8 cd
5% CO_2	1.5 ab	39.8 d	68.8 e	80.8 ef	99.4 f

($P<0.001$, l.s.d. = 19.3)

In figure 3 and 4 the development of cavities and browning is shown separately. Figure 3 shows that at standard storage condition pears of harvest 4 and 5 exhibits a limited rise in the incidence of cavities indicating that the choice of latest harvest date (harvest 3) was good. High CO_2-level already induces cavities in pears from the second and the third pick, and also in all picks at much higher levels, confirming the observation of the previous year that CO_2 plays an important role in the initiation of brown heart. The same holds more or less for internal browning (figure 4)

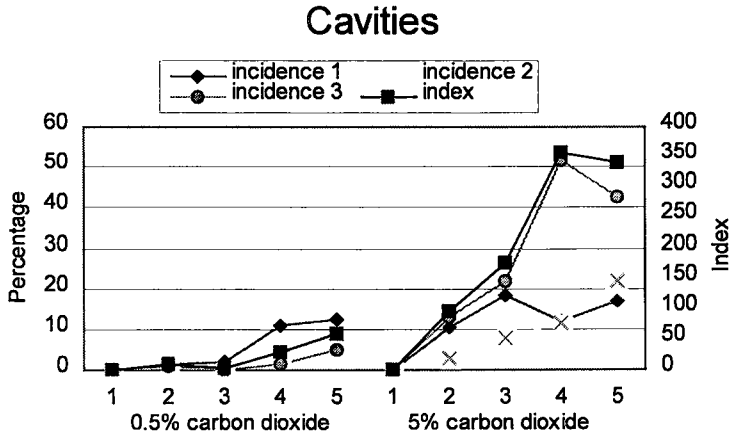

Figure 3. Incidence of cavities of class 1, 2 and 3 and cavity index in Conference pears from 5 harvest dates, stored at 0.5 and 5.0% CO_2, both at -0.5 °C and 2% O_2 (average of 7 orchards).

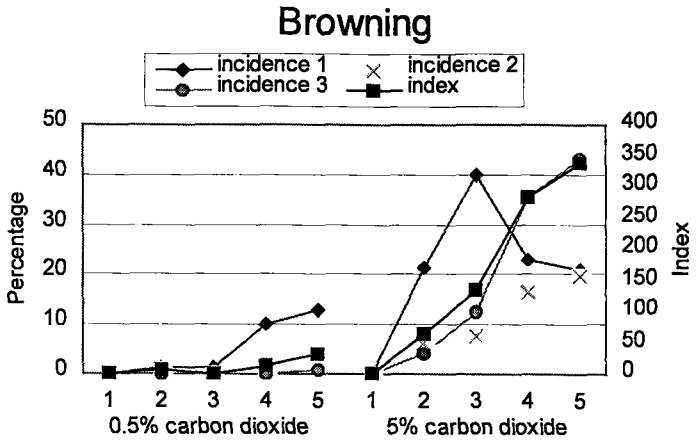

Figure 4. Incidence of browning of class 1, 2 and 3 and browning index in Conference pears from 5 harvest dates, stored at 0.5 and 5.0% CO_2, both at -0.5 °C and 2% O_2 (average of CO_2 7 orchards).

Loss of fruit weight

Loss of fruit weight (average 3.7%) - measured as difference in weight of the boxes before and after storage - was similar in both objects (table 3). This means that, just as in the previous year, the water loss was small and not related to treatment or harvest date.

Table 3. Loss of weight in fruits from 5 harvest dates at two levels of CO_2 concentration in CA-storage.

	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5
0.5% CO_2	4.3	3.9	2.8	2.5	3.3
5% CO_2	2.5	6.1	3.2	3.7	3.4

(P = 0.243)

Fruit weight, ground colour, TSS and rot

From table 4 it might be concluded that the incidence of rot is more or less independent from picking date and storage condition except for the latest pick stored at high CO_2. Since, however, this last observation is in line with the observation of the previous year - showing increased incidence of rot with picking date and with CO_2-level - the conclusion cannot be refuted that rot increases with picking date and is higher at high CO_2.

Table 4. Incidence of rot after storage until April as a function picking time and storage condition.

	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5
0.5% CO_2	1.2 a	1.9 a	1.5 a	1.7 a	2.8 a
5% CO_2	1.4 a	2.7 a	2.0 a	1.2 a	12.5 b

P = 0.027, l.s.d. = 4.9

Table 5 shows in addition the increase in fruit weight as a function of harvest date. Mean fruit weight was similar as in 1997. Loss of fruit weight on the basis of single fruits is higher than indicated in table 3, at least partly because larger fruits show more rot and are represented less in the sample after storage. Contrary to the previous year, there was no decrease of green ground colour with harvest date, not at harvest and not after storage. The level of sugars was slightly lower than in 1997 and showed less increase with harvest date. The acid level (data not shown) was very low but after storage significantly (though slightly) higher after storage at 5.0 %CO_2 (0.08 compared to 0.07% malic acid).

Table 5. Mean fruit weight, green ground colour and TSS of fruits from different harvest dates as an average of both storage conditions (low and high carbon dioxide) at harvest and after storage and rot incidence after storage.

	Fruit weight		Green ground colour		TSS		Rot
	Harvest	Storage	Harvest	Storage	harvest	Storage	Storage
Pick 1	175 a	160 a	-15.6	-13.4 bc	11.0	12.3	1.3 a
Pick 2	193 a	175 a	-14.8	-12.6 ab	11.5	12.3	2.3 a
Pick 3	225 b	198 b	-14.8	-14.1 c	11.3	12.0	1.8 a
Pick 4	230 b	211 b	-15.9	-12.3 a	11.6	12.1	1.5 a
Pick 5	233 b	215 b	-15.0	-13.4 bc	11.7	12.1	7.7 b
F test	< 0.001	<0.001	N.S.	0.002	N.S.	N.S.	0.002
l.s.d.	30	21		0.9			3.5

Yellow ground colour

There was a tendency that the level of yellow pigments (b-value) increased during storage and that this increase was lower at 0.5% CO_2 (table 6).

Table 6.Yellow ground colour at harvest and after storage at two levels of CO_2 at 5 harvest date as an average of samples from 7 orchards.

	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5	F test	l.s.d.
At harvest	36.6 a	36.4 a	37.8 b	36.9 ab	39.9 c	<0.001	1.1
0.5% CO_2	34.2 b	34.1 b	34.8 b	34.5 b	37.2 c	0.005	1.2
5.0% CO_2	32.2 a	32.1 a	34.8 b	33.9 b	36.3 c		

Firmness

Firmness decreased with picking date and during storage (table 7). There is a tendency that loss of firmness was higher in pears stored at 5.0% CO_2 due to lower firmness values in later picked fruit. These results differ widely from those in the previous year, but in both years firmness seems to decrease faster at the high % CO_2.

Table 7. Firmness at harvest and after storage at two levels of CO_2 of fruits from 5 harvest dates as an average of 7 orchards.

	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5	F test	l.s.d.
At harvest	7.3 d	6.9 c	6.4 b	5.8 a	5.9 a	<0.001	0.3
0.5% CO_2	6.7 e	6.1 d	5.9 cd	5.6 bc	5.3 b	0.034	0.3
5.0% CO_2	6.7 e	6.5 e	6.0 d	5.3 b	4.9 a		

Comparison between CA-storage at 2, 3 and 4% O_2

Cavities and browning

In this experiment the incidence of cavities (fig. 5) and browning (fig. 6) was almost negligible. Almost certainly this is a consequence of the longer duration of cool storage (21 compared to 7 days in the preceding year).

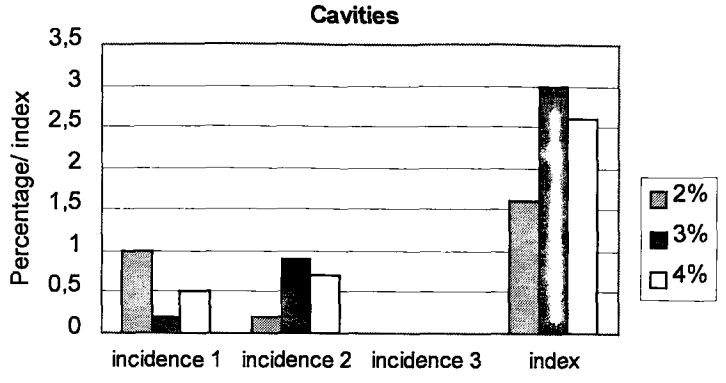

Fig. 5. Influence of % O_2 (2, 3 and 4) on the incidence of cavities of class 1, 2 and 3 and cavity index in Conference pears from 5 harvest dates, stored at 0.5 % CO_2 and -0.5 °C (average of 7 orchards).

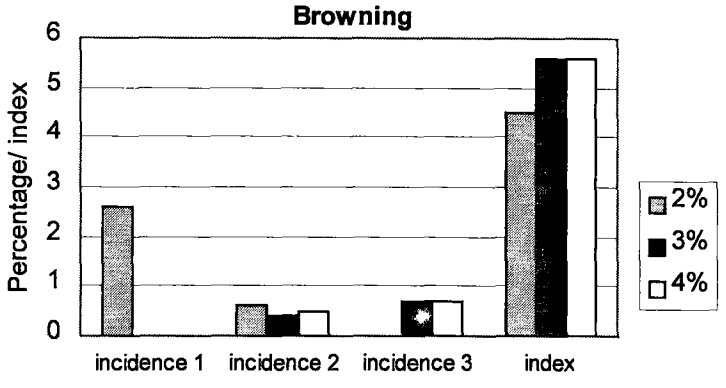

Fig. 6. Influence of % O_2 (2, 3 and 4) on the incidence of internal browning of class 1, 2 and 3 and cavity index in Conference pears from 5 harvest dates, stored at 0.5 % CO_2 and -0.5 °C (average of 7 orchards).

Loss of fruit weight

There was a tendency of higher water loss in later picked fruit, but % O_2 did have no influence (table 9)

Table 8. Percentage of loss of fruit weight after storage at 3 levels of oxygen of fruits from 3 picking dates.

	2% Oxygen	3% Oxygen	4% Oxygen	Average
Pick 3	1.4	3.1	2.8	2.4
Pick 4	3.4	2.5	2.6	2.8
Pick 5	2.9	3.6	4.0	3.5
F test				N.S.

Firmness, rot and total disorders
As expected, the firmness of stored pears was influenced by picking date (table 9). Differences between oxygen levels, however, were low with only a tendency for a higher firmness loss at higher % O_2. Both incidence of rot and of total disorders was higher at the latest picking date but was not influenced by % oxygen.

Table 9. Influence of % oxygen during storage on total disorders, firmness and % rotted fruits in fruits of pick 3, 4 and 5 (average of 7 orchards)

Pick	2% oxygen	3% oxygen	4% oxygen	Mean
	Total disorders			P<0.001
Pick 3	2.5	2.5	2.3	2.4 a
Pick 4	2.5	1.6	2.3	2.1 a
Pick 5	9.4	5.8	6.1	7.1 b
	Rot			P=0.003
Pick 3	2.0	2.3	2.1	2.1 a
Pick 4	2.1	1.4	2.3	1.9 a
Pick 5	5.7	4.6	4.9	5.1 b
	Firmness (kg) (p			P<0.001
Pick 3	5.8	5.7	5.8	5.8 c
Pick 4	5.6	5.6	5.4	5.5 b
Pick 5	5.3	5.0	5.0	5.1 a

Fruit weight and yellow and green ground colour
Oxygen concentration only showed significant positive influence on the b-value, representing the level of yellow pigments (table 10). Green ground colour and fruit weight were not affected.

Table 10. The Influence of oxygen concentration during storage on green and yellow ground colour after storage as an average for pick 3, 4 and 5 and of 7 orchards.

% oxygen	Green ground colour	Yellow ground colour
2 %	-10.9	35.4 a
3 %	-10.9	35.4 a
4 %	-10.9	36.8 b
F test	N.S.	<0.001

Table 11 shows that, considering pick 3, 4 and 5, picking date had a significant influence on yellow ground colour whereas but not on green ground colour and fruit weight.

Table 11. Fruit weight and green and yellow ground colour at harvest and after storage in fruit of pick 3, 4 and 5, as an average of 3 oxygen levels in storage.

	Weight (gram)		Green ground colour		Yellow ground colour	
	Harvest	Stored	harvest	stored	Harvest	Stored
Pick 3	225	203	-14.8 a	-11.0 b	37.8 a	34.7 a
Pick 4	230	213	-15.9 b	-11.5 b	36.9 a	35.7 b
Pick 5	233	224	-15.0 ab	-10.1 a	39.9 b	37.3 c
F test	N.S.	N.S.	0.033	0.003	<0.001	<0.001

Conclusion and continuation
Higher oxygen concentration significantly affects fruit quality (esp. Ground color) and higher carbon dioxide is no longer useful for for the model. Further experiments are not useful.

Task 4. Climate and orchard factors

Objectives
The objectives within this task are
(1) to find a possible relation of brown heart with (micro)climate
(2) to study the influence of nutrition, especially the elements N, K and Ca
(3) to study the influence of fruit load and
(4) to find a possible relation with position of the fruit in the tree

Material and Methods
Climate data (temperature, rainfall and RV) from 7 orchards were either collected in the orchard itself ('Mety' station) or obtained from nearby meteorological stations.

Nutrition was varied in two orchards in the region 'Zeeland' by applying extra doses of Ca, N or K, K by spraying a solution of 10 gr/l potassium sulfate 11 times between June 4 and August 9, Ca by spraying a solution of 7 gr/l calcium chloride id., and N by broadcasting two times 60 kg/ka, a total of 120 kg/ha.

Fruit load was varied in two orchards in the region 'Zeeland' either by removing after the regular June drop 1/3 or 2/3 of all pears in normal bearing trees, or by selecting trees naturally showing a bearing of 2/3 or 1/3 of normal level.

Fruit position was studied by sampling five different positions ('top'(15%), 'high' (15-30%), 'inner', 'north-east' and 'south-west') according to figure 7 at two orchards in the region 'Zeeland'.

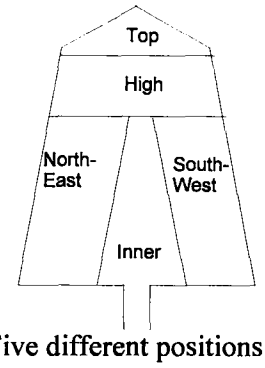

Figure 7: Five different positions in the tree.

For picking and storage see tasks 2 and 3.

Results and Discussion
Climate data
Climate data of the 7 orchards or meteorological stations close to these orchards where collected and sent to partner 1 for modelling purposes (ATO-DLO, H.A.G.M v/d Boogaard).

Nutrition
Table 12 shows that the treatment in fact did not affect the nutrient levels in the fruit. Table 13 and 14 show that there was also no indication of any influence of the treatment on the incidence of cavities or internal browning.

Table 12. The level of major nutrients and % dry matter in pears from pick 3 as influenced by an extra dosage of K, Ca or N as compared to the control (mean from two orchards).

	Ca	K	Mg	N	P	%dm
Control	5,96	131,1	7,40	60,7	10,72	14,1
+ K	6,25	142,0	7,04	58,7	10,84	14,3
+ Ca	5,32	120,3	6,60	57,2	10,18	14,2
+ N	6,19	146,9	7,27	62,4	12,11	14,2
F pr	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Gem.	5,93	135,1	7,08	59,7	10,96	14,2

Table 13. Incidence of cavities in relation to the nutrient level; cavities scored in 3 classes of severity and transformed into the cavity index.

Fruit load	Cavities class 1	Cavities class 2	Cavities class 3	Cavity index
Control	3.3	8.6	6.7	69.6
+ Potassium	4.1	7.5	8.0	74.5
+ Calcium	4.4	6.6	7.8	70.7
+ Nitrogen	5.7	6.8	6.5	65.3
F test	N.S.	N.S.	N.S.	N.S.
l.s.d.				

Table 14. Incidence of internal browning in relation to the nutrient level; browning scored in 3 classes of severity and transformed into the browning index.

Fruit load	Browning class 1	Browning class 2	Browning class 3	Browning index
Control	8.2	5.6	5.1	55.8
+ Potassium	6.5	7.6	4.5	56.5
+ Calcium	6.1	6.9	4.4	52.9
+ Nitrogen	5.2	5.5	8.2	71.2
F test	N.S.	N.S.	N.S.	N.S.
l.s.d.				

Loss of weight
Mean loss of water was 4% in this experiment. Water loss from the N treatment was significantly higher than from the control (6.2 and 3.1 % respectively).

Other quality characteristics
Other quality characteristics did not differ between treatments and controls.

Fruit load

Diseases
I this experiment, less than 1% of the fruits developed rot.

Cavities and internal browning
Variation in natural bearing was only available from one orchard. Without any statistical backing, the impression is that the cavities of class 2 and 3 and the cavity index are increased by lower bearing, both naturally and by hand thinning (table 15). The same holds more or less for internal browning (table 16). These effects are more clearly shown in figs 8 and 9. These conclusions strengthen the conclusions of the previous year and ask for one larger experiment unequivocally confirming the effects.

Table 15. Incidence of cavities in relation to fruit load either using natural variation or induced by hand thinning; cavities scored in 3 classes of severity and transformed into the cavity index (average of two orchards; data between brackets from one orchard only).

Fruit load	Cavities class 1	Cavities class 2	Cavities class 3	Cavity index
Control	(7.0) 5.8	(0.8) 1.4	(0.8) 1.6	(14.3) 19.7
- 1/3 natural	(9.9)	(3.8)	(5.4)	(53.6)
- 2/3 natural	(7.2)	(4.6)	(4.2)	(46.2)
- 1/3 thinning	(7.0) 8.1	(1.6) 3.5	(2.7) 6.7	(28.5) 58.8
- 2/3 thinning	(6.2) 5.3	(2.6) 2.8	(2.6) 4.2	(29.9) 39.2
F test	N.S.	N.S.	N.S.	N.S.
l.s.d.				

Table 16. Incidence of internal browning in relation to fruit load either using natural variation or induced by hand thinning; browning scored in 3 classes of severity and transformed into the browning index (average of two orchards; data between brackets from one orchard only).

Fruit load	Browning class 1	Browning class 2	Browning class 3	Browning index
Control	(6.2) 4.4 a	(0.3) 1.0	(0.0) 0.7	(7.0) 11.3
- 1/3 natural	(10.1)	(2.0)	(0.0)	(16.1)
- 2/3 natural	(10.3)	(1.9)	(0.8)	(20.7)
- 1/3 thinning	(8.2) 11.7 b	(1.1) 2.8	(0.0) 0.9	(11.5) 25.3
- 2/3 thinning	(3.9) 4.7 a	(1.0) 2.6	(0.7) 0.5	(10.9) 15.3
F test	0.03	N.S.	N.S.	N.S.
l.s.d.	5.9			

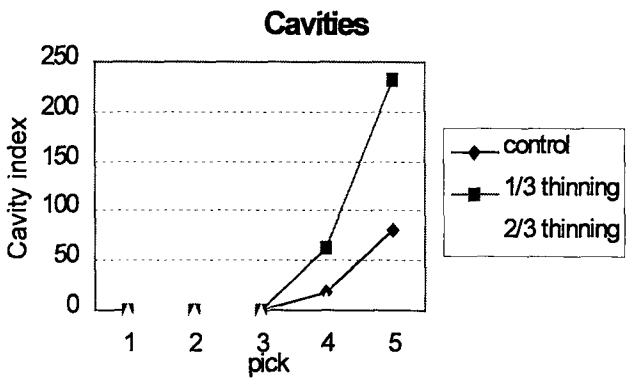

Fig. 8. Influence of degree of thinning on the incidence of cavities in pears (average of two orchards)

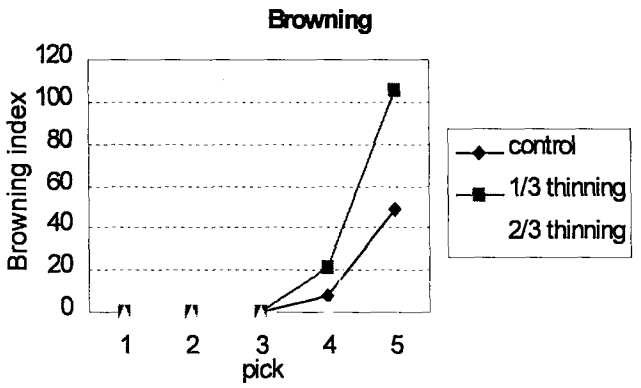

Fig. 9. Influence of degree of thinning on the incidence of browning in pears (average of two orchards).

Fruit weight, loss of weight and firmness
Water loss during storage (average of 3.5%) was not influenced by the degree of bearing. Table 17 shows that - contrary to the previous year - fruit weight was much higher in trees with lower natural degree of bearing and only slightly increased by hand thinning. 'Sugar' level increased slightly with decreasing degree of bearing and increased in all treatments by about 1% during storage. Firmness tended to be higher at lower degree of bearing. In all aspects the severest hand thinning produced the largest differences. Table 18 shows clearly that a lower degree of bearing results in a less advanced stage of starch degradation. This might be the result either of a real arrears or of a higher total level of starch at the same stage of chemical degradation.
Differences between the objects in ground colour were negligible.

Table 17. Fruit weight, TSS and firmness at harvest and after storage at different degrees of fruit bearing either by natural variation or by hand thinning; data between brackets refer to one orchard showing natural variation in fruit load (all data refer to the average of 5 picking dates).

	Fruit weight		TSS (%Brix)		Firmness	
	At harvest	After storage	At harvest	After storage	At harvest	After storage
Control	(207) 192	(177) 173 a	(11.2) 11.2 a	(12.3) 12.4 a	(6.6) 6.6	(5.8) 5.6a
- 1/3 natural	(215)	(216)	(11.3)	(12.2)	(6.8)	(6.0)
- 2/3 natural	(240)	(239)	(11.7)	(12.8)	(6.7)	(5.8)
- 1/3 thinning	(206) 201	(186) 193 b	(11.6) 11.8 b	(12.4) 12.6 a	(6.8) 6.7	(5.8) 5.8 ab
- 2/3 thinning	(215) 209	(200) 195 b	(12.1) 12.2 c	(13.1) 13.2 b	(6.8) 6.7	(5.9) 5.9 b
F test	N.S.	0.007	< 0.001	0.001	N.S.	0.041
l.s.d.		14	0.2	0.4		0.2

Table 18. Starch degradation stage at harvest and after storage; data between brackets refer to one orchard showing natural variation in fruit load (averages of 5 picking dates).

	Starch
	At harvest
Control	(4.8) 5.5 c
- 1/3 natural	(4.1)
- 2/3 natural	(3.7)
- 1/3 thinning	(4.3) 4.1 b
- 2/3 thinning	(3.1) 3.5 a
F test	< 0.001
l.s.d.	0.4

Fruit position

loss of weight
The mean loss of weight in this experiment was 4.2% without any effect of treatment or picking time.

sum of disorders
Table 19 shows that as, a function of time, the incidence of disorders increased first in the positions 'top' and 'high'. At normal picking date (3) these positions would already be too risky. This is the same as in the preceding year. In contrast with the preceding year, however, fruits from inner position develop high number of disorders in pick 4 and fruits from all positions show very high % disorders in pick 5. Figs 10 and 11 illustrate these effects as well.

Table 19. Sum of disorders (% of pears affected) in 5 different positions at 5 harvest dates (average of two orchards).

Position	Pick 1	Pick 2	Pick 3	Pick 4	Pick 5	Mean
Top	0.0	0.0	5.9	3.9	61.1	14.2
High	0.0	0.0	4.4	5.0	80.4	18.0
North-East	1.1	0.0	0.1	1.4	62.8	13.1
South-West	0.2	0.0	0.2	2.6	61.5	12.9
Inner	0.0	0.2	0.5	11.8	65.7	15.6
F test	< 0.001 (pluk)					N.S. (object)
Mean	0.3 a	0.0 a	2.2 a	5.0 a	66.3 b	

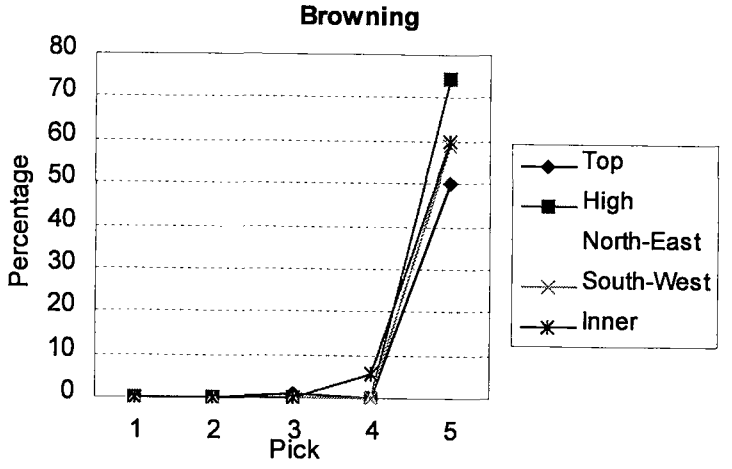

Figure 10. Percentage of fruits from different positions and at different harvest dates showing internal browning after storage (% of pears affected).

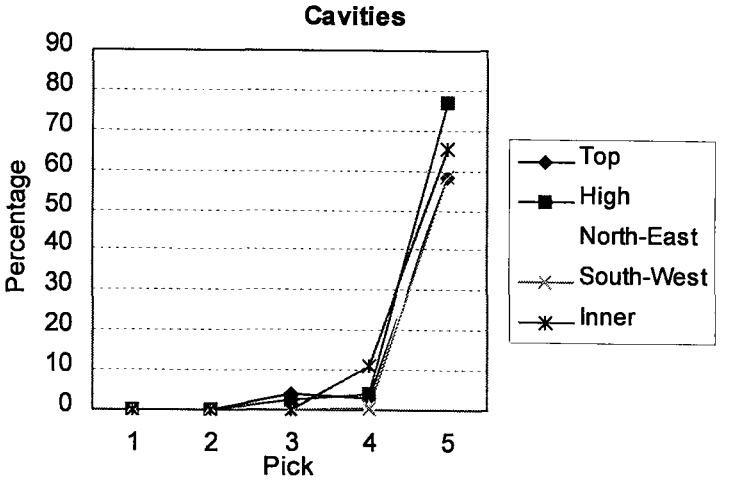

Figure 11. Percentage of fruits from different positions and at different harvest dates showing cavities after storage (% of pears affected).

Fruit weight

Fruits from the inner position differed in more than one aspects form fruit form all other positions (fig. 20). They had lower fruit weight (tendency), higher firmness (tendency) and lower TSS (significantly), both at harvest and after storage.

Table 20. Fruit weight, TSS and firmness at harvest and after storage (average of 5 picking dates).

	Fruit weight		TSS (%Brix)		Firmness	
	At harvest	After storage	At harvest	After storage	At harvest	After storage
Top	190	167	11.6 b	12.5 c	6.5	5.7
High	192	171	11.5 b	12.4 bc	6.5	5.6
North-East	194	170	11.4 b	12.1 ab	6.4	5.6
South-West	192	170	11.5 b	12.2 abc	6.4	5.7
Inner	180	155	10.9 a	11.9 a	6.8	5.9
F test	N.S.	N.S.	0.045	0.005	N.S.	N.S.

Ground colour

Table 21 shows that, in contrast to the preceding year, there were no significant differences in ground colour, nor at harvest nor after storage, between fruit from different positions.

Table 21. Green and yellow ground colour as measured by a- and b-value (L*a*b with Minolta chromameter) for fruits from 5 different positions at harvest and after storage (average of 5 picking dates).

	Green ground colour		Yellow ground colour	
	At harvest	Stored	At harvest	Stored
Top	-15.0	-11.7	36.9	34.1
High	-14.5	-12.0	36.7	33.8
North-East	-14.4	-12.2	36.3	33.6
South-West	-14.6	-12.2	36.5	33.8
Inner	-14.8	-12.4	36.3	33.2
F test	N.S.	N.S.	N.S.	N.S.

Conclusions and continuation

The climate aspects needs further data collecting. Nutrition seems also in the second year to have no relation with the risk of brown heart and this aspect should not be continues. The effect of position is clear (higher risk in the top) and needs no further experimentation. The effect of degree of fruit load is important enough to try to confirm the observed tendencies (lower load, higher risk of brown heart) in a following year.

Task 5. Post harvest treatments

Objectives

The objectives of this task are

(1) to develop storage scenario's that minimize the risk of brown heart and

(2) to find treatments that give an early indication of the sensitivity of pears to the development of brown heart in storage.

Material and Methods

Fruits from 7 orchards were used to do the following experiments (cultivation and harvest is described under task 1 and 2):

(1) At all harvest dates, after 7 days cooling at -0.5 °C, storage was continued at -0.5 °C, 2% O_2 and 0.5 or 5.0% CO_2. Fruits from pick 3, 4 and 5 were placed at cooling conditions for 7, 21, 33, 50 or 80 days before being moved to -0.5 °C, 2% O_2 or 0.5% CO_2. Fruits were stored till March 23-26, 1999. Incidence of brown heart, and fruit quality were measured after warming-up during one day.

(2) Pears from all picks from 7 orchards were placed at combinations of the following conditions: 1 or 2 days at 18°C and 18 or 17 at 4 °C, 2 % O_2 and 5% CO_2 and 4 or 5 days at 18 °C and 15 or 14 days at 4 °C, 2 % O_2 and 0.5% CO_2 The total duration of the test was always 19 days.

Mild scenario's

Disorders

Table 22 shows that in this experiment, as in the preceding year, the incidence of disorders is very much reduced in fruit from later picks by postponing the start of CA conditions more than 7 days. Previous results showed that 7 days represented the worst case.

Table 22. Influence of length of cooling period before starting CA-conditions on the level of disorders (% of fruits) in fruit from three harvest dates.

Cooling days	Pick 3	Pick 4	Pick 5	Average
7	4.2 a	19.7 b	31.8 c	18.5
21	2.5 a	2.5 a	9.4 a	4.8
33	4.9 a	1.8 a	5.0 a	3.9
50	1.9 a	2.0 a	8.7 a	4.2
80	3.0 a	1.1 a	6.7 a	3.6
Average	3.3	5.4	12.3	

P = 0.005

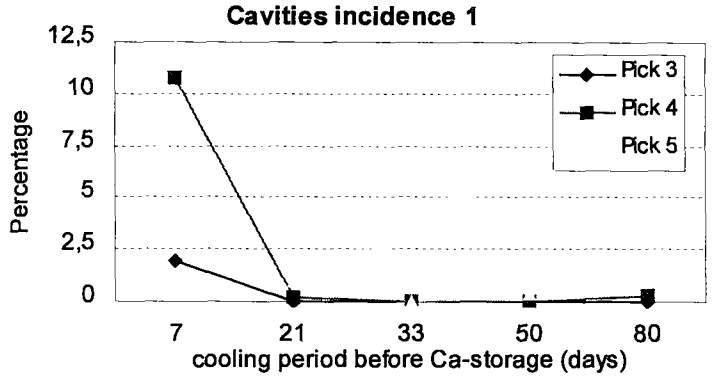

Fig. 12. The incidence of cavities of class 1 as a function of picking time and length of cooling period before start of CA (mean of 7 orchards).

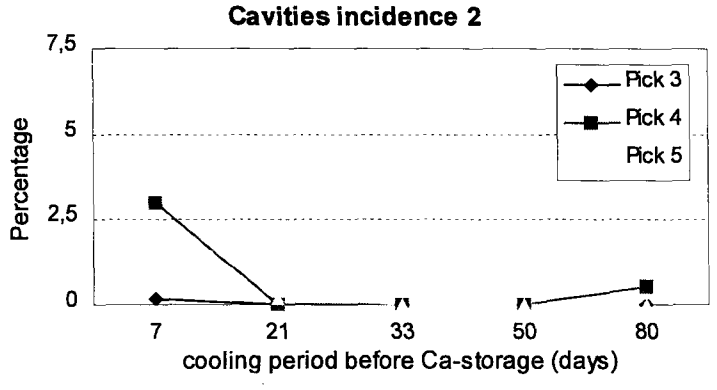

Fig. 13. The incidence of cavities of class 2 as a function of picking time and length of cooling period before start of CA (mean of 7 orchards).

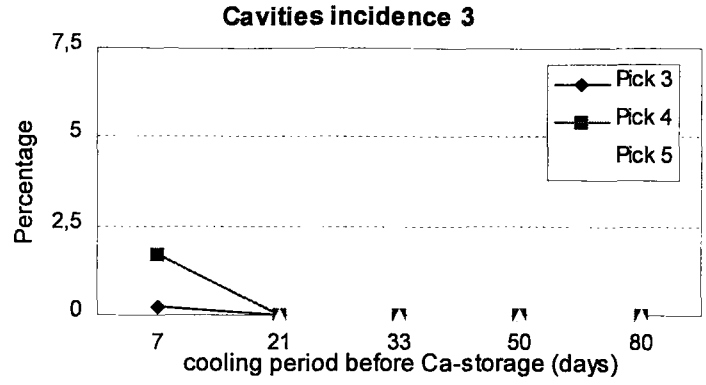

Fig. 14. The incidence of cavities of class 3 as a function of picking time and length of cooling period before start of CA (mean of 7 orchards).

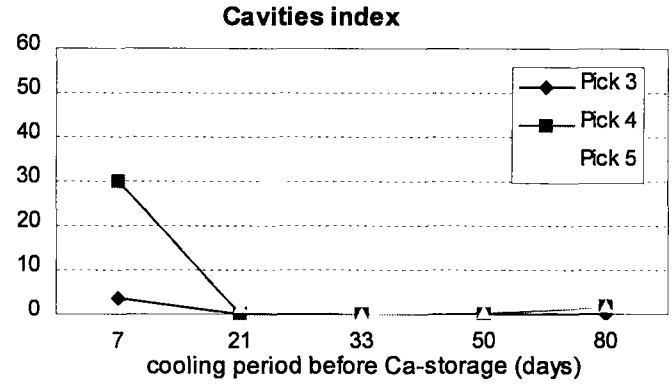

Fig. 15. The cavity index as a function of picking time and length of cooling period before start of CA (mean of 7 orchards).

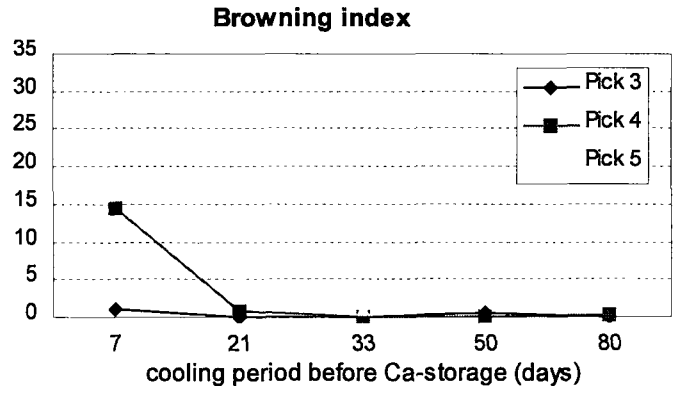

Fig. 16. The browning index as a function of picking time and length of cooling period before start of CA (mean of 7 orchards)

Fruit weight
There were no significant differences between fruit weight at harvest and after storage, due to treatments.

Fruit weight, green ground colour, TSS and rot
Table 23 shows the small changes in the four characteristics due to picking date and the changes due to storage.

Table 23. Fruit weight, green ground colour (a-value), TSS at harvest and after storage and rot after storage in fruits of pick 3, 4 and 5 (average of 7 orchards and all cooling periods).

	Weight		a-value		TSS		Rot
	Harvest	stored	harvest	stored	Harvest	stored	Stored
Pick 3	225	202	-14.8 a	-11.7 b	11.3	12.4	2.5 a
Pick 4	230	210	-15.9 b	-10.9 a	11.6	12.3	1.6 a
Pick 5	233	218	-15.0 ab	-11.7 b	11.7	12.4	4.8 b
F test	N.S.	N.S.	0.033	0.023	N.S.	N.S.	0.004

Firmness and yellow ground colour
Firmness decreased with picking date and during storage while yellow ground colour increased with picking date but decreased during storage (table 24)

Table 24. Firmness and yellow ground colour (b-value) at harvest and after in fruits of pick 3, 4 and 5 (average of 7 orchards and all cooling periods).

	Firmness		b-value	
	Harvest	stored	Harvest	Stored
Pick 3	6.4 b	5.8 c	37.8 a	34.7 a
Pick 4	5.8 a	5.6 b	36.9 a	35.3 b
Pick 5	5.9 a	5.1 a	39.9 b	37.3 c
F test	<0.001	<0.001	<0.001	<0.001

Influence of length of the cooling period
Table 25 shows that green ground colour was lower at periods longer than 7 days compared to 7 days cooling before CA but that there was no further decrease after 21 days. Both yellow ground colour and TSS increased with longer cooling periods. An explanation in the case of 'sugars' is not easy! There was no significant influence of treatment on loss of weight (water).

Table 25. Fruit weight, ground color (a and b), TSS, and loss of weight as a function of the length of cooling period before start of CA conditions (average of 7 orchards).

Days cooling before CA	Fruit weight	a-value	b-value	TSS %brix	Loss of weight %
7	206	-12.8 c	35.2 a	12.0 a	3.1
21	209	-10.9 a	35.4 a	12.3 b	2.6
33	213	-11.0 ab	35.2 a	12.5 c	3.4
50	210	-11.8 b	36.2 b	12.5 c	3.4
80	211	-10.7 a	36.8 b	12.5 c	4.4
F test	N.S.	<0.001	<0.001	0.032	N.S.

Stress test

The aim of a stress test is to indicate sensitive lots of fruit **before,** in a normal scenario, CA conditions are started i.e. within 21 days. This means that any test should take not more than 19 days in order to evaluate the result and support the decision. Possible factors that could induce early browning in the test in sensitive lots are temperature, O_2 and CO_2. In the present experiment the effect of these factors is combined with a period at room temperature after storage but preceding the test condition. Earlier experiments indicated that imposing stress conditions immediately after picking did not result in useful signals. Results of the preceding year showed that especially two scenario's produced useful results, one combing two days at room temperature followed by 5%CO_2, the other combining 4 days at room temperature with normal level, i.e. 0.5%, of CO_2 (table 25). Basically, the effect of the stress test should be directly related to the effect of storage including 7 days of cooling before CA. This means e.g. zero browning in the test in early picked fruit and so on.

Table 25. Four different scenarios in the tress test, combining different number of days at room temperature following picking with two CO_2 levels.

Days at 18°C Following pick	Storage condition		
	Temperature (°C)	%O_2	%CO_2
1	-0.5	2	5.0
2	-0.5	2	5.0
4	-0.5	2	0.5
5	-0.5	2	0.5

Table 26 shows the individual result for each orchard. Orchards with code 12 and 178 showed very small incidence of brown heart after storage. In general, the treatments including 5%CO_2 showed already browning symptoms in pears of pick 2 whereas those including 0.5%CO_2 gave symptoms only in pears from pick 3 and later.

Table 26. % of pears (by weight) affected in the test and % of pears (by number) affected by browning and/or cavities after storage for 7 orchards.

Pick	Days 18°C	Condition during stress-test	Orchard (code)							CC Pick 1-5
			12	33	77	78	81	177	178	
1	1	4°C + 2% O_2 + 5% CO_2	0	0	0	0	0	0	0	
	2	4°C + 2% O_2 + 5% CO_2	0	6.0	0.9	0	0.9	1.3	0	
	4	4°C + 2% O_2 + 0,5% CO_2	0	0	0	0	0	0	0	
	5	4°C + 2% O_2 + 0,5% CO_2	0	0	0	0	0	0	0	
		Werkelijk na CA-bewaring								
1	7	-0,5°C + 2% O_2 + 0,5% CO_2	0	0	0	0	0	0	0	
2	1	4°C + 2% O_2 + 5% CO_2	0	0	0	0	2.5	13.7	2.0	
	2	4°C + 2% O_2 + 5% CO_2	0	2.8	2.2	0	0	8.6	2.9	
	4	4°C + 2% O_2 + 0,5% CO_2	0	0	0	0	0	0	0	
	5	4°C + 2% O_2 + 0,5% CO_2	*	*	*	*	*	*	*	
		Werkelijk na CA-bewaring								
2	7	-0,5°C + 2% O_2 + 0,5% CO_2	0	0	1.3	0	2.4	24.1	0	
3	1	4°C + 2% O_2 + 5% CO_2	1.2	3.7	0	1.6	1.4	33.8	0	
	2	4°C + 2% O_2 + 5% CO_2	0	0	0	0	0	1.7	0	
	4	4°C + 2% O_2 + 0,5% CO_2	0	6.7	5.4	0	0	31.8	1.1	
	5	4°C + 2% O_2 + 0,5% CO_2	1.1	3.9	10.4	41.5	7.0	49.6	25.2	
		Werkelijk na CA-bewaring								
3	7	-0,5°C + 2% O_2 + 0,5% CO_2	0	4.7	0	0	0	11.5	1.3	
4	1	4°C + 2% O_2 + 5% CO_2	0	0	0	0	1.4	0	0	
	2	4°C + 2% O_2 + 5% CO_2	0	0	2.8	0	0	1.5	0	
	4	4°C + 2% O_2 + 0,5% CO_2	1.3	38.1	16.7	46.5	15.9	46.6	2.7	
	5	4°C + 2% O_2 + 0,5% CO_2	3.6	79.6	65.4	88.3	73.8	83.0	50.0	
		Werkelijk na CA-bewaring								
4	7	-0,5°C + 2% O_2 + 0,5% CO_2	0	18.3	18.3	30.8	6.8	48.9	1.6	
5	1	4°C + 2% O_2 + 5% CO_2	68.4	84.6	97.1	75.7	92.2	100	81.6	
	2	4°C + 2% O_2 + 5% CO_2	75.8	100	98.2	89.0	98.8	98.1	94.2	
	4	4°C + 2% O_2 + 0,5% CO_2	42.2	98.5	56.0	82.8	79.7	78.5	39.8	
	5	4°C + 2% O_2 + 0,5% CO_2	26.5	90.8	62.7	55.1	46.9	96.9	39.6	
		Werkelijk na CA-bewaring								
5	7	-0,5°C + 2% O_2 + 0,5% CO_2	7.1	52.8	22.4	17.3	35.9	64.4	1.5	

Table 27 shows for each picking date the coefficients of correlation between incidence of browning in the stress test (% of pears affected by weight) and incidence of brown heart after storage (% of pears affected by browning and/or cavities). Each single correlation coefficient refers to 7 orchards and is, thus, based on only 7 pairs of data.

Apparently all four stress scenarios produce reasonably good correlation with incidence of brown heart after storage in pears of pick 3 (the normal pick). Yet the combinations of 4 or 5 days cooling with low carbon dioxide seem slightly better.

Another way of analysing the result is to combine data from all picking dates. The result of that analysis shows that the treatment 5 days in room temperature followed by the standard CA condition of 4°C at 0.5%CO_2 gives the best variation explained (R^2) at a value of 65% (fig 17).

Table 27. Correlation between result of the stress tests (scored as % browning by weight) and of actual storage (scored as % of pears affected by browning and/or cavities for each picking date (7 orchards)

Days 18°C	CO_2	Pick	Storage at -0.5°C + + 2% O_2 + 0,5% CO_2				
			Pick 1	Pick 2	Pick 3	Pick 4	Pick 5
1	0.5	1	*	*	*	*	*
		2	*	**0.98**	**0.88**	**0.70**	**0.64**
		3	*	**0.99**	**0.94**	**0.80**	**0.73**
		4	*	-0.08	-0.25	-0.28	0.13
		5	*	**0.61**	0.52	0.52	**0.65**
2	5.0	1	*	-0.01	0.37	0.15	**0.64**
		2	*	**0.88**	**0.94**	**0.69**	**0.66**
		3	*	**0.99**	**0.92**	**0.78**	**0.67**
		4	*	0.37	0.22	0.40	0.22
		5	*	0.28	0.38	0.37	**0.61**
4	0.5	1	*	*	*	*	*
		2	*	*	*	*	*
		3	*	**0.96**	**0.96**	**0.81**	**0.79**
		4	*	0.49	0.59	**0.89**	**0.71**
		5	*	0.22	0.38	0.56	**0.77**
5	5.0	1	*	0.20	-0.22	-0.01	-0.45
		2	*	a	a	a	a
		3	*	**0.65**	0.55	**0.78**	0.24
		4	*	0.32	0.38	**0.69**	**0.62**
		5	*	**0.62**	**0.81**	**0.80**	**0.90**

* no correlation possible because of zero browning
a treatment not included in the test

Conclusions

The results of these experiments show
(1) that a ratter long cooling period before establishment of CA can prevent the occurrence of brown heart in pears picked at normal harvest date, without large effects on quality
(2) that there are perspectives of developing tests that can discriminate between sensitive and insensitive lots of fruit within the optimum cooling period of 21, before starting CA conditions.

Continuation

A number of questions remain to be solved i.e.

1. Can CO_2 be allowed to accumulate to a certain extent during the cooling period?
2. Can the stress test be further improved by changing the temperature during the CA-phase?
3. What is the effect of a short initial stress of O_2 (e.g. 5 days 0%) or CO_2 (e.g. 5 days 20%)

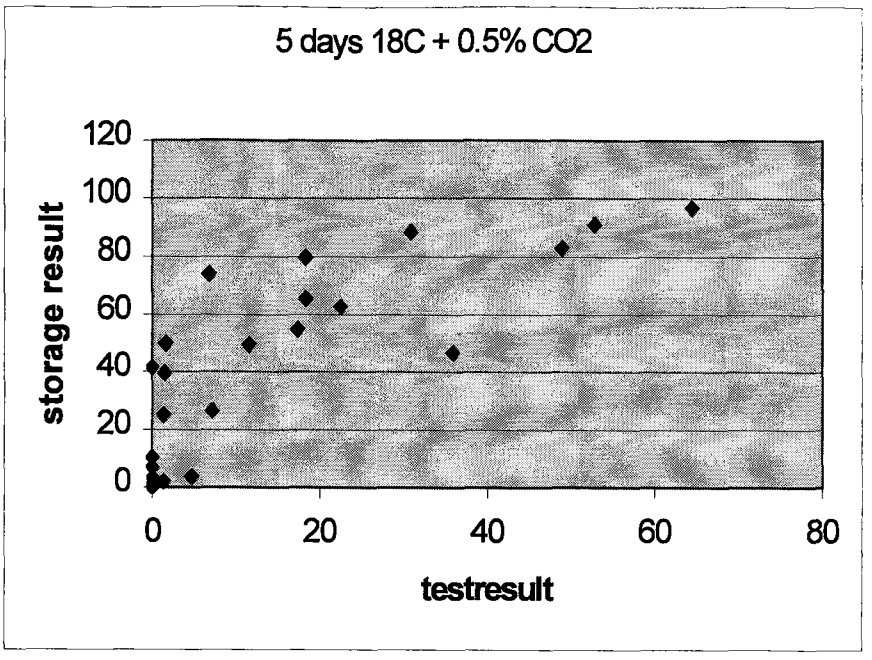

Figure 17. The relationship between the incidence of brown heart in the stress test and in normal storage combining data of fruit from all picking dates (R^2=65%)

Task 7 Destructive measurements

Objectives

1. To establish consequences of specific treatments or scenarios for other fruit quality characteristics (quality evaluation)
2. To establish internal factors that may be associated with or causally related with the observed phenomena of brown heart (nutrient concentration and vitamin C)

Material and Methods

Task 7a. Quality evaluation

Quality data have been given in all chapters and sections along with the effects of treatments with respect to brown heart (see chapter two for description)

Task 7b. Vitamin C

Vitamin C was determined in fresh mixed samples of 15-25 pears directly after sampling, both at harvest and after storage . The procedure was essentially according to routine used in RIKILT-DLO, using the total measurement (ascorbic acid plus dehydro ascorbic acid). Data were collected in four different experiments

1. Pick 1, 3 and 5 at harvest and after storage in the standard treatment in 7 orchards
2. In the fruit position experiment (pick 5)
3. In the nutrition experiment (pick 5)
4. In the fruit load experiment (pick 5)

Results and discussion

Table 28 shows the results of the evaluation of vitamin C concentration in time both in the orchard and after storage.

Table 28. concentration of total ascorbic acid in samples from 7 orchards in fruit form pick 1, 3 and 5 at harvest and after storage; between brackets subsamples from pears showing internal browning within the original sample.

Orchard (code)	Pick 1		Pick 3		Pick 5	
	at harvest	Stored	At harvest	Stored	At harvest	Stored
12	8.8	-	7.7	4.4	-	2.8
33	9.5	3.1	8.4	2.6	-	2.0 (2.0)
77	10.1	4.1	7.4	3.3	-	2.9 (2.1)
78	10.7	4.1	7.3	3.4	-	2.8 (2.3)
81	9.6	4.1	7.8	3.5	-	2.7 (2.7)
177	9.0	3.7	6.3	2.9	-	2.2
178	9.5	3.4	7.0	2.9	-	2.8 (2.4)
average	9.6	3.8	7.4	3.3	-	2.6

The following conclusions can be drawn.

1. The vitamin C concentration decreases significantly in the orchard during the picking period.
2. The vitamin C concentration decreases very strong during storage.
3. It seems that somewhere between 3.3 and 2.6 we have a critical concentration assuming that the vitamin C concentration is somehow related the incidence of brown heart.

Table 29 shows the ascorbic acid levels in the position experiment, the nutrition experiment and the fruit load experiment. There are no significant differences in any of the three experiments.

Position experiment

The higher incidence of brown heart in fruits form the position high and inner is not associated with differences in total ascorbic acid concentration.

Nutrients

There is also no indication of any influence of nutrients. It might be interesting to test a possible negative effect of N. This would fit into the overall picture that N has a negative influence on quality characteristics in general.

Fruit load

The increase in incidence of brown heart in trees with low degree of bearing is also not accompanied by a lower total ascorbic acid level.

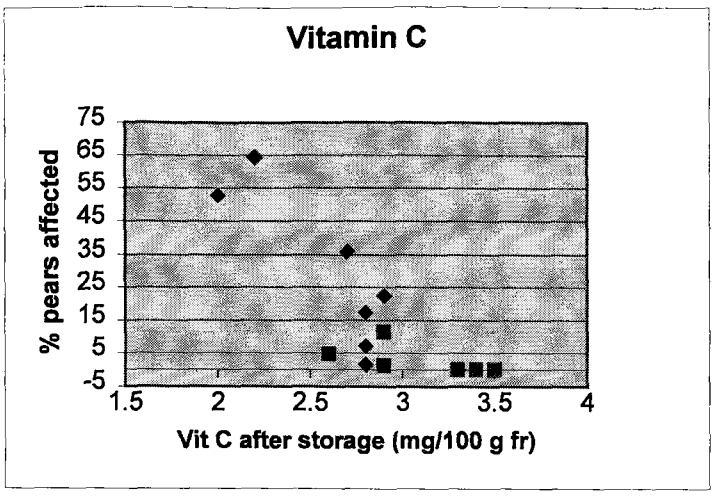

Fig 18. Vitamin C concentration in pears after storage from 7 orchards from pick three (blue) and pick 5 (purple) in relation to the incidence of brown heart.

Table 29. total ascorbic acid concentration (mg/100 gram) in pears of pick 5 after storage depending on position in the tree, treatment with nutrients and level of fruit bearing.

Treatment	Orchard	Vit. C	Treatment	Orchard	Vit. C	Treatment	Orchard	Vit. C
Position			*Mineral*			*Fruit load*		
Top	77	3.0	Control	78	3.2	Normal	17	3.0
	78	2.9		87	3.2		78	2.7
High	77	2.7	Potasium	78	3.1	-1/3 nat.	17	2.8
	78	2.7		87	3.0		78	-
N-O	77	2.8	Calcium	78	3.0	-2/3 nat.	17	3.1
	78	2.7		87	3.0		78	-
S-W	77	2.8	Nitrogen	78	2.6	-1/3 thin.	17	3.3
	78	2.8		87	-		78	3.0
Inner	77	2.9				-2/3 thin.	17	2.8
	78	-					78	3.1

Task 7c. Mineral composition

Objectives

The aim of determining the nutrient status of the fruits is to detect any possible relation of concentration of a particular nutrient and the incidence of brown heart. In order to detect any such relation this has been studied in this project for the second year.

Material and methods

Fruits from 7 orchards from pick 3 were used for analysis of Ca, K, Mg, N and P.

Results

Data of the relationship between nutrient concentration and incidence of brown heart in pick 3 is shown in table 30. The only relationship that might have some meaning and might be interesting for further exploration is that between Ca and brown heart

Table 30. Concentration of nutrients (mg/100 gram fresh weight) and % dry weight at harvest of 7 orchards and incidence of cavities and internal browning in fruits from pick 3 after standard CA-storage.

Orchard	Ca	K	Mg	N	P	%dw	Browning	Cavities
12	7,91	117,64	6,58	50,51	12,03	13,2	1.0	1.0
33	6,89	153,27	7,48	64,90	14,45	14,5	8.6	14.2
77	7,28	146,25	7,09	57,79	11,64	12,9	5.5	6.0
78	5,78	130,98	6,65	57,50	10,28	13,3	5.9	8.3
81	5,86	133,24	6,87	58,31	12,97	11,8	7.0	5.5
177	6,36	135,55	6,36	62,06	11,76	12,7	18.1	26.3
178	6,56	136,25	7,57	69,64	13,25	12,5	0.9	0.9

Conclusions

A possible negative relationship of calcium concentration in the fruit with risk of brown heart can not be excluded.

Continuation

In the third year samples of pick 3 will be analyzed for their concentration of nutrients.

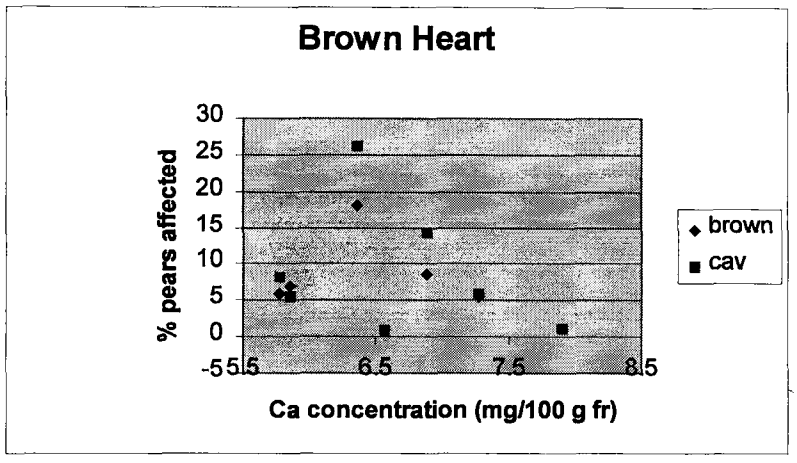

Fig. 19. Relationship between Ca concentration in fruits from pick 3 from 7 orchards and the incidence internal browning (blue) and cavities (purple).

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 5 total cost: (50%)	138,8 kECU	*EC contribution to partner no. 5:*	69.4	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

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Scientific team
Team leader: Prof. dr. ir. Bart Nicolaï
Other scientific staff:, dr. ir. Bert Verlinden, ir Ann Schenk, ir Ann Peirs, ir. Wendy Schotsmans

Objectives
The main objective of the project is the optimisation of the quality of Conference pears, being the most important pear grown within Europe, and the reduction of losses during storage by preventing the development of disorders resulting in Brown Heart in pears. Conference pears are cultivated in all European countries. Brown Heart is a disorder, which causes problems throughout Europe. However, there is difference in incidence of the disorder between countries. Pears grown in the north-western part of Europe show the disorder more often and more severe then pears grown in the Southern part of Europe. Comparison of pears grown in both regions, in different climates, should give sufficient data for thorough conclusions. The key element in the project is the development of technology, which enables a rapid measurement and decision about post-harvest treatments and optimal storage conditions. The main advantage of such a technology is that it leads to an accurate advice, based on characteristics of products of the same harvest, rather then doing research on products from one year to reveal advice on products harvested the next year (with the uncertainty that product physiology might have changed).
Three pathways are most promising towards such an approach, and will be the main objectives of the project:

1. Development of a predictive model based on registered variances in orchard and weather conditions of a broad group of European countries;
2. Development of a predictive model based on a rapid assessment of pear physiology (respiration rate, fermentation rate, resistance to gas diffusion);
3. Testing and implementation of post-harvest treatments, which improve the storability of the harvested pears, leading to pears with a better quality

The models will be used for advice on optimal storage and pre-storage treatments, and for predictions on maximum storability. In addition to adapting storage conditions to changes in pear physiology, it might be possible to increase storability, or reduce the risk of brown heart, by specific post-harvest treatments. Physiological and biochemical measurements within the project are expected to elucidate the processes, which have to be increased or reduced in order to develop these treatments. An additional part of the project is a fourth objective:

4. Development of a non-destructive detection system for Brown Hearts. A non-destructive method would facilitate the use of the same pears for several measurements.

Actions in the project

Task 1:	Cultivation of pears
Task 2:	Harvest of pears
Task 3:	Storage of pears
Task 4:	Variation in climate and orchard characteristics
Task 6:	Measuring gas exchange and diffusion rates
Task 7a:	Destructive measurements, Quality evaluation
Task 9:	Modelling
Task 11:	Dissemination of results

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1998 TO 31-05-1999

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-months:	1
No. of man-month devoted already to the task:	0,5

Objectives

The objective is to produce pears under different climate and orchard conditions, in orchards with varying susceptibility for Brown Heart.

Material and methods

Two orchards were used, one in Velm, the other in Zellik. According to the owner the orchard of Zellik was more susceptible to Brown Heart, the results from the season 97-98 confirmed this.

Results and Discussion

The pears were grown by the owners of the orchards in a normal way, specifics are shown in Table 2.

Task 2: Harvest of pears

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-months:	4
No. of man-month devoted already to the task:	2

Objectives

The objective of this task is to investigate the influence of harvest date on the development of Brown Heart. Different harvest dates are needed in order to get a variance of developmental stages. The dates themselves have to be determined using the same or a comparable method for all partners.

Material and methods

The optimal harvest date was predicted on the basis of firmness, soluble solid content and starch index measured starting at the end of July with weekly intervals. Firmness was measured with a penetrometer using the small plunjer, soluble solid content with a digital refractometer. The starch index was determined with the Eurofru chart. Pears were harvested on the optimal harvest date, 1 week before and 1 week after the optimal harvest date. For each harvest date different trees were used and complete trees were picked.

Results and Discussion

Prediction of optimal harvest date:

Starting from 29/07/98, each week pears were picked at the orchard of Velm. Firmness (Figure 1) decreased faster than in 1997, soluble solid content (Figure 2) was already a little higher than the previous year, and starch index (Figure 3) was a lot higher. From these measurements the Streif index was calculated (Figure 4). With these data and data from previous years the optimal harvest date for long time storage was determined as being 31/08/98.

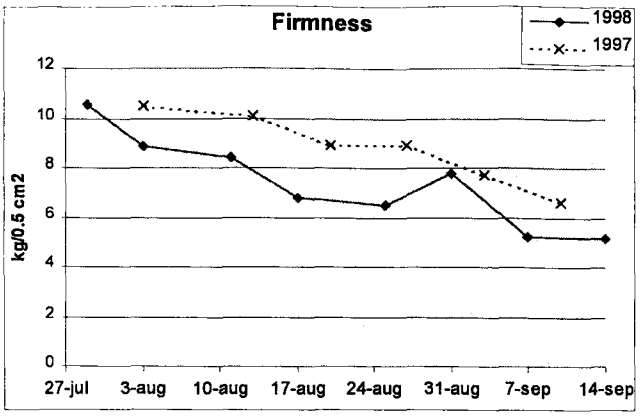


Figure 1: Firmness evolution of Conference pears during growth

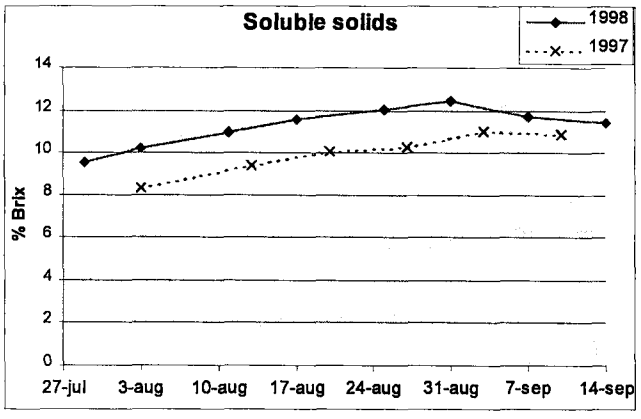


Figure 2: Soluble solids content evolution of Conference pears during growth

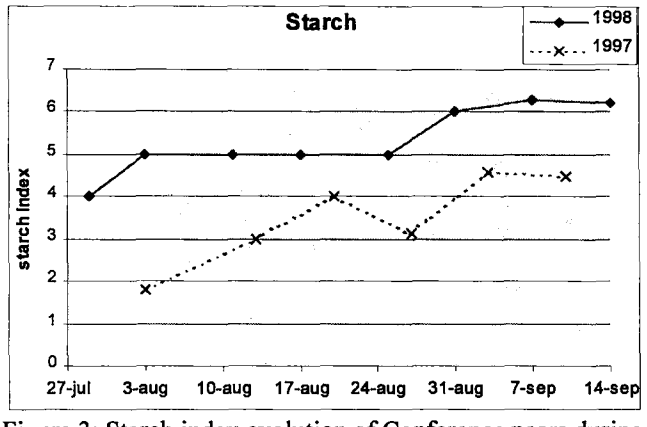


Figure 3: Starch index evolution of Conference pears during growth

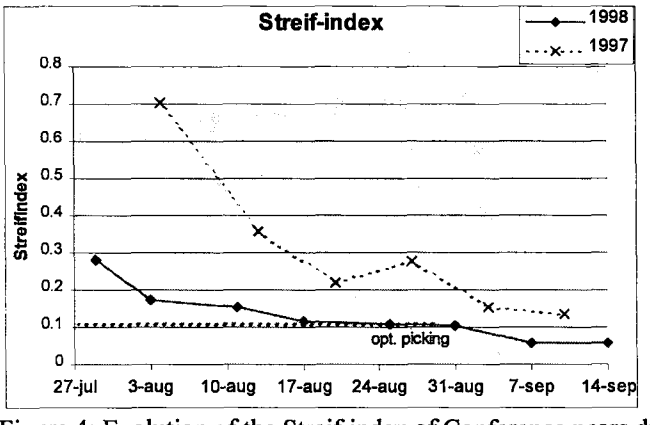


Figure 4: Evolution of the Streif index of Conference pears during growth

Harvest date:

The harvest dates are shown in Table 2. The pears for the brown heart evaluation were harvested at the 3 different dates. At harvest an initial quality evaluation was performed (Table 1) to have a reference to compare the results of the storage experiment with. The pears for respiration and diffusion measurements were harvested at the second and third harvest date.

Table 1: Quality parameters at harvest.

Orchard	Harvest	Diameter (mm)	Weight (g)	Firmness (kg/0.5cm²)	Soluble solids (%Brix)	Brown Heart	Cavities
Velm	I	64.23	228.65	7.79	11.94	0	0
Velm	II	68.19	272.52	7.88	12.59	0	0
Velm	III	68.51	293.63	7.51	12.33	0	0
Zellik	I	64.06	207.60	7.36	11.43	0	0
Zellik	II	66.68	219.44	7.36	11.59	0	0
Zellik	III	69.20	249.56	7.98	10.88	0	0

Task 3: Storage of pears

Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-months: 4
No. of man-month devoted already to the task: 1.5

Objectives

The pears are stored to provide material for the measurements throughout the year for task 6 and 7a.

Material and methods

After picking the pears were stored for 1 week at -0.5°C, 95% RH in air. Then, half of the pears were stored at 2% O_2 and 0.7% CO_2 (control storage conditions) and the other half at 2% O_2, 5% CO_2 (Brown Heart inducing conditions) both at -0.5°C. For each CA-condition 4 containers were used, one for each Brown Heart evaluation during the storage.

Results and Discussion

The containers were opened shortly to put the pears in at the different harvest dates, after closing the containers, storage conditions were reached within 4 hours. After 4 different storage periods 1 container of each CA-condition was opened to provide fruit for task 6 and 7a.

Task 4: Variation in climate and orchard characteristics

Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-months: 1
No. of man-month devoted already to the task: 0,5

Objectives

The objective is to relate the meteorological data with the pear characteristics and occurrence of Brown Heart.

Material and methods

The co-ordinates of the orchard were provided by the geology department of the KULeuven. The orchard at the location Velm has its own weather stations. For the orchard of Zellik data of a nearby (Brussegem, < 1 km) meteorological station was used.

Results and Discussion

Orchard characteristics are shown in Table 2. Temperature, relative humidity, radiation and rainfall were measured and logged every hour. The data are available for Task 9 and have been sent to the ATO-DLO.

Table 2: Orchard characteristics

Characteristic	Velm	Zellik
Location	50°46' NB, 5°07' OL	50°52' NB, 4°16' OL
Soil	Aba	Aba
Plant Year	1991	1981
Full bloom 1997	09/04/1997	09/04/1997
Full bloom 1998	10/04/1998	15/04/1998
Fertilisers 1997-1998	30 E N (111 kg/ha Ammoniumnitrate) 45E P (100 kg/ha triple super phosphate) 100E K (200 kg/ha potasium sulfate)	68 E N (250 kg/ha Ammoniumnitrate) 128 E K (213 kg/ha Chloor potasium) 103 E Mg (367 kg/ha kieseriet)
Fertilisers 1998-1999	30E N (111 kg/ha ammoniumnitrate) 45E P (100 kg/ha triple super phosphate) 50E K (100 kg/ha potasium sulfate)	41E N (150 kg/ha ammoniumnitrate) 128E K (304 kg/ha patentkali)
Foliar fertilisers 1997-1998	7 kg/ha ureum 9 kg/ha monokalifosfate 26 kg/ha calcium nitrate	63 kg/ha ureum 1.02 kg/ha monoammoniumphosphate 0.75 kg/ha solubor 0.58 kg/ha Mantrac 6 kg/ha monokaliphosphate 7.13 kg/ha magnesiumphosphate 20.37 kg/ha potasium nitrate
Foliar fertilisers 1998-1999	18 kg/ha ureum 95kg/ha monokaliphosphate 15 kg/ha calcium nitrate	21.02 kg/ha ureum 1 kg/ha monoammoniumphosphate 0.48 kg/ha solubor 0.29 kg/ha Mantrac 15.46 kg/ha monokaliphosphate 5 kg/ha magnesiumsulfate 15 kg/ha potasium nitrate
Harvest I (1997)	8/09/97	8/09/97
Harvest II (1997)	16/09/97	16/09/97
Harvest III (1997)	22/09/97	22/09/97
Harvest I (1998)	24/08/98	26/08/98
Harvest II (1998)	31/08/98	31/08/98
Harvest III (1998)	07/09/98	07/09/98
Evaluation 1 (1997)	6-7/11/97	6-7/11/97
Evaluation 2 (1997)	22-23/12/97	22-23/12/97
Evaluation 3 (1997)	4-6/02/98	4-6/02/98
Evaluation 4 (1997)	11-12/03/98	11-12/03/98
Evaluation 1 (1998)	26-27/10/98	26-27/10/98
Evaluation 2 (1998)	30/11/98	30/11/98
Evaluation 3 (1998)	04-05/01/99	04-05/01/99
Evaluation 4 (1998)	22/03/99	22/03/99

Task 6: Measuring gas exchange and diffusion rates

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-months:	7
No. of man-month devoted already to the task:	3

Objectives

Testing the methodology for measuring gas exchange and diffusion resistance. Determination of respiration rates and diffusion resistance values under normal and Brown Heart inducing storage conditions during the storage period for the different harvest dates.

Material and methods

Gas exchange rates

The respiration rate of the pears was measured under several temperature and gas conditions as shown in Table 3.

Table 3: Gas conditions for respiration measurements

Temperature (°C)	O_2 concentration (%)	CO_2 concentration (%)
2	0	0
	0.8	1
	3	5
	8	
	20.8	

The gas mixtures were prepared using a gas-mixing device with four channels. Each run four different gas mixtures can be prepared. The pears were put in respiration flasks of approximately 1.2 litre. The experimental set-up for the respiration measurements is shown in Figure 5. Four flasks are connected together on one channel coming from the gas-mixing device. The pears were allowed to equilibrate with the chosen atmosphere while the respiration container was flushed with the particular gas mixture. Before the first flask and after the last, water flasks were connected. The first water flask ensured that the atmosphere was humid, the last functioned as a water lock to ensure that the surrounding air can not flow into the flask in case the flow should stop. After 48 hours the respiration containers were closed and the concentrations of O_2 and CO_2 were measured using a CP-2002P micro-GC from Chrompack. After about 16 hours the concentrations of O_2 and CO_2 were measured again. Respiration rates were calculated from the concentration changes.

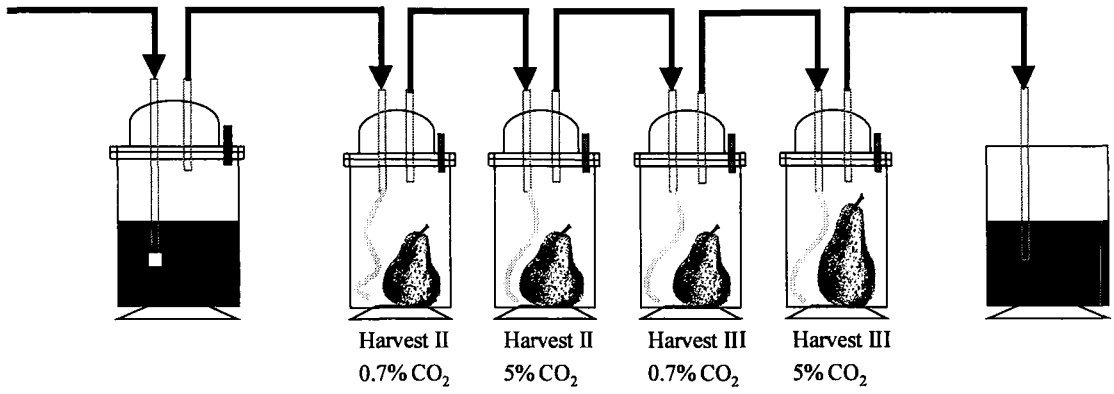


Figure 5: Experimental setup respiration

Diffusion resistance

Efflux-method: After the respiration experiment an amount of neon was injected into the flask. The fruit was stored under this high neon concentration (0.5-2.5kPa) over night until equilibrium was reached. In the morning the final equilibrium concentration was measured. This concentration is considered as the initial concentration inside the fruit when this is placed in a second, neon free flask. After specific time intervals a sample of the atmosphere in the flask was measured using a CP-2002P micro-GC from Chrompack. After this the fresh weight and the under water weight were measured. Diffusion resistance was only measured the 1st, 2nd and 3rd evaluation, so not at harvest due to lack of time.

Results and Discussion

Gas exchange rates

The 4 pears in a series of flasks had a different history regarding harvest date and storage conditions. The results of the respiration measurements were therefor statistically analysed to see if any influence of the different parameters (CO_2 concentration during the respiration measurements, storage time, harvest date) could be found. As it shows in Table 4 no significant influence was found for storage time, harvest date and storage conditions at a 95% significance level, therefor the data were pooled in one dataset to estimate the Michaelis Menten parameters to describe O_2 consumption and CO_2 production rates.

Table 4: Statistical analysis of respiration measurements ($\alpha = 0.05$).

Factor	P-value for O_2 consumption	P-value for CO_2 production
CO_2 level for respiration	0.0322	0.0001
Storage time	0.1553	0.2725
Harvest date	0.1005	0.0857
Storage conditions	0.5104	0.1704

Different models were used for O_2 consumption rate, a model with competitive inhibition, one for an uncompetitive type of inhibition, one for a non-competitive type of inhibition and one for a combination of the competitive and uncompetitive type. After evaluating the results the non-competitive type of inhibition (Equation 1) was considered the best with Vm_{O2} the maximum O_2 consumption rate (nmol/kg.s), Km_{O2} and Km_{CO2} (kPa) the Michaelis Menten constants, C_{O2} and C_{CO2} respectively the O_2 and CO_2 concentrations in kPa.

$$V_{O_2} = \frac{Vm_{O_2} * C_{O_2}}{\left(Km_{O_2} + C_{O_2}\right) * \left(1 + \frac{C_{CO_2}}{Kmn_{CO_2}}\right)}$$

Equation 1: Model for non-competitive inhibition of O_2 consumption by CO_2.

The total CO_2 production is a sum of oxidative and fermentative CO_2 production using the model given in Equation 2 with RQox the ratio between oxidative CO_2 production and O_2 uptake, Vmf_{CO2} (nmol/kg.s) the maximum fermentative CO_2 production and Kmf_{CO2} (kPa) the Michaelis Menten constant for the inhibition of CO_2 production by O_2.

$$V_{CO_2} = RQ_{ox} * V_{O_2} + \frac{Vmf_{CO_2}}{1 + C_{O_2}/Kmf_{O_2}}$$

Equation 2: Model for CO_2 production as a sum of oxidative and fermentative CO_2 production.

Table 5: Fitted parameter values for the respiration model

Parameter	Estimate	Standard Error
Vm_{O2} (nmol/kg.s)	23.5	1.2
Km_{O2} (kPa)	2.2	0.4
Kmn_{CO2} (kPa)	7.7	1.5
Rqox (-)	0.731	0.018
Vmf_{CO2} (nmol/kg.s)	16.0	0.6
Kmf_{O2} (kPa)	0.32	0.05

The estimated parameters are given in table 5and the model was fitted on the experimental data.

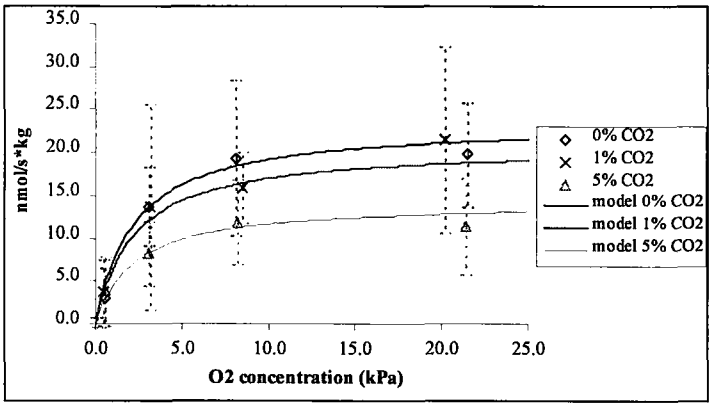


Figure 6: Oxygen consumption as a function of the chosen O_2 concentration at different CO_2 concentrations at 2°C (the symbols represent mean O_2 consumption rates and the dotted lines are the standard errors).

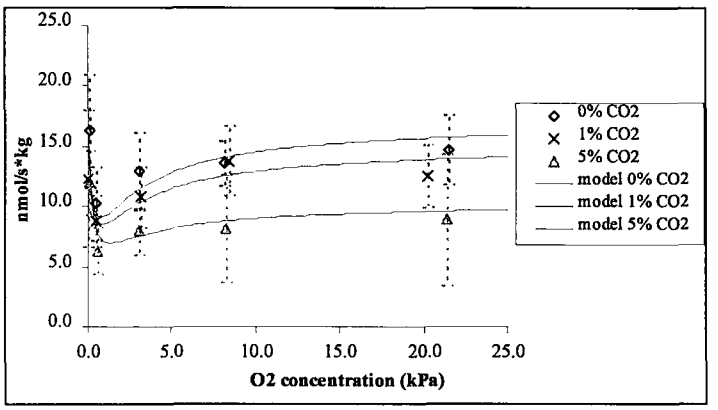


Figure 7: CO_2 production as a function of the chosen O_2 concentration at different CO_2 concentrations at 2°C (the symbols represent mean CO_2 production rates and the dotted lines are the standard errors).

Diffusion resistance

About 220 pears were measured during the 2nd and 3rd evaluation after the respiration measurements were done. Due to problems with the method and the GC measurements only 87 pears could be used for further analysis. For these pears linear trend lines were drawn as is shown in Figure 8.

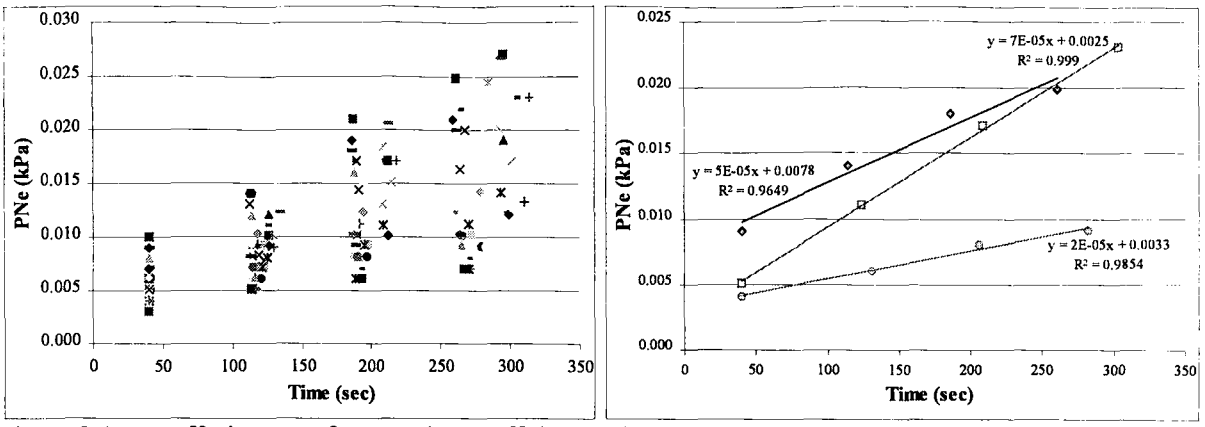


Figure 8: Neon effusion out of pears. Some off the trend lines and the corresponding trend line equations are displayed.

Using the slope of these trend lines the resistance was calculated using the following formula.

$$R = \frac{C_i(0)A}{V_e\, dC_e(t)/dt}$$

Equation 3: Calculation of the resistance to gas effusion using the linear method by Banks.

In Table 6 the effusion resistance values for the different evaluations are displayed, there are no significant differences between them.

Table 6: Effusion resistance values

Evaluation	Resistance to neon effusion	Resistance to O_2 effusion	Resistance to CO_2 effusion
2	400000±200000	500000	600000
3	320000±140000	400000	500000

The standard deviations are very big. We also looked for correlations between the resistance and the parameters in the formula, they should be independent (Figure 9).

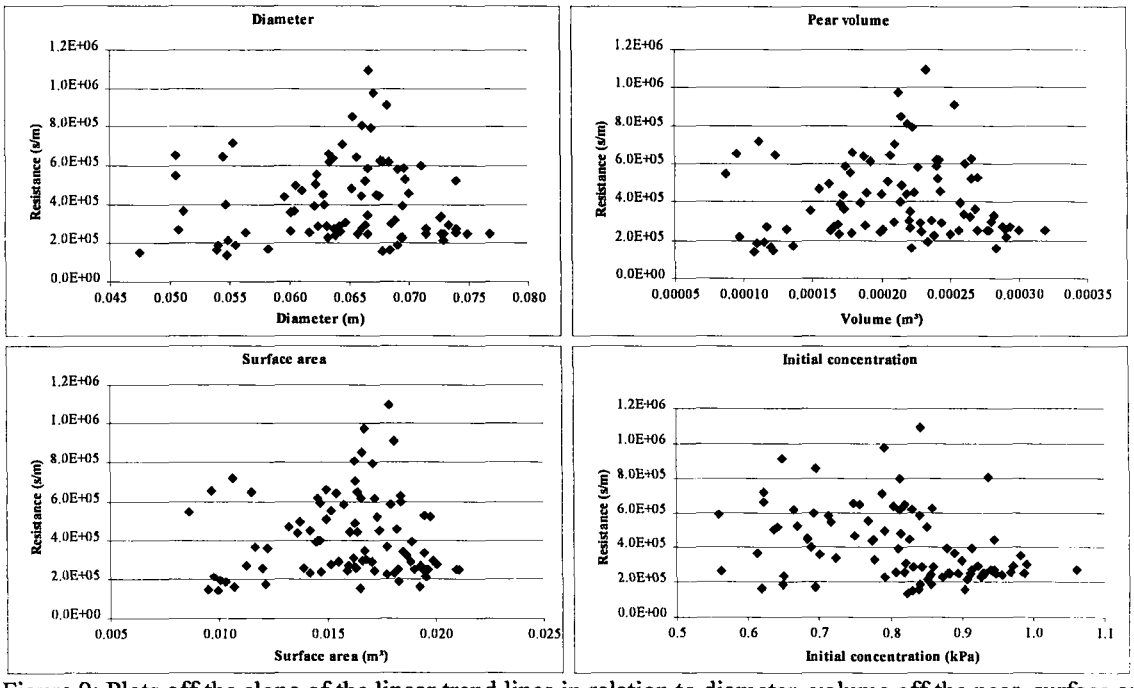
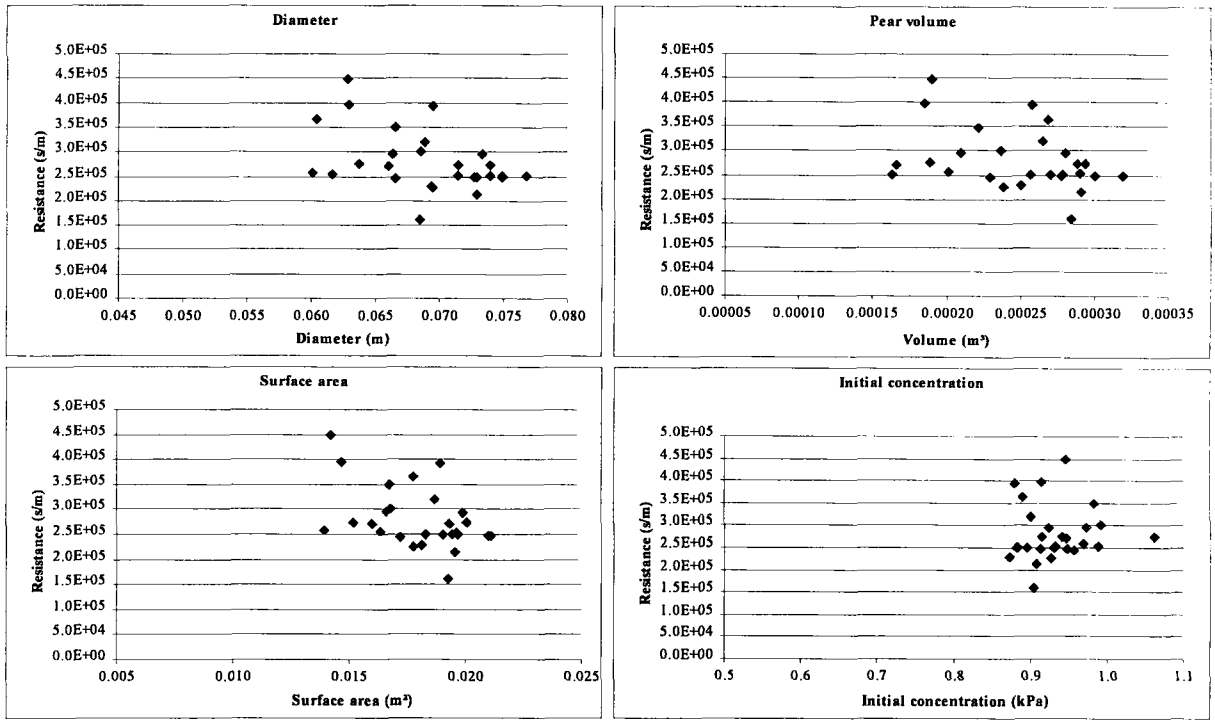


Figure 9: Plots off the slope of the linear trend lines in relation to diameter, volume off the pear, surface area and the initial gas concentration.

There does not seem to be any correlation but it is clear that the variance is very large. In the plot of resistance versus initial concentrations, the variance is much smaller when the initial concentration is higher. Therefore we looked at the resistance values for initial concentrations larger then 8.5 kPa. The variance is a lot smaller now.

Table 7: Effusion resistance values with Ci > 0.85

Evaluation	Resistance to neon effusion	Resistance to O_2 effusion	Resistance to CO_2 effusion
2	270000±60000	340000	400000
3	320000±70000	400000	470000



Diffusion measurements using the efflux method are very difficult and there is a lot of variance.

Task 7a: Destructive measurements, quality evaluation

Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-months: 4
No. of man-month devoted already to the task: 2

Objectives

To evaluate the quality changes and Brown Heart occurrence during storage for different storage conditions and periods, different orchards and different harvest dates.

Material and methods

Selection of pears

For each harvest date trees were randomly selected and completely picked at the chosen harvest date. All the pears of one orchard were than gathered and randomly 80 pears were selected for each storage condition, evaluation combination. The rest of the pears were evaluated at harvest.

Quality evaluation method

The following characteristics were measured: weight, diameter, ground colour (Eurofru colour chart), firmness (penetrometer) and soluble solid content (digital refractometer).
After measuring the different quality characteristics the occurrence of brown spots and cavities was assessed using the picture chart delivered by partner 4. The pear was sliced in two from stem to blossom end and compared with a range of pictures. Each pear was classified on a scale from 0 to 4 for both brown spots and cavities.

Results and Discussion

Harvest date, orchard, storage period, storage conditions

At this date, all four quality-evaluations were carried out and statistical analysis (ANOVA) showed a clear effect of harvest date, orchard, storage period and storage conditions (see Table 8) on the occurrence of brown heart and cavities.

Table 8: Statistical analysis of brown heart measurements ($\alpha = 0.05$).

Factor	P-value for brown spots	P-value for cavities
Harvest date	0.0001	0.0001
Orchard	0.0001	0.0001
Storage period	0.0001	0.0001
Storage conditions	0.0001	0.0001

As shown in Figure 10 later picked pears show more severe occurrence of brown spots and the pears from Zellik were more sensitive than Velm.

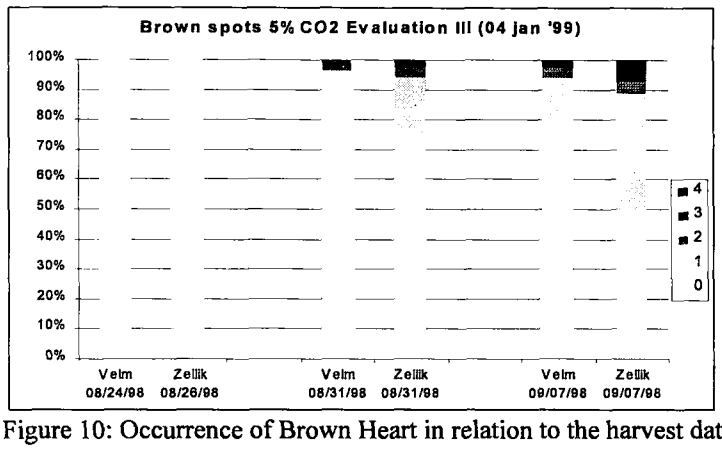


Figure 10: Occurrence of Brown Heart in relation to the harvest date and the orchard.

The effect of storage period and conditions is shown in Figure 11 for brown spots and in Figure 12 for cavities. The longer pears were stored the more likely they develop Brown Heart. The Brown Heart inducing storage conditions (2% O_2, 5% CO_2) had more browning and cavities then the control storage conditions.

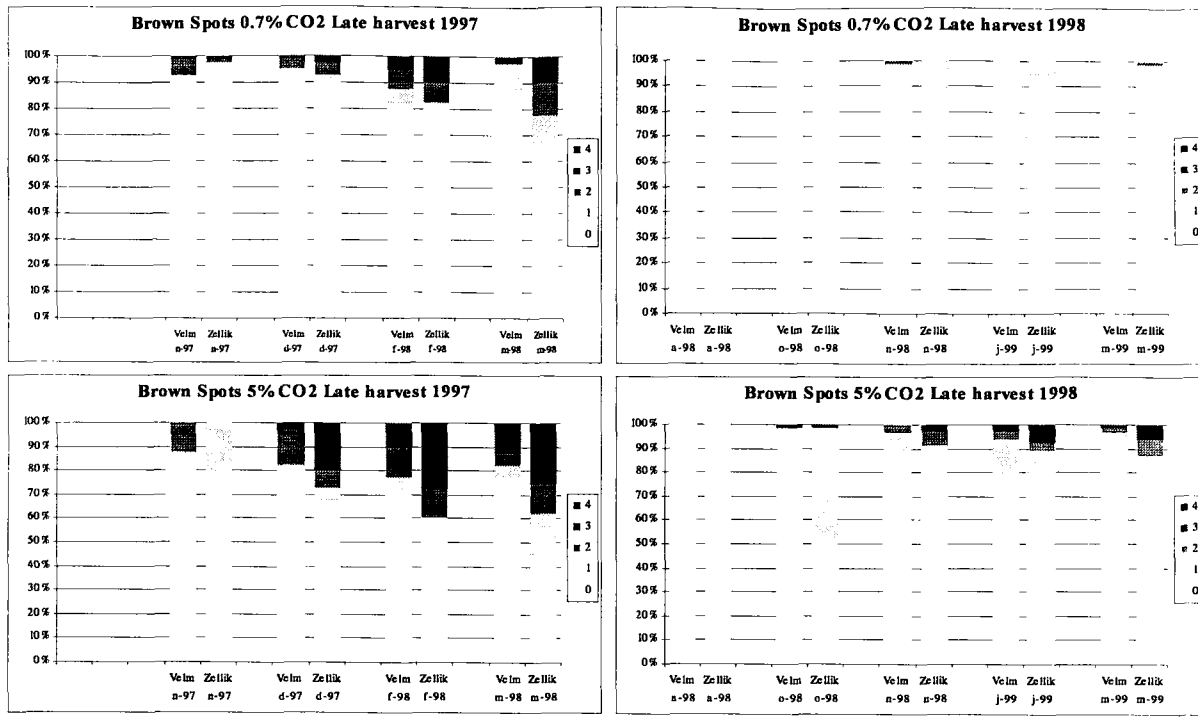


Figure 11: Brown spot occurrence.

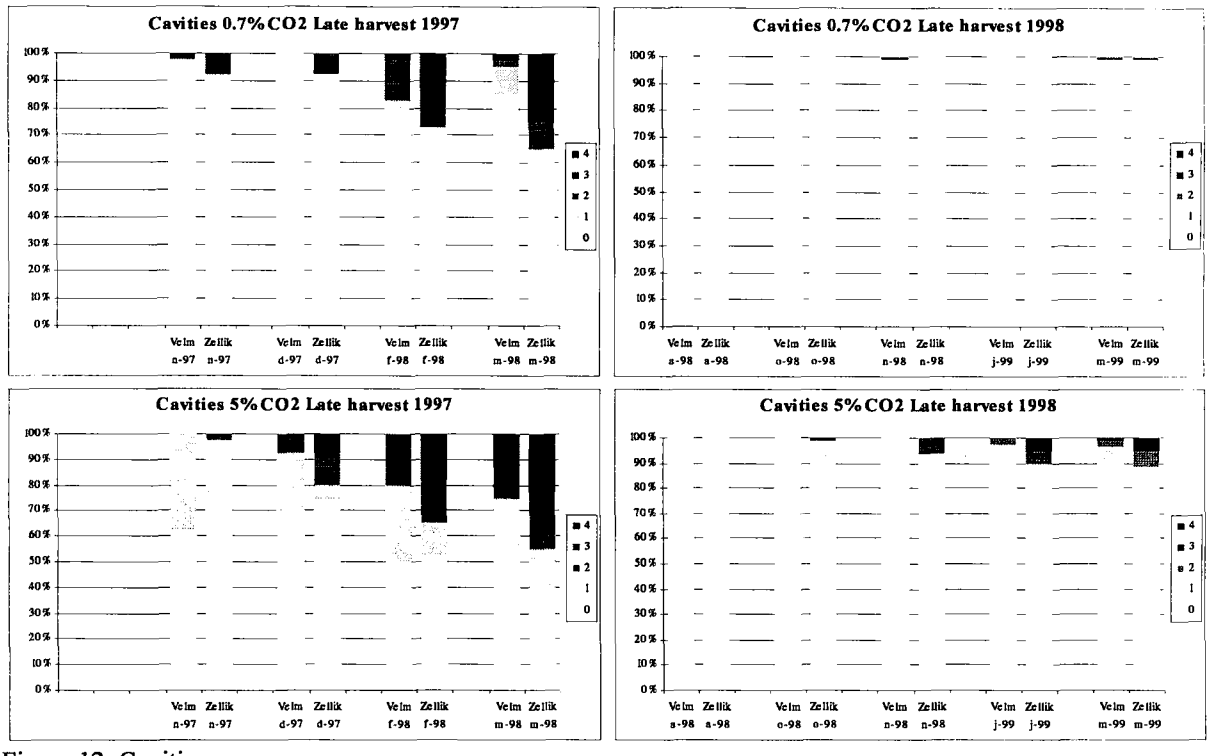


Figure 12: Cavities occurrence.

Figure 11 and Figure 12 also give a comparison between the 2 seasons (1997-1998 and 1998-1999). When data of this year were compared to data from last year there was a clear difference. Some examples for brown spots are shown in Figure 11 while Figure 12 gives some examples for cavities. In the season 1998-1999 the extent of brown heart occurrence was much smaller. In some cases the amount off infected pears was the same as but not as severe as during the season 1997-1998.

When evaluating this difference between the two seasons we have to remember that there have been some storage problems during 1997-1998.
Correlations can also be found between brown heart occurrence and other quality attributes.

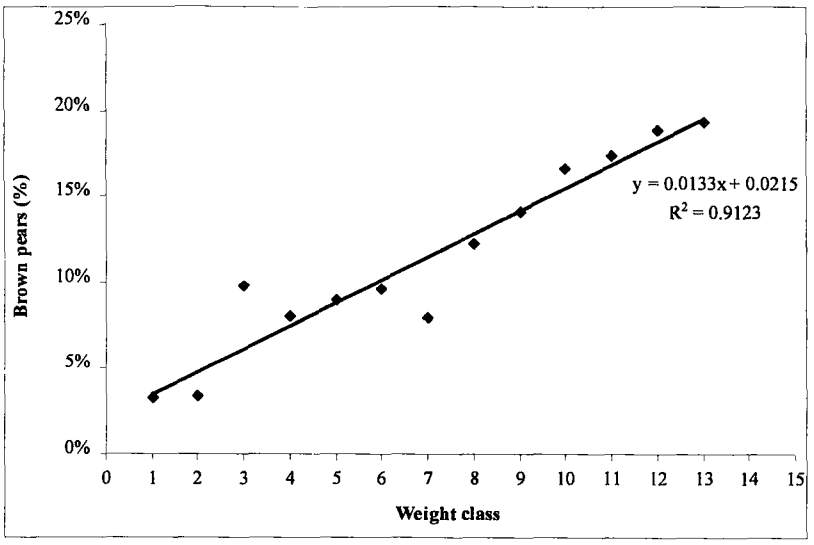


Figure 13: Percentage brown pears in function of weight classes. 1: less then 80 g., 2: 81-100 g, 3: 101-120 g, 4: 121-140 g, 5: 141-160 g, 6: 161-180 g,...

In Figure 13 the percentage of brown pears was plotted as a function of weight classes and we found a liner trend here, when the same plot is made for diameter classes a quadratic trend is found (Figure 14) which is logical because there exists a quadratic relation between the weight and diameter of a pear.
If we simplify the shape of a pear and consider it a cone, the weight equals the volume times the density. The volume of a cone equals $R^2*h*\pi/3$ with R the largest diameter and h the height. So the weight of a cone in proportional to the power of the largest diameter.

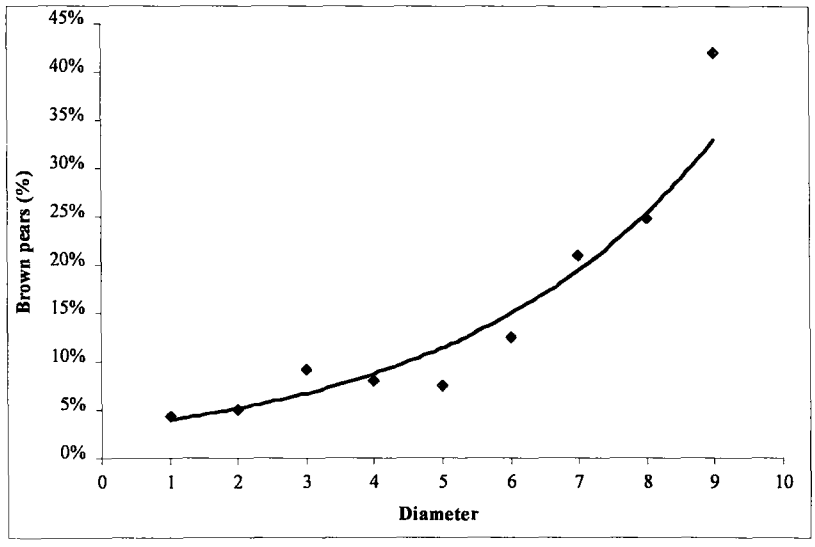


Figure 14: Percentage brown pears in function of diameter classes. 1: less then 45 mm., 2: 45-50 mm, 3: 50-55 mm, 4: 55-60 mm, 5: 60-65 mm, 6: 65-70 mm,...

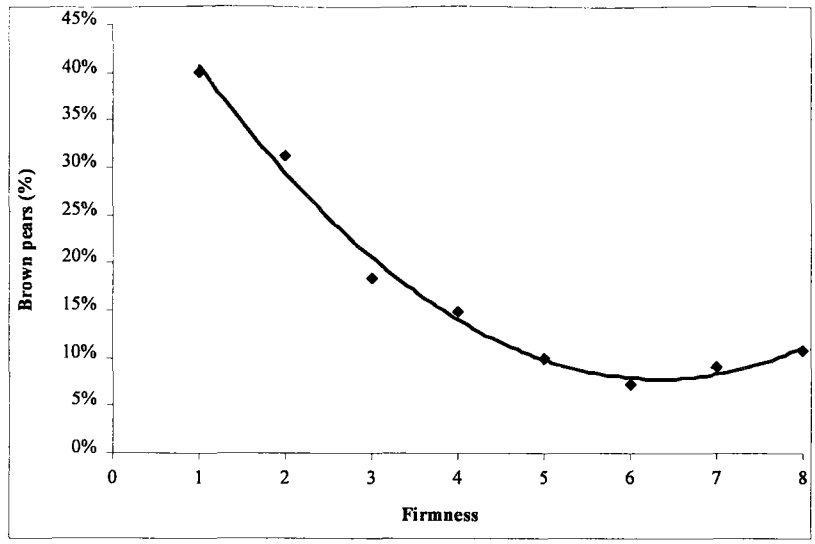


Figure 15: Percentage brown pears in function of firmness classes. 1: less then 4 kg., 2: 4-5 kg, 3: 5-6 kg, 4: 6-7 kg, 5: 7-8 kg, 6:8-9 kg,...

There is also a strong correlation between Brown Heart occurrence and firmness (Figure 15). The firmer the pears the less likely they get the disorder.

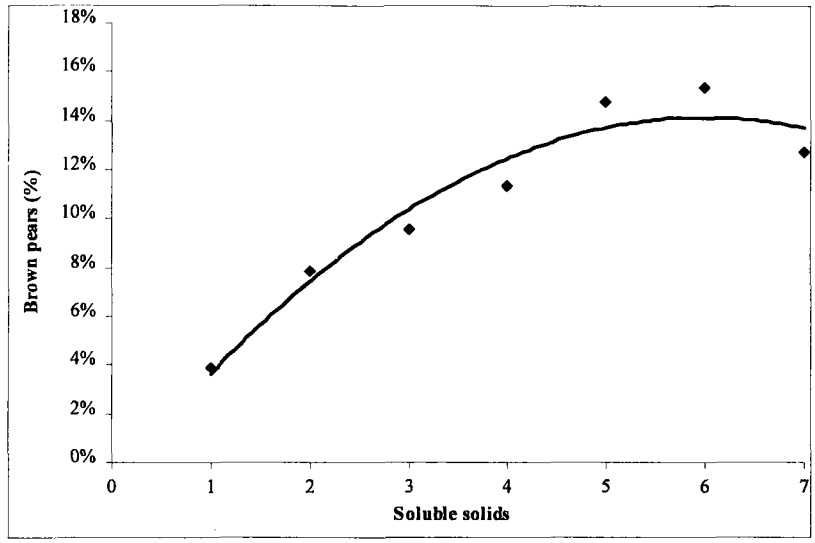


Figure 16: Percentage brown pears in function of soluble solids classes. 1: less then 11 %Brix, 2: 11-11.5 %Brix, 3: 11.5-12 %Brix, 4: 12-12.5 %Brix...

There is also a quadratic correlation between soluble solids and occurrence of Brown Heart (Figure 16).

Task 9: Modelling

Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-months:	4
No. of man-month devoted already to the task:	1

Objectives

The objective is to build predictive models, which can be used in practice to know in advance how long and under which conditions a certain set of pears can be stored before developing the disorder.

Material and methods

Materials
The data gathered in Task 7a form the season 1997-98 was used in this modelling task. On each pear a series of measurements was carried out. Each pear was weighed (g) and the largest diameter (mm) was measured with a calliper. The soluble solids content (SSC) was measured with a refractometer (PR-101, ATAGO, Japan). A Magness-Taylor penetrometer with a 8mm probe was used to measure the firmness of the fruit. Finally, each pear was cut in two and the absence or presence of the disorder was coded with 0 and 1, respectively.

Statistical analysis
Logistic regression
Logistic regression is a statistical method to analyse binary and binomial response data. It is based on the construction of a statistical model describing the relationship between the observed response and explanatory variables, also called independent variables. The dependence of the probability of disorder on explanatory variables is modelled as follows

$$\text{logit}(\pi_i) = \log\left(\frac{\pi_i}{1-\pi_i}\right) = \alpha + \beta' \mathbf{x}_i \qquad (1)$$

where
π_i is the probability to develop the disorder given the set of explanatory variables,
α is the intercept parameter,
β is the vector of slope parameters and,
$\mathbf{x}_i$ is the vector of explanatory variables.

The logit function transforms the probability scale from the range (0,1) to $(-\infty, +\infty)$. Other transformations are possible, but the logit transformation leads to coefficients β interpretable in terms of odds ratios (a measure of association, widely used especially in epidemiology). All statistical analyses in this report were performed using the SAS/STAT software, version 6.11 (SAS Institute Inc., Cary NC. USA).

Dependent and independent variables
The absence and presence of the storage disorder was coded, respectively, with 0 and 1. In the first two predictive models the occurrence of internal brown and occurrence of cavities are considered as two separate disorders. However, in reality both phenomena are expected to be coupled, and thus, also a joint model for brown and cavities is constructed. The factor picking date was coded with the cell reference coding system. The second picking date (pears picked at the optimal time) is chosen as reference. The other picking dates are compared to this reference. Since the factor picking date has three levels, 2 dummy design variables were used. The continuous variable weight was polychotomised. Three weight classes were defined: <100g, between 100 and 170g and >170g. The class of the lightest pears was taken as reference class and again two dummy variables were used.

The explanatory variables were divided into two groups. The first group contained all the external variables like conservation time, oxygen and carbon dioxide concentration, temperature and picking date. The second group contained the intrinsic variables: sugar content, hardness, size, weight and colour. For both response variables, brown and cavity, models were built with the external variables and with the variables of both groups, to investigate whether the addition of intrinsic parameters improved the model.
All possible first order interaction terms were used in the model selection procedure and, to avoid multicollinearity problems, all variables introduced in the model with a quadratic main effect were first mean-centred.

Model selection criteria
The set of 2016 observations was randomly divided into a calibration set (1816 observations) and a validation set (200 observations). In order to select an appropriate subset of important explanatory variables, the calibration set was used to generate 10 different data sets of 1616 observations. The sets were constructed by a random elimination of 200 observations of the calibration set. On each of the 10 data sets and on the complete calibration set a stepwise, a backward and a forward multiple logistic regression procedure was performed. The most important variables were selected from this. Biologically important variables (e.g., temperature) were entered manually in the model.

Results and Discussion

Models for internal brown
Only external explanatory variables
In Table 9 the model parameter estimates, the odds ratios and the 95% lower and upper Wald confidence limits are given.
The picking date is a very important variable in this model. Picking I is the dummy variable standing for the early picking date. A change from optimal to early picking time corresponds to an odds ratio of 0.089, indicating that internal brown occurs about ten times as often (in terms of odds) for optimal picking time than for early picking time. Picking II is the dummy variable for the late picking time. The odds for brown increase by a factor 3.8 for the over mature picked pears compared to the optimally picked ones. Since all the other variables left in the model occur in interaction terms, the interpretation of their odds ratios and their coefficients is not straightforward anymore. These odds ratios are indicated by an asterisk in Table 9. The variable temperature, for instance, occurs as main effect but also in interaction with the carbon dioxide concentration: the coefficient of temperature is a function of the carbon dioxide concentration ($0.00933+0.1494\times CO_2$). For a fixed temperature, the effect of temperature on brown will increase for higher CO_2 concentrations, and this will cause more brown spots. In a similar way, oxygen, carbon dioxide and temperature interact. The coefficient of carbon equals $0.14-0.13\times O_2$ $+0.15\times$temperature. For a fixed temperature equal to 0°C and a low O_2 concentration, a positive relation exists between the CO_2 concentration and the incidence of internal browning (higher CO_2 concentration lead to more browning). For higher O_2 levels, this relation becomes negative. No physiological explanation could be found for this statistical relationship.
The interpretation of the oxygen variable is even more complex. Figure 17 shows the value of the oxygen coefficient as a function of the carbon dioxide concentration and the storage time. The oxygen concentration is always negatively related to the incidence of brown heart whatever the carbon dioxide concentration or the storage time may be. However, during the first month the influence of oxygen increases (decreasing coefficient) to reach maximal importance around 4 months. Afterwards the oxygen influence on internal browning decreases again. Independent of the storage time, the influence of oxygen on brown increases with the carbon dioxide concentration. Finally, the analysis of the storage effect on brown is far more complicated. The presence of a mean and a quadratic effect (see Table 9) indicates that brown increases during the first storage

period and decreases afterwards. This may be an indication for the fact that brown is replaced by cavities. The cavity model later on will illustrate that cavity is linearly related to storage time.

External and intrinsic explanatory variables
In order to investigate the role of some intrinsic fruit parameters in the development of internal browning, a model is built with both the external and the intrinsic parameters. The same heterogenity factor as in model 1 was used to correct for overdispersion. Table 10 summarises the model. The main effect for temperature and storage time do not meet the 5% level of significance for entry in the model, but because of the 'hierarchical principle' it is recommended to add insignificant main effects to the model when significant higher order main and interaction effects are included in the model. Most of the information about temperature is enclosed in the interaction terms between temperature and carbon dioxide. Softer pears are more influential to internal browning than harder ones: an increase of hardness with one unit increases the odds of browning with 39%. The influence of picking date and storage time is the same as described earlier. Important to note is also the fact that pears with a weight between 100 and 170g are 3 times more susceptible to browning than lighter pears ($<$100g) but 4 times less susceptible than heavy pears ($>$170g).
The analysis of the oxygen leads to similar results as those obtained in the analysis of model 1. Figure 18 shows the odds ratio for oxygen as a function of storage time and carbon dioxide concentration. In this plot is shown how the odds for internal brown changes for a 1% increase in oxygen concentration. For a storage time of 50 days and a carbon dioxide concentration of 0.7% the odds for internal browning will be about 3 times less when oxygen is increased with 1%. For long storage times and low carbon dioxide concentrations the odds ratio is almost equal to one. An increase in oxygen will then have very low influence on the development of internal brown. Table 10 also shows the rather complicated effect of carbon dioxide concentration on the probability of browning. The estimate of the main effect parameter is positive but incorporating interaction terms, the effect of carbon dioxide increases with decreasing oxygen concentration and decreases with decreasing temperatures and increasing colour value. The model classifies up to 85% of the calibration pears in the right class, whereas 83% of the validation pears are classified correctly. Again a compromise has to be found between sensitivity and specificity depending on the purpose the model is used for. The model for internal browning lowers the value of -2 Log L from 1442 to 867. Inclusion of the intrinsic parameters decreases its value further to 821, indicating the importance of the intrinsic variables.

Models for cavities

Only external explanatory variables
Table 11 gives an overview of the important parameters for the model (heterogenity factor=1.414). The influence of temperature on cavities changes from a positive value for low to a negative value for high oxygen levels. Also here, oxygen concentration is negatively related to the occurrence of cavities. This model classifies up to 84% of the pears correctly, depending on the choice of the cut off value.

External and intrinsic explanatory variables
The odds ratios for picking date and pear weight are comparable to those of the model for internal browning. Pears containing less sugar seem tend to contain more often cavities (see Table 12). The storage time is positively related to the occurrence of cavities, but this relationship is weakened for higher carbon dioxide concentrations. The odds ratio for oxygen is a function of colour and temperature, but within the range of those variables, oxygen has an odds ratio (for a unit increase) lower than 1. Carbon dioxide interacts with both the storage time and the hardness. The prediction performance of this model is 86% and 83% of the pears from the validation set are sorted correctly.

Both values hold for a cut off value of 0.5. A 59 units decrease in -2 Log L value shows that the addition of the intrinsic parameters improves the model.
Figure 19 shows the probability of having internal brown (upper plane) and cavities (lower plane) as a function of the storage time and the oxygen concentration. In this plot, based on the models with only external parameters, the temperature (0°C), the carbon dioxide concentration (1%) and the picking date (optimal) are held constant. Important to note is the quadratic and linear effect of time on respectively brown and cavities. Around 22 weeks (154 days) of storage the probability for brown is maximal (30%) and the incidence of cavity is equal to 13%. For low oxygen concentrations the quadratic storage time effect on brown can be seen. High oxygen concentrations (>2%) will not lead to core breakdown. In order to avoid core breakdown, controlled atmosphere storage conditions with high oxygen levels are recommended, but this will cause a faster quality decay of the stored product. The decrease in brown after 150 days can be an indication that brown disappears while cavities show up more often. This hypothesis is investigated in the next experiment.

Conclusion

Although there is not always a simple interpretation of the effects of the variables in the models built, there are some general tendencies uncovered by the models. A low oxygen concentration will always cause more brown and cavities. Over mature picked pears are far more susceptible to core breakdown. Heavier pears will develop 3 to 4 times more cavities and internal brown than light pears. The storage time is quadratically related to brown and linearly to cavity. This might be an indication that brown disappears in favour of cavities. This hypothesis is made stronger by the last experiment where it is shown that a pear will first develop brown spots before cavities occur. Since the picking time is confounded with cooling period preceding the controlled atmosphere conditions, further research will be carried out to separate both variables. The role of carbon dioxide is complex because of the interaction terms. But in general, a higher carbon dioxide concentration corresponds to more brown and cavities.
Inclusion of the intrinsic parameters improves the models. However, the functionality of variables like sugar and hardness is doubtful, since both variables require a destructive test. Their importance would increase if non-destructive tests could be used to predict hardness and sugar content.
The models can also be used to classify the pears in having core breakdown or not. Depending on the cut off value the classification performances varied between 80% and 86%.

Table 9: Summary of statistical analysis for model for internal browning: external parameters as explanatory variables.

Variable	Parameter estimate	Wald Confidence limits Lower	Upper	Odds ratio
Intercept	0.6414	0.0394	1.2435	-
Carbon dioxide	0.1444	0.0166	0.2722	1.155*
Storage time	0.000622	-0.00258	0.00382	1.001*
Oxygen	-1.3515	-1.7700	-0.9330	0.259*
Picking I	-2.4176	-2.9937	-1.8416	0.089
Picking II	1.3294	0.9655	1.6933	3.779
Temperature	0.00933	-0.3404	0.3590	1.009*
Storage time^2	-0.00014	-0.00019	-0.00008	1.000*
Storage time.Oxygen	0.00373	0.00235	0.00512	1.004*
Storage time^2.Oxygen	0.000087	0.000055	0.000119	1.000*
Oxygen.Carbon dioxide	-0.1333	-0.2217	-0.0450	0.875*
Temperature.Carbon dioxide	0.1494	0.0457	0.2531	1.161*

-2 Log L: 1442 intercept only, 867 intercept and covariates. AIC: 1444 intercept only, 891 intercept and covariates. Hosmer and Lemeshow Goodness of Fit Test: 11.43 with 7 df, p=0.12. All variables entered in the model met the 0.05 level of significance, unless stated else. * = not directly interpretable odds ratio's.

Table 10: Summary of statistical analysis for model for internal browning: external and intrinsic parameters as explanatory variables.

Variable	Parameter estimate	Wald Confidence limits Lower	Upper	Odds ratio
Intercept	1.2496	-1.9946	4.4938	-
Carbon dioxide	0.3337	0.1492	0.5183	1.396*
Storage time	0.00343	-0.00027	0.00713	1.003*
Oxygen	-1.5450	-1.9960	-1.0940	0.213*
Picking I	-2.7571	-3.3793	-2.1348	0.063
Picking II	1.4213	1.0388	1.8038	4.143
Temperature	-0.0112	-0.3936	0.3713	0.989*
Storage time^2	-0.00017	-0.00023	-0.00011	1.000*
Storage time.Oxygen	0.00405	0.00254	0.00557	1.004*
Storage time^2.Oxygen	0.000099	0.000064	0.000133	1.000*
Oxygen.Carbon dioxide	-0.0913	-0.1860	0.00345	0.913*
Temperature.Carbon dioxide	0.1858	0.0747	0.2970	1.204*
Colour	0.2995	0.0665	0.5325	1.349*
Hardness	0.3298	0.1309	0.5287	1.391
Sugar	-0.2555	-0.4376	-0.0734	0.775
WeightI	1.0817	0.4162	1.7471	2.950
WeightII	1.4320	0.7021	2.1619	4.187
Carbon dioxide.Colour	-0.1214	-0.1965	-0.0463	0.886*

-2 Log L: 1442 intercept only, 821 intercept and covariates. AIC: 1444 intercept only, 857 intercept and covariates. Hosmer and Lemeshow Goodness of Fit Test: 7.6 with 7 df, p=0.37. All variables entered in the model met the 0.05 level of significance, unless stated else. * = not directly interpretable odds ratio's.

Table 11: Summary of statistical analysis for model for cavity development: external parameters as explanatory variables.

Variable	Parameter estimate	Wald Confidence limits Lower	Upper	Odds ratio
Intercept	-1.0828	-1.4051	-0.7605	-
Storage time	0.00697	0.00439	0.00956	1.007
Oxygen	-0.3505	-0.4817	-0.2194	0.704*
Picking I	-2.3669	-3.1331	-1.6007	0.094
Picking II	1.4410	1.0806	1.8014	4.225
Temperature	0.2706	-0.0847	0.6259	1.311*
Temperature.Oxygen	-0.2465	-0.4755	-0.0174	0.782*

-2 Log L: 1219 intercept only, 820 intercept and covariates. AIC: 1221 intercept only, 834 intercept and covariates. Hosmer and Lemeshow Goodness of Fit Test: 3.55 with 8 df, p=0.89. All variables entered in the model met the 0.05 level of significance, unless stated else. * = not directly interpretable odds ratio's.

Table 12: Summary of statistical analysis for model for cavity development: external and intrinsic parameters as explanatory variables.

Variable	Parameter estimate	Wald Confidence limits Lower	Upper	Odds ratio
Intercept	-2.9756	-6.6281	0.6768	-
Carbon dioxide	0.9245	0.4264	1.4226	2.521*
Storage time	0.0224	0.0145	0.0303	1.023*
Oxygen	-0.7891	-1.1458	-0.4325	0.454*
Picking I	-2.6021	-3.4286	-1.7755	0.074
Picking II	1.5068	1.1268	1.8868	4.512
Temperature	0.1929	-0.1996	0.5854	1.213*
Storage time.Colour	-0.00407	-0.00634	-0.00179	0.996*
Carbon dioxide.Hardness	-0.1553	-0.2422	-0.0683	0.856*
Oxygen.Colour	0.0890	0.0243	0.1537	1.093*
Temperature.Oxygen	-0.1949	-0.4534	0.0636	0.823*
Colour	0.3205	0.1134	0.5275	1.378*
Hardness	0.7196	0.3928	1.0464	2.054*
Sugar	-0.2211	-0.4126	-0.0295	0.802
WeightI	0.5702	-0.0664	1.2067	1.769
WeightII	0.9817	0.2660	1.6975	2.669
Storage time.Carbon dioxide	-0.00161	-0.00293	-0.00028	0.998*

-2 Log L: 1219 intercept only, 761 intercept and covariates. AIC: 1221 intercept only, 795 intercept and covariates. Hosmer and Lemeshow Goodness of Fit Test: 5.36 with 7 df, p=0.61. All variables entered in the model met the 0.05 level of significance, unless stated else. * = not directly interpretable odds ratio's.

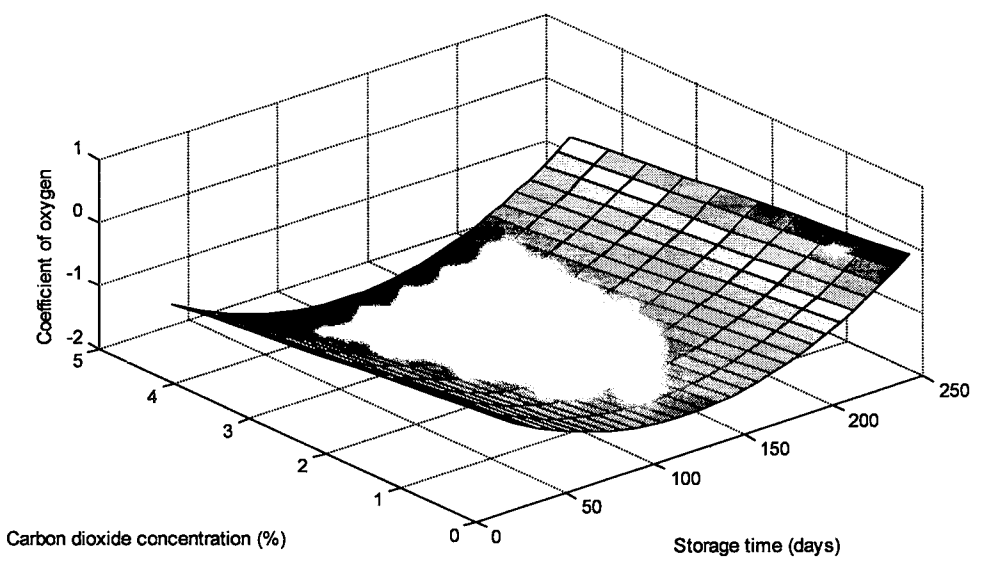


Figure 17: Value of the oxygen coefficient as a function of the carbon dioxide concentration and the storage time.

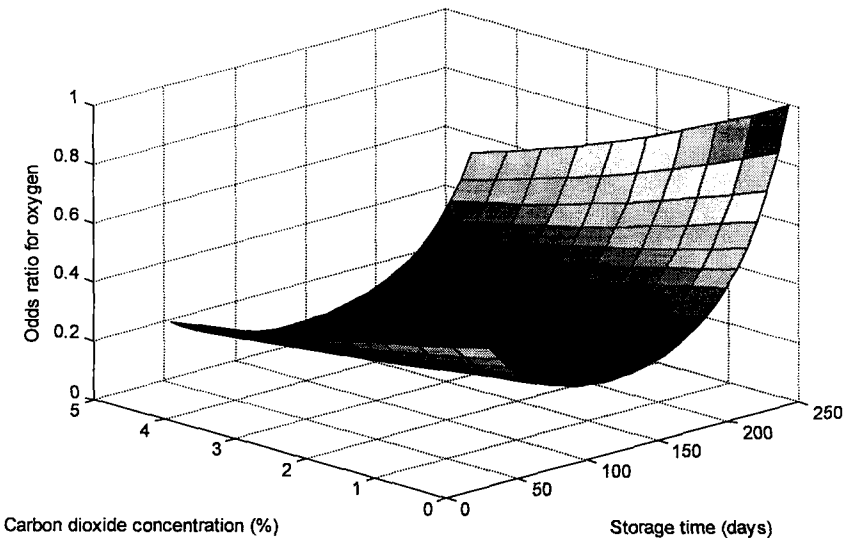


Figure 18: Odds ratio for oxygen as a function of storage time and carbon dioxide concentration.

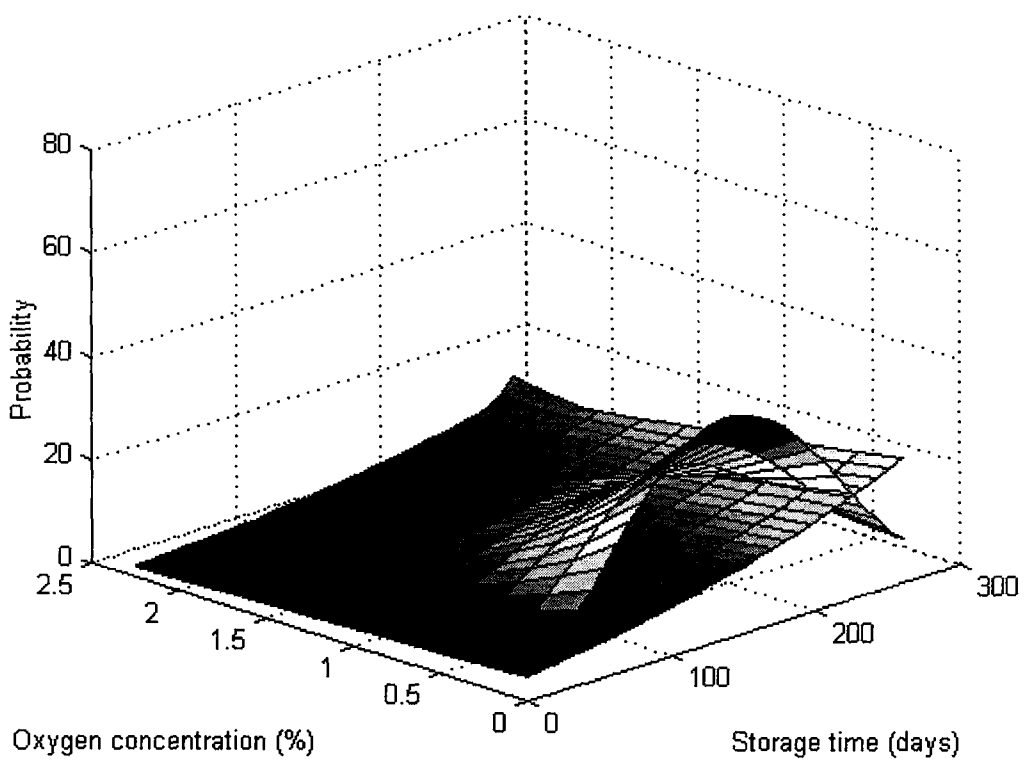


Figure 19: Probability for incidence of internal browning (upper plane) and cavities (lower plane) as a function of storage time and oxygen concentration. (temperature = 0°C, carbon dioxide concentration = 1% and picking date = optimal)

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997 TO 31-05-1998

The experimental set-up and measurement of the diffusion resistance gave some problems. The were some problems with measuring the neon concentrations by means of the micro-GC. Unfortunately the

data were analysed after the measurement period due to lack off time so the missing values were not spotted until than and experiments could not be repeated.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

Tasks 1 to 4 will be continued in a similar way as in the previous periods. However, the early harvest dates will not be included any more as agreed in the last progress meeting. For the storage of pears more effort will be placed on optimal storage conditions.
Tasks 6 and 7a will be continued.
Task 9: The logistic prediction models will be validated.

E. DISSEMINATION

Scientific publications:

Lammertyn J., Aerts M., Verlinden B.E., De Baerdemaeker J. and Nicolaï B. (1999). Modelling factors influencing core breakdown disorder in *Pyrus communis* cv. Conference: a logistic regression approach. Submitted.

Lammertyn J., Aerts M., Verlinden B., De Baerdemaeker J. and Nicolaï B. (1998). Relation between core breakdown disorder and storage conditions of *Pyrus communis* cv. Conference. XXV International Horticultural Congress, Brussels, Belgium, 2-7 August 1998. *Acta Horticulturae* (in press)

Lammertyn J., Aerts M., Verlinden B., De Baerdemaeker J. and Nicolaï B. 1998. Logistic regression for modelling factors influencing core breakdown disorder in Pyrus communis cv. Conference. EurAgEng 98, Oslo, Norway, 23-27 August 1998. CD-rom in press.

Publications in professional journals

Lammertyn J. en Schenk A., 1998a. Hol en bruin bij Conference. De Boer en de Tuinder (108) 39:12

Lammertyn J. en Schenk A., 1998b. Bewaaronderzoek bij Conference: bruin slaat op hol. Belgische fruitrevue (50) 11:5-9

Schenk A. en Lammertyn J., 1998. "Holle peren"-special. Fruitteeltnieuws Berichten uit wetenschap en praktijk (11) 18:16-17

Presentation at workshops

Voorlichtingsdag fruitteelt 16/01/99

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-98 to 31-05-99

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 6 total cost: (100%)	196,2 kECU	*EC contribution to partner no. 6:*	197,1	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

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A. PARTNER INFORMATION

Name and address

Name of the institute : University Hohenheim, Institut für Obstbau, Bavendorf (UHOH)
Address: D 88213 Ravensburg
country: Germany

Scientific team

Dr. Josef Streif, team leader
Ing. Claudia Rabus, PhD student
Ing. Haibo Xuan, PhD student
Ing. Adriano Saquet, PhD student

Objectives

The main objective of the project is the quality improvement of pears and the reduction of losses during storage by preventing brown heart disorders.
Brown heart is a fruit disorder, which exists in Conference pears throughout Europe, but the disorder seems to appear more severe in Nothern countries than in Southern. Comparisons of pears grown in different climates should give information about the causes of the disorder. The key element in the project is the development of technology which enables a decission about optimal storage treatments following specific growing conditions. Three pathways are promissing to approach the brown heart problem:

- Development of a predictive model based on registered variances in orchard and weather conditions of a broad group of European countries.
- Development of a predictive model based on a rapid asssessment of pear physiology (respiration rate, fermentation rate, resistance to gas diffusion).
- Testing and implementation of a post-harvest treatments, which improve the storability of the harvested pears, leading to pears with a better quality.

Actions in the project

The following tasks were carried out by partner 6 in the described period:

Task 1	Cultivation of pears
Task 2	Harvest of pears
Task 3	Storage of pears
Task 4	Registration of climate and orchard factors
Task 5	Postharvest treatments
Task 6	Gas exchange and diffusion measurement
Task 7	a: Fruit quality evaluation
	b: Mineral analysis
	c: Biochemical analysis

B. RESEARCH ACTIVITIES DURING THE PERIOD 01-06-1998 TO 31-05-1999

Task 1:	**Cultivation of pears**
Duration:	48 months
Current Status:	24months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	1

Objectives

Testing the influence of growing conditions such as climate, site conditions and crop load of two different orchards for susceptibility of Conference pears under CA storage.

Material and methods

In 1998/99 the same orchards and experimental design were used as in the previous year.

Tab. 1: Orchard specifications used for the experiments

		subsets	trees /set	area (ha)	age (year)	rootstock	distance (m)	formation	special treatments
Orchard I	Exp. Station	3	12	0,29	19	quince A	4x2	central axis	hand thinning
Orchard II	H. Knäple	3	4	0,29	32	seedling	4x6	central axis	not thinned

In 1998 the cropping behaviour of the pear trees was very good and regulary. The cultivation program was normal without any treatments of growth regulators. Fruit thinning was done in orchard I, only.

Task 2 **Harvest of pears**
Duration: 48 months
Current Status: 24 months to completion
Total estimated Man-month: 4
No. of man-month devoted already to the task: 2

Objectives

According to the data of the first year the harvest date seems strongly related to the occurence of brown heart in 'Conference' pears. Therefore fruits were picked at different dates in order to get a variation in physiological and biochemical fruit characteristics such as flesh firmness, soluble solids, starch content, respiration, ethylene production, etc.

Material and methods

The method for determination of optimum picking date and picking procedure was the same as in 1997. Samples for determination of optimum picking date were taken 3 times in weekly intervals at August 31, September 07, and 14. The second pick should be at the estimated optimum date. Progress in maturity was monitored observing changes in firmness (hand-penetrometer), starch conversion (scale 1-10) and soluble solid content (hand-refractometer). Special interest was given to firmness value which should be between 6-6.5 kg/0.5 cm^2 at optimum picking date. From the observed parameters the 'Streif-index' was calculated.

Results and Discussion

The changes in the fruit characteristics used for ripening determination during three different picking dates are shown in table 2 and the values of Streif-Index of the last two years in figure 1. According to these results there was no difference in ripening development between orchard I and orchard II, but the ripening index was significantly lower in 1998 compared with 1997. It means that 1998 the fruits ripened 1 week earlier which was caused by a earlier bloom in April 1998, too. When we assume an optimum ripening index of 0.14 - 0.12 for the beginning of picking of Conference pears, then we started with the harvest of the pears 1 week to late in 1998. This is reflected in the firmness values, too. This could also result in higher amounts of disordered fruits at the end of storage periode 1998, which we could not realize, however (see results task 3).

Table 2: Fruit quality and ripening parameters of Conference pears from two different orchards at three picking dates

Orchard I								
harvest date	storage cond. %CO2+%O2	size mm	firmness kg/0.5cm2	solubl sol. %	acidity mval	starch 1-10	colour CIE a	ripening Index
I (31-Aug.)	begin	60,70	6,02	12,35	1,85	4,50	-17,64	0,11
I (07-Sept.)	begin	65,50	5,52	13,15	1,82	5,00	-16,95	0,08
II (14-Sept.)	begin	69,67	5,50	13,77	1,59	7,00	-15,60	0,06
Orchard II								
harvest date	storage cond. %CO2+%O2	size mm	firmness kg/0.5cm2	solubl sol. %	acidity mval	starch 1-10	colour CIE a	ripening Index
I (31-Aug.)	begin	63,83	6,43	12,87	2,01	4,17	-16,14	0,13
I (07-Sept.)	begin	60,17	5,45	12,37	1,81	6,17	-16,08	0,08
II (14-Sept.)	begin	63,67	5,38	12,63	1,84	8,83	-15,21	0,05

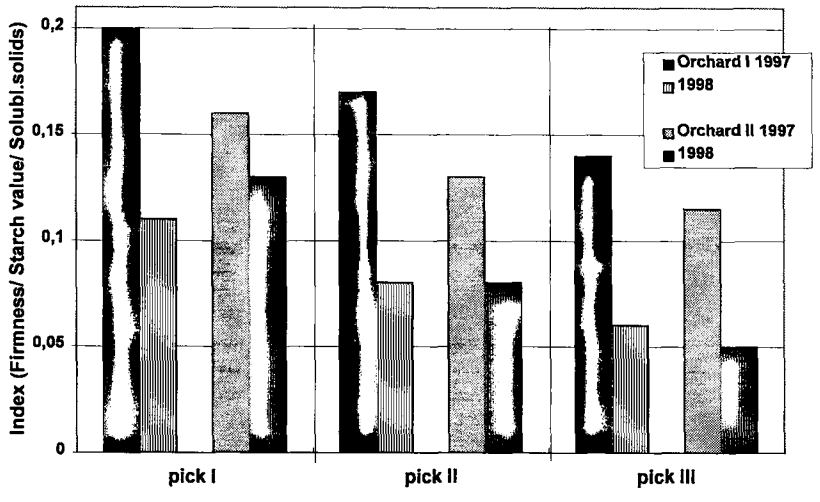

Fig.1: Ripening index at harvest in 1997 and 1998

Conclusions

In 1998 the pears ripened about 1 week earlier than 1997. Harvesting of pears with 1 week interval resulted in fruits with different quality and ripening characteristics. It was possible to characterize the picking dates, when we use fruit firmess alone or the calculated ripening index of firmness, starch conversion and soluble solids content,

Task 3	**Storage of pears**
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	2

Objectives

Pears from different orchards and picking dates were stored under various CA storage conditions in order to study the influence of CO_2 and O_2 on the occurence of core browning in the course of storage periode.

Material and methods

Storage devices

During the storage periode 1998/99 the same storage technique was used as 1997. The CA conditions were slightly modified, however:

Temperatures:	-0.5 to -1 °C:
CA I (conditions avoiding core browning):	2.0 % 0_2 plus 0.7 % CO_2
CA II (conditions provoking core browning):	2.0 % 0_2 plus 5.0 % CO_2

Fruit material:

Fruits of 2 orchards with variations in crop load and picking dates were harvested, selected for homogenious size, colour and maturity and brought as soon as possible under the adopted CA-conditons. For 2 different CA-concentrations, 3 replications and 5 sampling dates (including harvest) in one box with 15 kg fruits each were stored.

The observations of browning disorders were intensified in the first three months of storage period in order to detect the very first beginning of damages.

Therefore, fruit samples were taken from the store after 1, 2, 3 and 6 months. The assessment of flesh browning and cavities were judged according to a picture card with 5 stages (0-4) of damages, the same as used in the previous year.

Results and Discussion

In 1998/99 the duration of storage periode was 6 months in total. After this time core browning disorders were developed mainly under high CO_2 condition in combinations with pick 3 (see Fig. 2) Already after 3 months the treatment with 5 % CO_2 has shown the maximum index of core browning, whereas after 6 months the cavity index still increased. Under low CO_2 (0,7 %) brown heart was developed after 6 months, only. In general, the risk for brown heart seems much lower in 1998/99 compaired with the occurence of this disorder in 1997/98 (see Fig. 3). Nevertheless, the strong relationship between the incidence of browning disorders on the one hand and the picking date and the amount of CO_2 concentration in the store atmosphere on the other hand could be observed as in the year before.

Table. 3: Incidence of core browning and cavities in Conference pears during 6 months of storage (all fruits disordered with highest intensity = 100)

sampling date	storage cond. %CO2+%O2		total nr. of fruits	healthy fruits %	browning index	cavity index
1 month storage	CO2 0,7+2 O2	pick I	74	100,0	0,00	0,00
		pick II	65	99,5	0,17	0,00
		pickIII	62	100,0	0,00	0,00
	CO2 5+2 O2	pick I	67	99,5	0,00	0,00
		pick II	66	99,0	0,17	0,00
		pickIII	65	99,0	0,17	0,00
2 months storage	CO2 0,7+2 O2	pick I	69	99,5	0,00	0,00
		pick II	66	99,5	0,00	0,00
		pickIII	60	100,0	0,00	0,00
	CO2 5+2 O2	pick I	73	99,5	0,15	0,15
		pick II	67	97,7	0,17	0,66
		pickIII	63	92,0	0,35	2,30
3 months storage	CO2 0,7+2 O2	pick I	40	99,2	0,00	0,00
		pick II	34	95,2	0,00	0,00
		pickIII	38	99,1	0,00	0,00
	CO2 5+2 O2	pick I	69	96,2	0,81	0,32
		pick II	67	91,6	1,49	1,33
		pickIII	63	73,1	5,64	6,00
6 months storage	CO2 0,7+2 O2	pick I	74	99,5	0,75	0,00
		pick II	66	99,0	0,51	0,00
		pickIII	60	95,0	3,89	0,37
	CO2 5+2 O2	pick I	68	99,5	0,00	0,33
		pick II	65	93,9	1,37	1,71
		pickIII	61	72,4	5,43	10,14

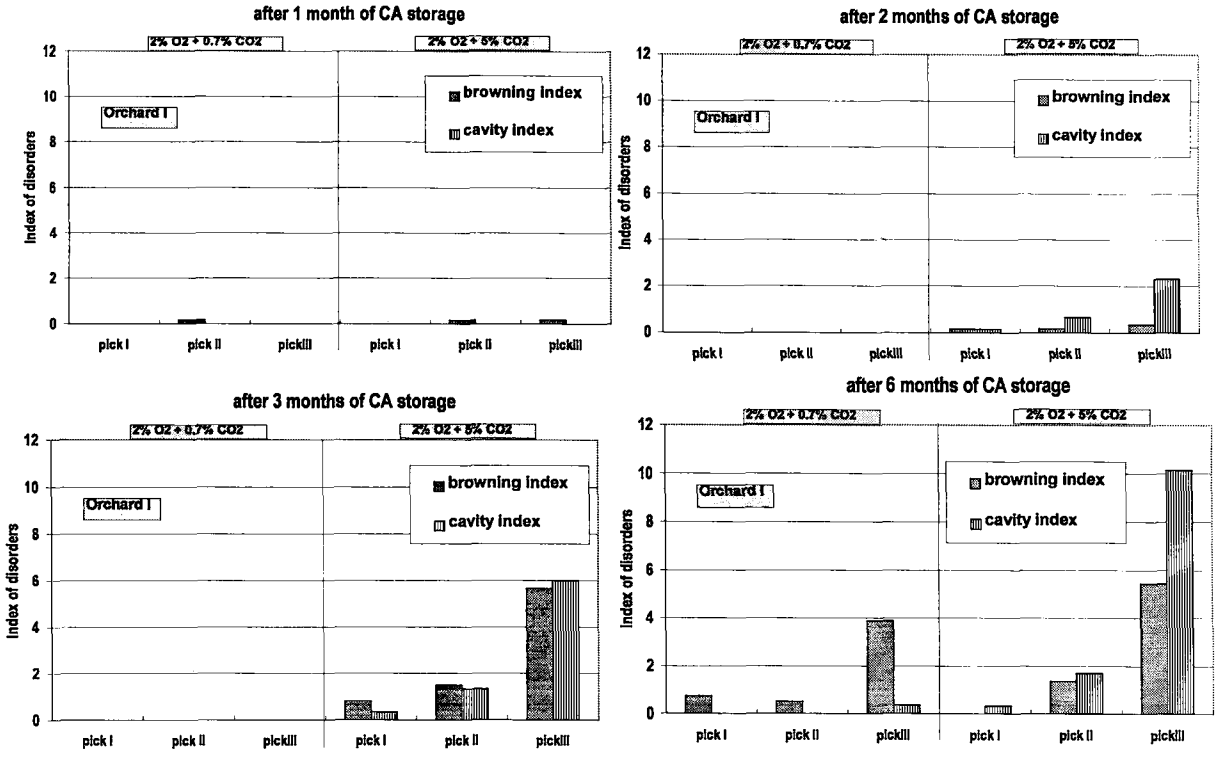

Fig. 2: Incidence of brown heart and cavities in Conference pears of different picking dates, storage conditions and after various storage time

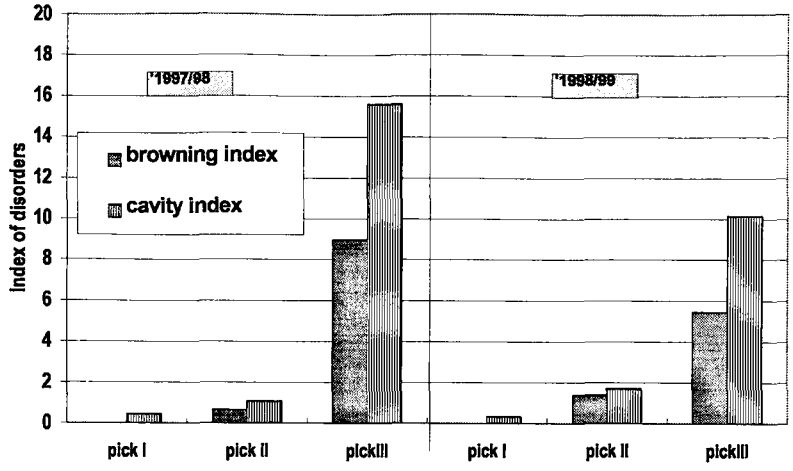

Fig.3: Comparison of browning disorders in Conference pears at the end of CA storage in 1997 and 1998

Task 4	**Climate and orchard characteristics**
Duration:	48 months
Current Status:	24months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	1

Objectives

Site conditions and climate variation may influence the occurrence of brown heart The objective of this task is to collect meteorological data of various pear orchards under different site conditions and to relate them with the occurence of disorders of pears from trees with different crop load.

Material and methods

Climate characteristics:

The measurement of climate characteristics in both orchards was the same as described for the previous year.

Adjustment of crop load:

To prove the fruits originated from trees with different cropping behaviour, we had to adjust the amount of fruits to a high, normal, and low crop. From each tree the size of canopy was calculated and the number of fruits per tree were assessed according to the method from Prof. Winter (described in the last report). The final amount of fruits were regulated by hand thinning.

Results and Discussion

The *climate characteristics* as means of the months from May to September are seen in Table 3. In general, 1998 the temperature was somewhat higher compared with 1997, mainly in June, whereas the temperature in September was lower.

Table 3: Data of climate characteristics as means of the months May - September 1998 of orchard I and II and compaired with the data of 1997

Orchard I	Temperature (in 2m)			Humidity (%)			Radiation	Precip.
Month	Average	Max.	Min.	Average	Max.	Min.	(W m^{-2})	(mm)
1997								
May	13,2	20,7	6,8	67	92	40	4970	54
June	15,7	21,8	11,0	74	93	49	4256	187
July	16,7	23,1	11,6	76	95	50	4270	107
August	19,0	26,8	12,9	73	94	44	4422	73
September	14,5	22,4	8,7	77	95	47	3568	32
Mean 1997	15,8	23,0	10,2	73	94	46	21485	453
1998								
May	14,4	21,2	8,0	65	89	42	4914	49
June	17,3	24,1	11,3	68	92	42	4857	92
July	18,0	24,0	13,0	71	92	46	4282	74
August	18,3	25,4	12,4	67	91	41	4429	50
September	13,1	18,9	9,3	80	94	53	2642 !!	151
mean 1998	16,2	22,7	10,8	70	92	45	21123	417
Orchard II								
Mean 1998	16,1	23,5	9,4	67,5				440,2
Mean 1997	16,2	24,9	9,0	78,4				335,0

The effect of *various crop loads* in respect to the occurence of disorders was very clear (Figure 4). The lower the crop load the higher the occurance of brown heart and especially the occurence of cavities.

Conclusions

The meteorological data as means of days for each orchard will be used also for network modelling together with harvest dates and storage disorders.

Low crop load seems to be an factor which can provoke more browning disorders in fruits which was observed in the last season, too.

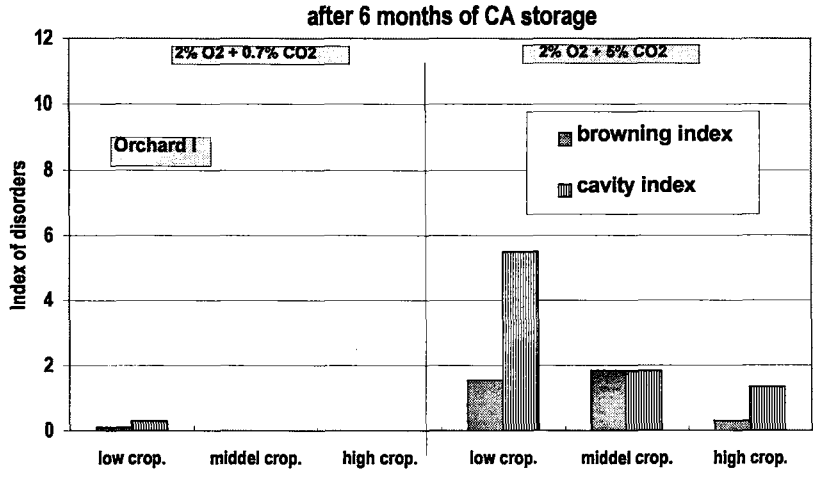

Fig. 4: Browning disorders of Conference pears from trees with different crop load after 6 months of CA-storage in low and high CO_2 concentrations

Task 5	**Postharvest treatments**
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	1

Objectives

The aims of this task are:

- to determine the preventive effect of various prestorage treatments
- to develop a rapid test in order to determine the susceptibility of pears to browning disorders before storage.

Our special aim was to examine which CA regime and which time of storage periode is most favorable for the induction of browning disorders.

Material and methods

Storage regimes

During 6 months pear fruits were kept under 3 different storage regimes: O_2 concentration was 2 % in each treatment. The CO_2 concentration was 3 % in the average of the whole storage period but in treatment A with an continuous increase from 1,5 % at the beginning up to 4,5 % CO_2 at the end of the 6 months storage periode. Storage regime B was the opposite: a decrease from 4,5 % to 1,5 % CO_2 during the storage period and regime C was constant 3 % CO_2. Fruit samples of the different treatments were taken each 2 months from the store and inspected for brown heart and cavity disorders.

Results and Discussion

The storage results as occurence of core browning and cavities after different periods are shown in Figure 5. Although the CO_2 concentration of all 3 storage regimes was the same on an average, the initial low CO_2 concentration resulted in lower amounts of damaged fruits during all sampling dates compared with the other two treatments. Specially regime B, with a high CO_2 concentration, promoted the occurence of more and earlier browning disorders.

Conclusions

Special attention for the appearance of first symptoms of browning must be payed to the conditions during the early storage periode.

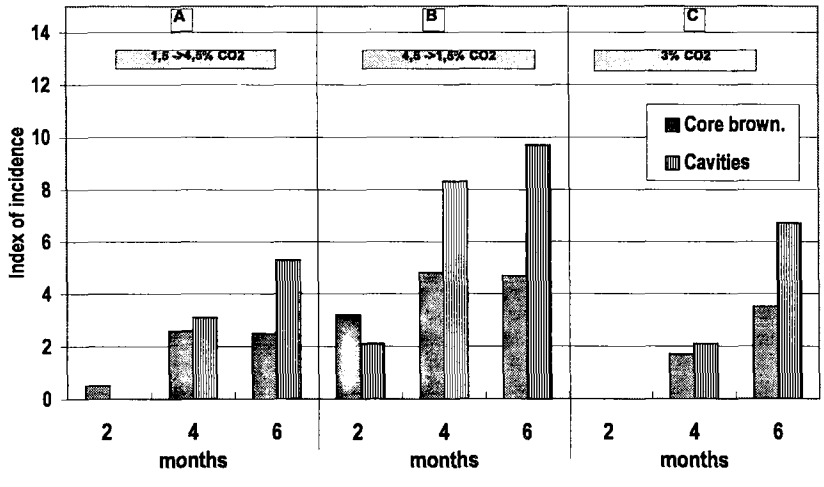

Fig. 5: Occurence of browning disorders in Conference pears after 2, 4, and 6 months of CA storage in shifting CO_2 concentrations

Task 6	**Gas exchange and diffusion measurements**
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	16
No. of man-month devoted already to the task:	8

Objectives

The *measurement of respiration* rates includes both O_2 uptake and CO_2 production under a range of CO_2 and O_2 concentrations. The O_2 concentrations should include 0% (maximum fermentative CO_2 production) and 21 % (normal air).

Measurement of gas diffussion can be used for information about the gas exchange between internal and external atmosphere and for calculation of internal gas concentrations.

Ethylene production of pears from different picking date and storage conditions can reflect the ripening inhibition.

Material and methods

Respiration and ethylene measurements:

O_2 uptake and CO_2- and ethylene- production were done by headspace GC anlysis at different times from the beginning to the end of the storage periode of the pears. The methods of measurement were the same as in the previous year.

Diffusion measurement:

Different methods of gas diffusion measurements were tested:

A: The penetration of neon from the core of the fruit through the skin was measured. Neon was used as gas which is not involved in the fruit metabolism. Neon was flushed through the core of the fruit continously with needels injected in the core and connected with an medicinal infusion device (Fig. 6 A).

B: The porosity of a disk from the fruit tissue. Air is pressed by a pumpe (0.3 bar) through the disk. According to the porosity of the tissue the air streams through the disk and displaces the water in the upper part of the messuring device, which is collected and measured (Fig. 6 B).

C: Gas diffusion of ethylene through a disc of fruit tissue with or without skin. The disc is placed in a special device of gas-tight connected syringes. The penetration of gas from one side with higher concentration to the other side of the tissue is measured (Fig 6 C).

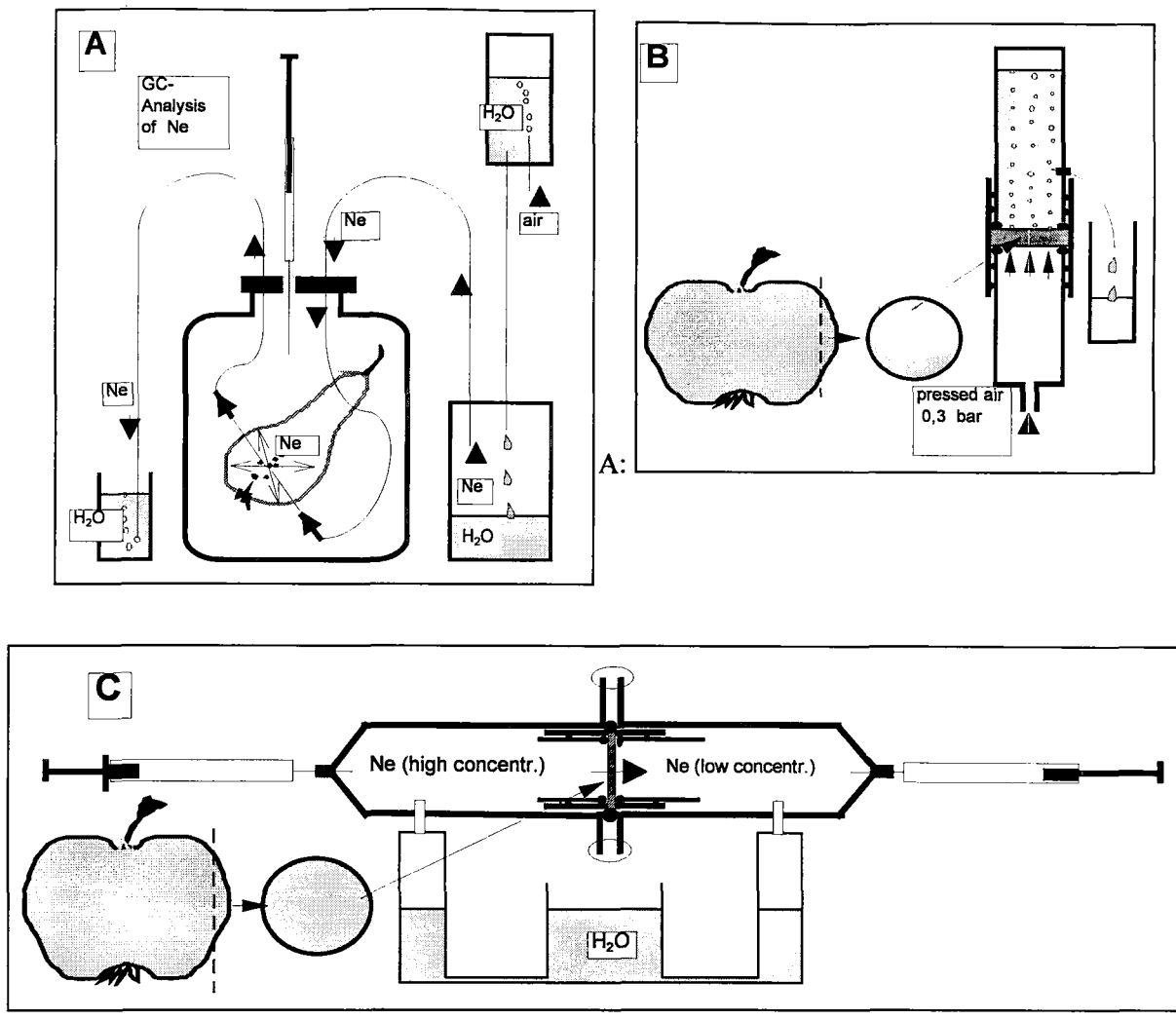

Fig. 6: Different devices for diffusion measurement. For explanation see text in *'material and methods'*

Results and Discussion

Respiration measurements: For the first 3 months of storage we obtained reliable data of respiration measurement (see Fig. 7). The 4th serie of measurement could not performed because of technical problems with the Micro-GC. Obvious differences between the different treatments can't be observed. But respiration and RQ of fruits from pick 3 were always somewhat higher than that of fruits from the earlier picks, however.

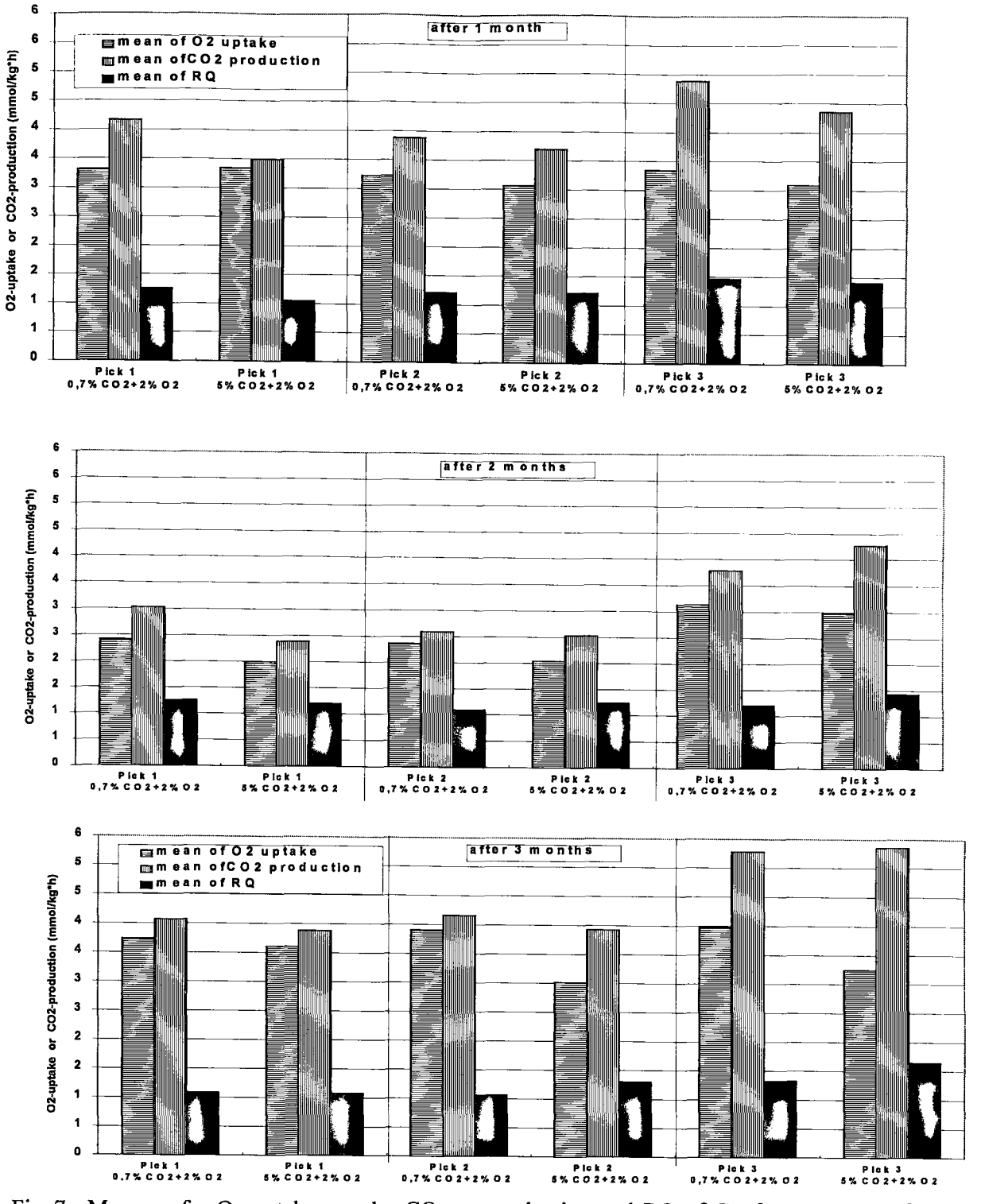

Fig. 7: Mean of O_2-uptake and CO_2-production and RQ of Conference pears after 1, 2, 3 months of CA-storage and various O_2-concentrations at 1°C for 5 days

Ethylene production: Ethylene production was measured together with respiration. The data for the sampling date 'after 3 months' are shown in Figure 8. At harvest and after 1 month of CA-storage no ethylene could be detected. In the following storage periode the amount of ethylene production was highly influenced by the O_2 concentration in the respiration jars. The CO_2 concenration which was applicated in the previous CA storage history (0.7% or 5% CO_2) seems to be also important. Obvious effects of storage conditions which are avoiding or provoking browning disorders can't be detected in a changed behaviour of ethylene production.

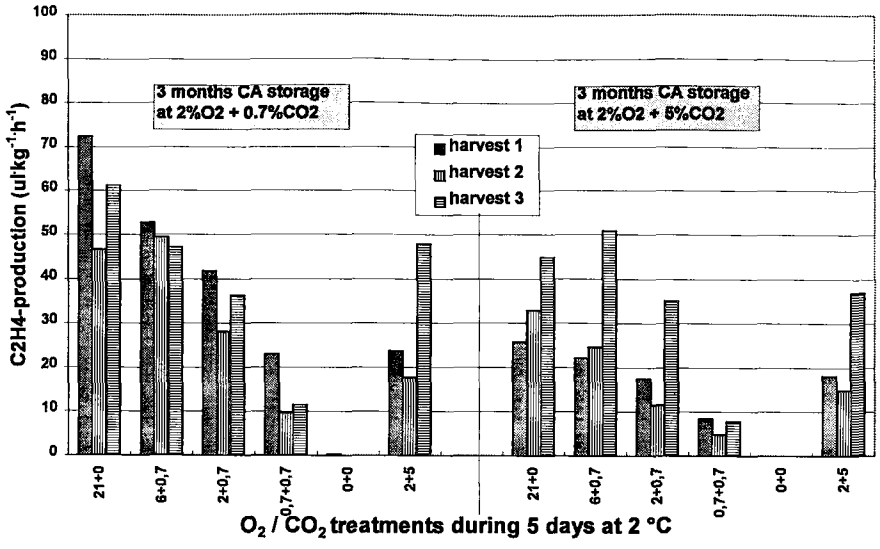

Fig.8: Ethylene production of Conference pears after 3 months of CA storage followed by different CO_2/O_2 treatments

Gas diffusion: Different methods for testing the permeability of fruit tissue gave similar results. In figure 9 and 10 the results for two apple cultivars (Braeburn and Jonagold) in comparison to Conference pear are given. With both methods Conference tissue shows a much lower permeability for gases than apple tissue. Between the apple tissue exist remarkable differences, however. Braeburn apples show a lower diffusion rate which is related to the higher susceptibility for browning disorders. More investigations are necessary to clarify the influence of internal gas conditions on occurence of brown heart.

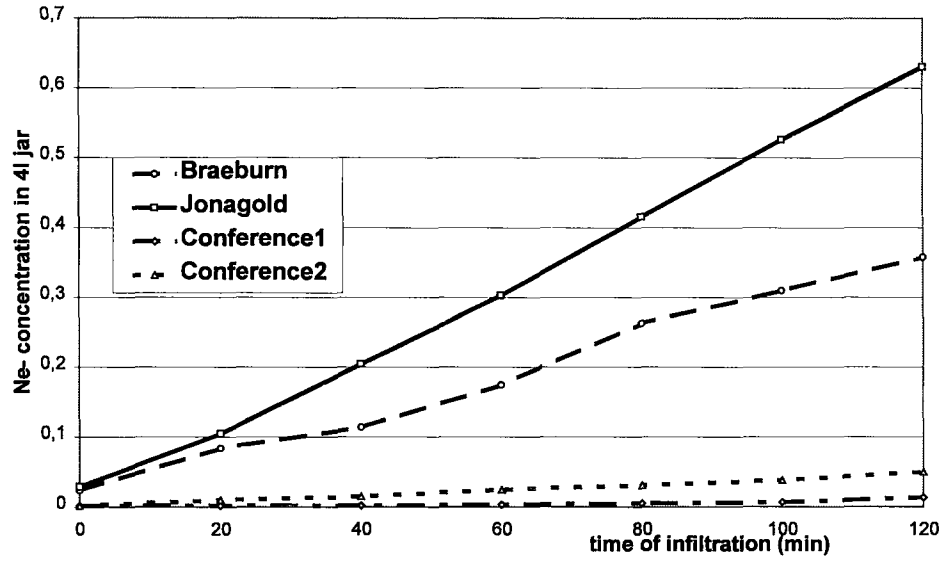

Fig. 9: Increase of Ne-concentration as the result of gas diffusion through the fruit tissue (methode A)

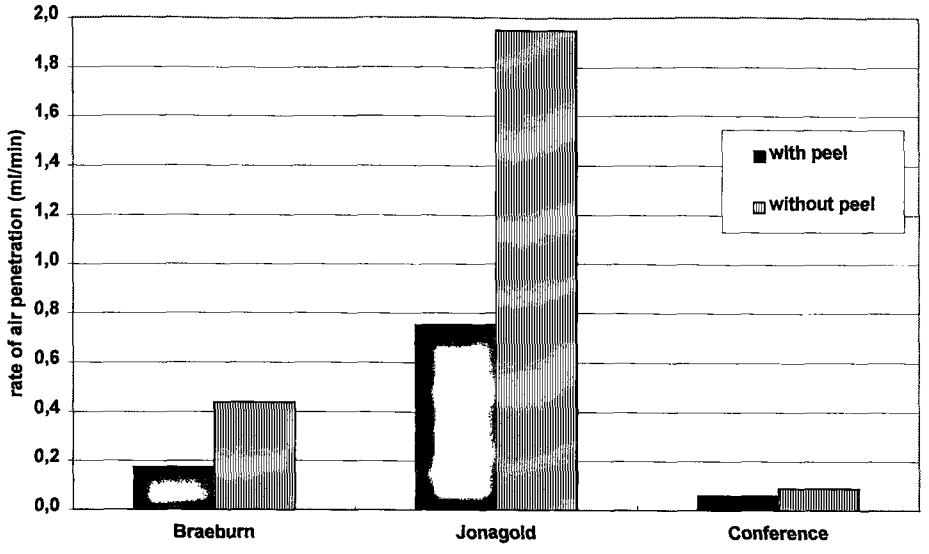

Fig. 10: Penetration of air through a disc (diameter 25 mm) of fruit tissue at a pressure difference of 0.3 bar (methode B)

Conclusions:
Measurement of respiration and gas diffusion must be performed during the whole storage periode to get more sure information on the effect of browning disorders.

Task 7	**a: Fruit quality evaluation** **b: Mineral analysis** **c: Biochemical analysis**
Duration:	48 months
Current Status:	24 months to completion
Total estimated Man-month:	26
No. of man-month devoted already to the task:	13

Objectives
Destructive measurements will accompany the different measurement during storage period in order to increase essential knowledge on different aspects of quality and browning disorders.
Several biochemical pathways are expected to be involved in the development of brown heart in pears. The measurement of fermentative metabolites can help to understand damages of tissues by toxic substances. Different enzymes, involved in the peroxid scavenging processes, are likely involved in the onset of brown heart. The role of energy metabolism for maintenance the cell integrity and the avoidance of browning disorders will be investigated, too.

Material and methods

a: Fruit quality evaluation
Methods for evaluation of fruit quality (*ground colour of peel, flesh firmness, soluble solids content and acidity of fruit juice)* were the same as described for last year but the number of sampling dates were reduced.

b: Mineral analysis

Mineral analysis of K, Ca, Mg, P in the fruits were performed with the same methods as in the previous year. Additional we analysed separately the mineral content in the inner and outer part (without skin) of the fruit.

c: Biochemical analysis

Ethanol, acetaldehyde and ethylacetate: The determinations of these substances were done simultaneous by heat space GC analysis as described in the last year report.

Ascorbic and dehydroascorbic acid: Ascorbic acid and dehydroascorbic acid were separately determined in the inner and outer parts of the fruit cortex without peel by a fluorometric technique as described last year.

Membrane peroxidation: The level of lipid peroxidation in the Conference fruit tissue was measured in terms of malondialdehyde content (MDA, a secondary end product of lipid peroxidation) determined by the thiobarbituric acid (TBA) reaction with the basic procedure according to Packer (1968), Du and Bramlage (1992) and Hodges (1999).

Antioxidative potential (AP): The antioxidative potential (AP) of lipo- and hydrophile antioxidants in Conference pears was assayed according to the method of Chevolleau et al (1992), and a modified method of Hillebrandt and Schmitz (unpublished). The test solution consisted of 0.5% ß-carotine and 20 mg linolic acid.

Enzymatic antioxidants: The fruit material, frozen with liquid N_2, were used to determine the activity of glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX). For enzyme extraction phosphate buffer, containing EDTA and PVPP, was used.

- GR-activity was determined by following the oxidation of NADPH (Esterbauer and Grill, 1978).
- CAT activity was determined by following the consumption of H_2O_2 (Chance and Maehly, 1955).
- SOD activity was determined by quantifying the ability of enzyme extracts to inhibit light-induced conversion of nitroblue tetrazolium (NBT) to formazan (Beauchamp and Fridovich, 1971).
- APX activity was determined by following the decrease in absorption at 290 nm of an assay mixture containing ascorbate (Nakano und Asada, 1987).

The techniques and protocols for determination of CAT, SOD and APX were learned from partner 2 (IRTA) in Lleida, Spain.

Energy metabolism:

a: Pyridine nucleotide assessment: The activity of NAD(H) and NADP(H) was measured by a modified method according to Brinkman et al. (1973). Fruit material without skin and core was frozen in liquid nitrogen, freeze dried and powdered. For the extraction of the reduced pyridine nucleotides samples were homogenised in a solution of KOH in ethanol plus KCl and for the oxidised forms in a solution of trichloracetic acid (TCA) plus KCl. After incubation at 60°C samples were centrifugated, the supernatant was neutralised and photometrically measured at 600 nm. The reaction solution for NAD and NADH contained alcohol dehydrogenase (ADH) (24 Units) and the reaction mixture for NADP and NADPH isocitrat dehydrogenase (18 Units).

b: *ATP and ADP assessment*: The concentration of ATP and ADP was determined by a bioluminecence technique. This assay is based upon the measurement of a stable level of light produced in a reaction catalysed by the firefly luciferase enzyme. The formula for the light producing reaction is as follows:

$$\text{ATP} + \text{D-luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{oxyluciferin} + \text{AMP} + \text{Ppi} + CO_2 + \text{LIGHT}$$

For this measurement a chemical kit from Bio-Orbit was used. Freeze dried and powdered samples were homogenised in a solution of TCA plus EDTA and extracted for 30 min on ice. The

samples were centrifugated and the supernatant measured with a luminometer. ATP concentration was determined in a mixture of sample and ATP-monitoring-reagent (AMR). For ADP determination, sample together with pyruvate kinase was incubated for 30 min and thereafter AMR solution was added.

Results and Discussion

a: Fruit quality evaluation

The results of quality changes in Conference pears monitored at harvest and 4 times during 6 months of CA storage are shown in table 4. The results are very similar to those from lst year concerning the decrease in flesh firmness, acidity, and ground color and increase of soluble solids. Preservation of quality characteristics as influenced by storage conditions was better under higher CO_2 concentration (2% O_2 +5% CO_2) than under low CO_2 (2% O_2+0,7% CO_2) with the exception of acidity. It seems that problems with browning disorders are reflected in a faster loss of acidity.

b: Mineral analysis:

The mineral content of the pears was analysed at the end of July during the growth of the fruits and directly after the various harvest dates. The cortex tissue of the pears was also separated also for the mineral analysis of inner and outer parts. In general, for K, Ca and K:Ca-ratio no obvious correlation was found in respect to incidence of browning disorders.But it seems to exist a correlation between the occurence of disorders and the phosphorus content of pears from the last harvest date (see Fig. 11).

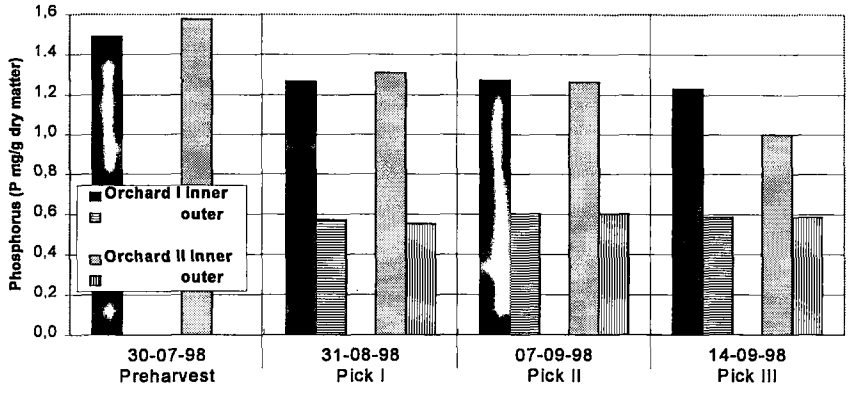

Fig. 11: Phosphorus content of different parts of cortex tissue from Conference fruits as influenced by picking date

c: Biochemical analysis

Ethanol, acetaldehyde and ethylacetate: Ethanol, acetaldehyde and ethylacetate content increased in the pear fruits with ongoing storage time (see Fig. 12 and 13). The concentrations of these substances were somewhat higher in fruits treated with 5 % CO_2 and in the internal part of the cortex than in 0,7 % CO_2 treated fruits and in the external part of the cortex. Ethanol and acetaldehyde production showed a very similar behaviour. Ethylacetate started to increase very rapidly after a delay of 2 months (data not shown). From former experiments with aroma analysis of pears we know, that ethylacetate is a typical component of volatiles in Conference pears and this substance is also produced in air stored fruits. But in apple fruits ethylacetate is poduced under low oxygen conditions, only.

Tab. 4: Quality parameters of Conference pears from different picking dates during 6 months of CA storage under various conditions (orchard I)

sampling date	harvest date	stor.cond. %CO2/%O2	size mm	firmness kg/0.5cm2	solubl sol. %	acidity mval	starch 1-10	colour CIE a	ripening Index
1	I	begin	60,70	6,02	12,35	1,85	4,50	-17,64	0,11
begin	II	begin	65,50	5,52	13,15	1,82	5,00	-16,95	0,08
	III	begin	69,67	5,50	13,77	1,59	7,00	-15,60	0,06
2	I	0,7/2	65,39	5,75	14,58	2,09		-16,10	
	I	5/2	62,11	5,61	14,18	1,72		-15,39	
after	II	0,7/2	66,67	5,42	13,88	2,49		-16,56	
1 months)	II	5/2	66,06	5,17	13,78	1,53		-15,95	
Oct.98	III	0,7/2	68,61	5,14	14,23	1,58		-15,81	
	III	5/2	66,33	5,08	14,42	1,59		-15,55	
3	I	0,7/2	65,39	5,98	14,19	1,63		-15,72	
	I	5/2	67,33	5,55	13,68	1,46		-16,22	
after	II	0,7/2	66,67	5,47	14,43	1,64		-16,36	
2 months)	II	5/2	66,89	5,59	13,92	1,63		-15,60	
Nov 98	III	0,7/2	66,06	5,24	15,32	2,02		-15,14	
	III	5/2	66,28	5,31	14,61	1,29		-15,40	
4	I	0,7/2	67,17	5,88	13,78	1,32		-15,76	
	I	5/2	66,06	5,74	13,94	1,46		-15,67	
after	II	0,7/2	68,22	5,61	13,56	1,31		-16,30	
3 months)	II	5/2	66,61	5,48	14,14	1,26		-15,75	
Dec.98	III	0,7/2	64,94	5,14	14,09	1,11		-15,69	
	III	5/2	68,06	5,30	14,24	1,17		-15,58	
5	I	0,7/2	67,17	5,18	13,28	0,97		-12,35	
	I	5/2	66,06	5,64	13,46	1,14		-12,87	
after	II	0,7/2	68,22	4,86	12,96	0,78		-12,00	
6 months)	II	5/2	66,61	5,23	13,51	0,93		-12,46	
March 99	III	0,7/2	64,94	4,44	13,61	0.66		-10,69	
	III	5/2	68,06	5,03	13,82	0,59		-12,80	

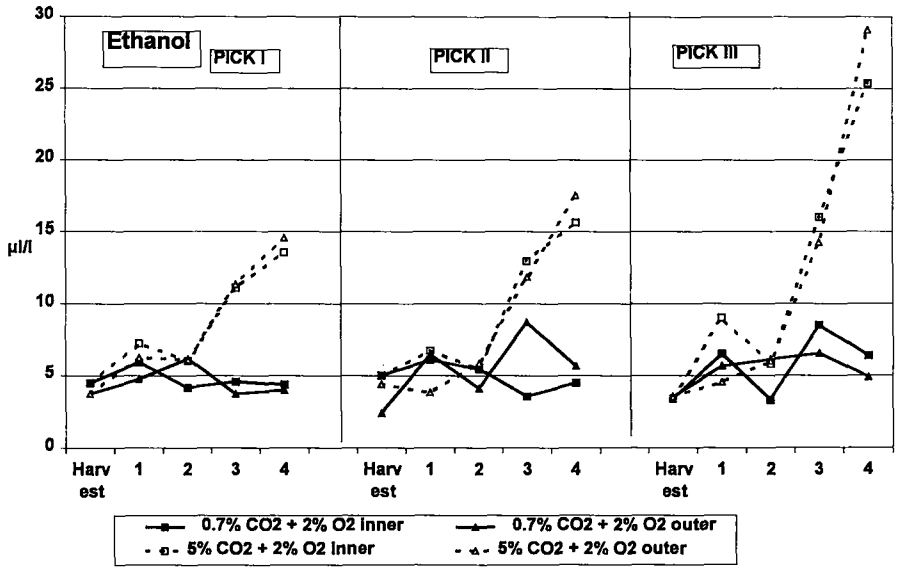

Fig. 12: Ethanol content in different parts of cortex tissue from Conference fruits as influenced by picking date and CA conditions

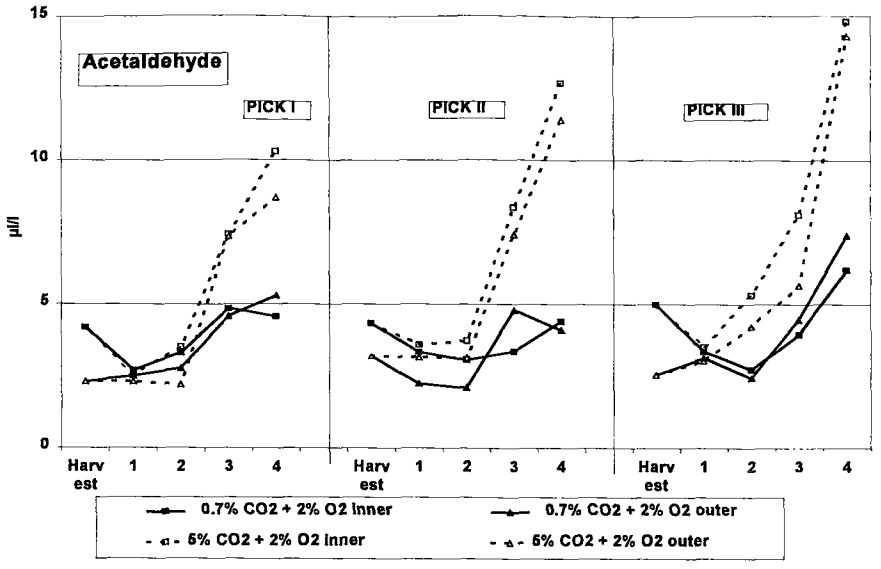

Fig. 13: Acetaldehyde content in different parts of cortex tissue from Conference fruits as influenced by picking date and CA conditions

Vitamin C and ascorbic acid content: Antioxidative substances like vitamin C may play an important part in the browning of fruit tissue. Pears show the browning disorders in a distinguished part of the fruit tissue. The outer part of fruit cortex, about 1 cm under the peel, remains normally free of browning disorders. Therefore we analysed separately both parts of tissue for vitamin C, ascorbate and DH-ascorbate content. The results are given in table 5. Treatments which show higher incidence of disorders seem to be related with lower vitamin C and lower ascorbic acid content, as it can be seen in general from the CA treatment 5% CO_2 + 2 % O_2 and from the late harvest date in special.

Antioxidative potential

Membrane peroxidation: The malon dialdehyde (MDA) content as a measure of membrane peroxidation decreased continously from first to last picking date. During the storage of six months the membrane peroxidation occured more or less, there was no difference between the two CO_2 storage atmospheres, however. Changes of MDA content seemed not related with the incidence of browning disorders, but more with the general ripening progress of the frits.

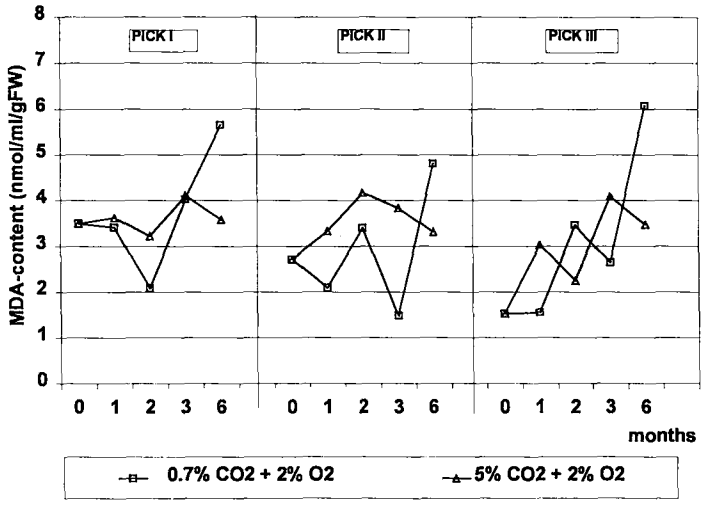

Fig. 14:
Changes of malon dialdehyde (MDA) content of Conference pears from different picking dates and CA-conditions

Tab. 5: Ascorbate, DH-ascorbate and vitamin C content in different part of fruit tissue from Conference

Ascorbate					
I. pick	harvest	1 month	2 months	3 months	4 months
0,7+2 inner	1,50	1,46	1,28	1,41	0,75
0,7+2 outer	1,98	1,88	1,52	1,45	1,28
5+2 inner	1,50	0,94	0,90	0,98	0,37
5+2 outer	1,98	1,60	1,78	1,50	0,47
II. pick					
0,7+2 inner	1,52	1,69	1,18	1,48	0,81
0,7+2 outer	1,96	1,27	1,65	1,24	1,34
5+2 inner	1,52	0,61	0,75	0,67	0,50
5+2 outer	1,96	1,38	1,02	1,20	0,49
III. pick					
0,7+2 inner	2,57	1,27	1,11	0,82	0,42
0,7+2 outer	2,78	2,45	1,81	1,58	0,83
5+2 inner	2,57	0,67	0,66	0,61	0,18
5+2 outer	2,78	1,93	1,25	1,16	0,12

DH-Ascorbate					
I. pick	harvest	1 month	2 months	3 months	4 months
0,7+2 inner	6,28	4,68	2,84	2,24	1,20
0,7+2 outer	5,50	4,78	3,59	1,99	0,97
5+2 inner	6,28	1,93	1,67	1,30	0,50
5+2 outer	5,50	3,35	1,98	1,60	0,89
II. pick					
0,7+2 inner	4,07	2,40	1,99	2,68	1,07
0,7+2 outer	3,19	3,08	2,06	1,62	1,46
5+2 inner	4,07	2,31	0,78	0,67	0,52
5+2 outer	3,19	1,98	1,25	0,34	0,76
III. pick					
0,7+2 inner	4,80	2,82	2,46	1,87	1,12
0,7+2 outer	5,30	3,06	2,33	1,77	1,07
5+2 inner	4,80	3,14	0,70	0,67	0,78
5+2 outer	5,30	2,73	1,11	0,42	1,25

Vitamin C					
I. pick	harvest	1 month	2 months	3 months	4 months
0,7+2 inner	7,78	6,14	4,12	3,65	1,95
0,7+2 outer	7,48	6,66	5,11	3,44	2,25
5+2 inner	7,78	2,87	2,57	2,28	0,87
5+2 outer	7,48	4,95	3,76	3,10	1,66
II. pick					
0,7+2 inner	5,59	4,09	3,17	4,16	1,88
0,7+2 outer	5,15	4,35	3,71	2,86	2,80
5+2 inner	5,59	2,92	1,53	1,34	1,02
5+2 outer	5,15	3,36	2,27	1,54	1,45
III. pick					
0,7+2 inner	7,37	4,09	3,57	2,69	1,54
0,7+2 outer	8,08	5,51	4,14	3,35	1,90
5+2 inner	7,37	3,81	1,36	1,28	0,96
5+2 outer	8,08	4,66	2,36	1,58	1,37

pears as influenced by picking date and CA conditions

Antioxidative potential: The changes of total content of lipo- and hydrophile antioxidants showed a remarkable relation to the occurence of browning disorders during the storage periode. After harvest, there was firstly an increase in AP and after that a decrease depending from the picking date and the applied CO_2 concentration of the fruits. The sooner the picking date and the lower the CO_2 concentration the slower the decrease of AP. The crucial period of increased browning disorders occured approximately at the first month or between the first and second months of pear storage. AP of non-enzymatical antioxidants was related with occurence of browning disorders.

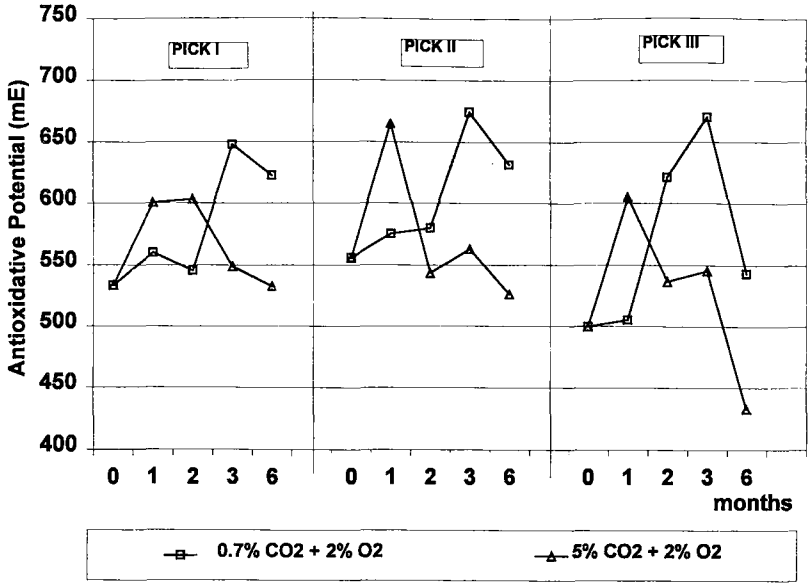

Fig. 15: Changes of antioxdative potential (AP) of Conference pears from different picking dates and CA-conditions

Enzymatic antioxidants:

SOD activity: The determination of superoxide dismutase (SOD) during the storage period showed first an increase and after that a decrease of activity, in general (see Fig. 16). At pick I, the changes of activity were only slight, but at pick II and specially at pick III, very pronounced. Fruits from pick III showed also higher differences in activity between 5% CO_2 and 0.7% CO_2. A relation between the occurence of disorders and SOD activity seems to exist.

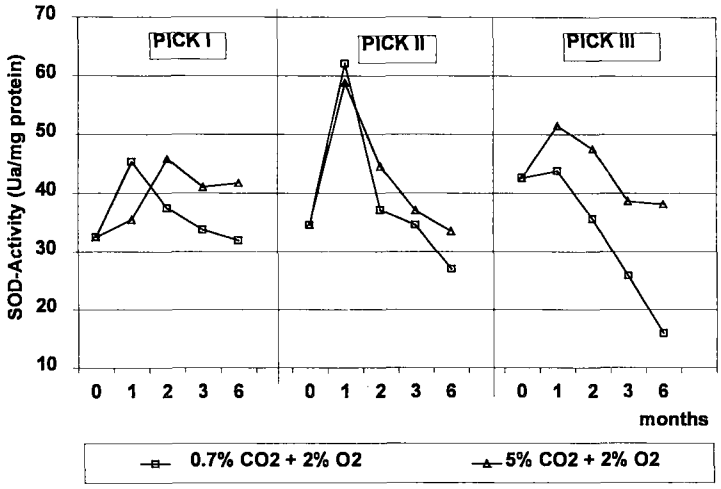

Fig. 16: Changes of superoxide dismutase (SAD) activity of Conference pears from different picking dates and CA-conditions

CAT activity: The activity of catalase (CAT) was reduced at the last pick in comparison with the previous picking dates. This behaviour of the CAT-activity persisted during the storage periode, without obvious differences between the high and low CO_2 treated fruits. In the moment it's hard to detect any correlation with the incidence of browning disorders and CAT.

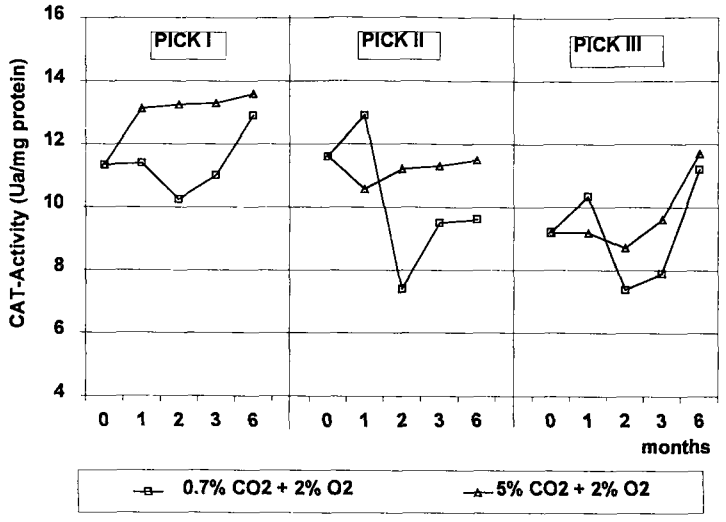

Fig. 17: Changes of catalase (CAT) activity of Conference pears from different picking dates and CA-conditions

APX activity: The initial values of ascorbate peroxidase activity (APX) were higher in less ripe fruits
and lower in more ripe fruits. In the first time after harvest the fruits from pick III in high CO_2 conditions exhibited a fast increase of APX activity which is conform with the sharp decrease of ascorbic acid content in this fruits.The other treatments showed only slight differences in the first 3 months of storage periode. After that all fruits treated with 5 % CO_2 showed tendentiously a faster decline in APX activity than 0.7 % CO_2 treated fruits, perhaps as a consequence of a lack of ascorbic acid which was substancially reduced, already.

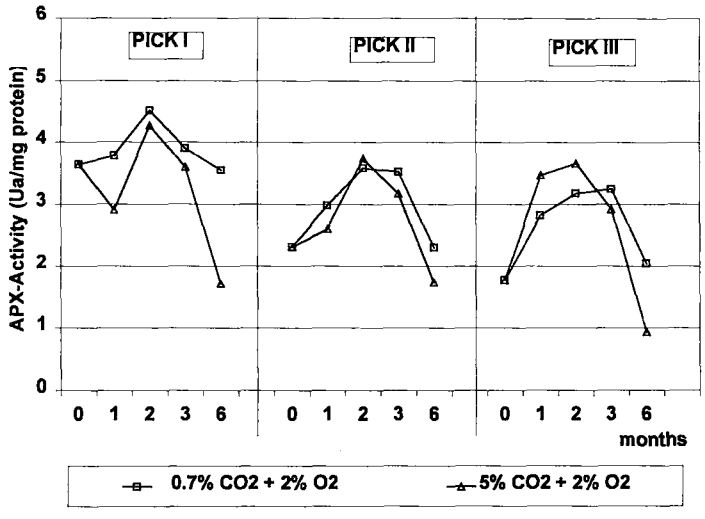

Fig. 18 Changes of ascorbate peroxidase (APX) activity of Conference pears from different picking dates and CA-conditions

GR activity: Glutathion reductase is also a detoxifying enzyme in the peroxid scavenging metabolism of plants. Under stress conditions (5% CO_2) the activity of GR should be reduced during storage time, which was partly confirmed by our results, mainly in fruits from pick 3,

which are more sensitive against CO_2 stress. GR showed some relationship to the incidence of browning disorders.

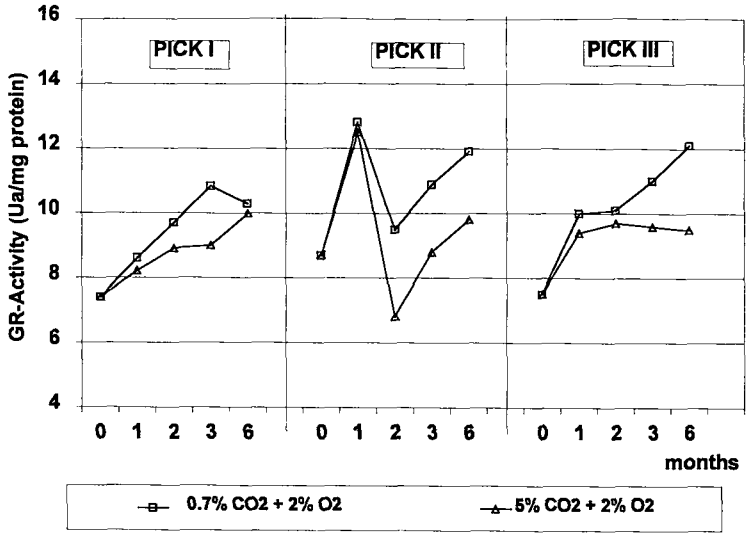

Fig. 19 Changes of glutathion reductase (GR) activity of Conference pears from different picking dates and CA-conditions

Energy metabolism

Pyridine nucleotides: The results of NAD(H) and NADP(H) are given in Fig. 20 and 21 A and B. The reduced forms of NAD and NADP accumulated during the storage time in all storage conditions and surprisingly also in cold storage at high oxygen concentration. Small differences were only observed between the different tested CA-conditions. After 8 days of shelf life at room temperature (20°C), a decrease in the content was noticed, probably due increased respiration activity and oxidation of these nucleotides. The contents of the oxidised NAD and NADP decreased during the storage period and increased slightly during the shelf life of fruits. The storage condition of 6 % CO_2 plus 0,5 % O_2 resulted in the lowest values of NADP. This condition showed also the lowest respiration rate and the highest incidence of disorders (data not shown). Even at shelf life condition the NADP content of this treatment did not increase.

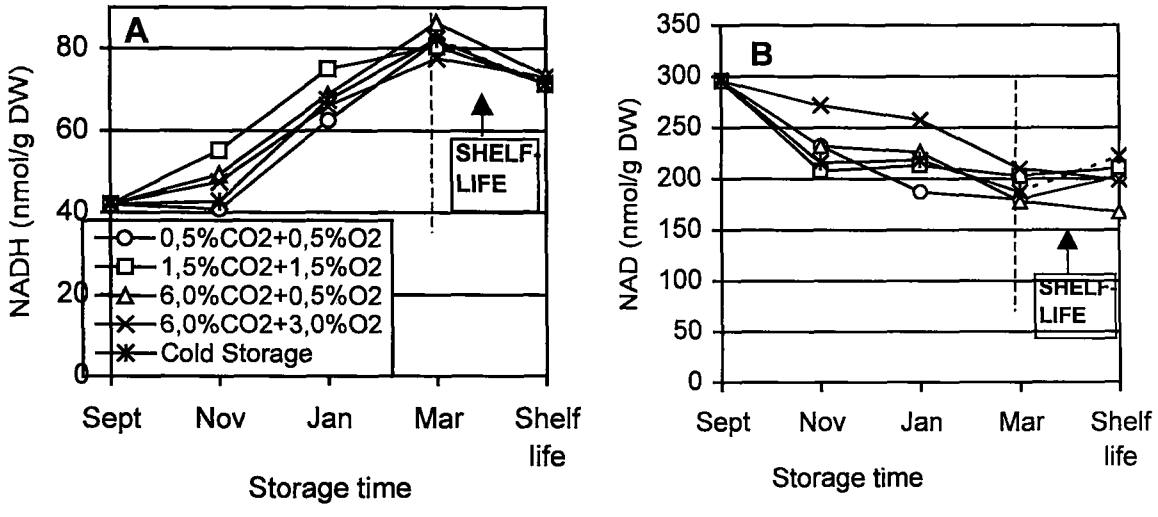

Fig. 20: Changes in the concentration of NADH (**A**) and NAD (**B**) of Conference pears under various CA-conditions

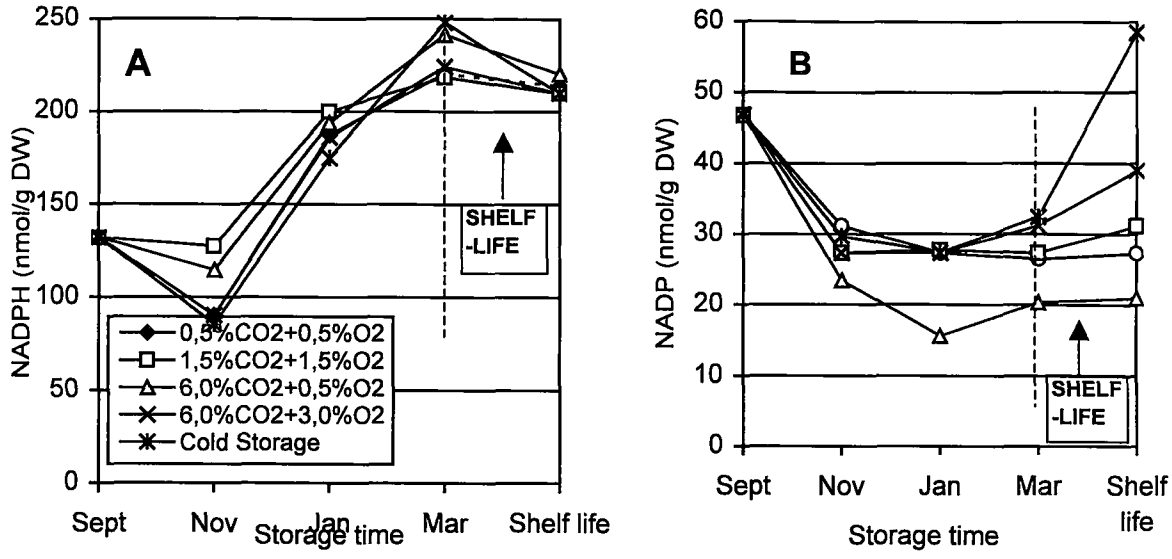

Fig. 21: Changes in the concentration of NADPH (**A**) and NADP (**B**) of Conference pears under various CA-conditions

ATP and ADP contents: Figure 22 shows the concentrations of ATP and ADP. In contrast to the results of NADH and NADPH the changes of ATP and ADP during the storage periode under various CA conditions are very obvious. The cold storage gave the highest values of ATP during all storage time followed by the CA condition of 1,5% CO_2 plus 1,5% O_2. The highest ATP production during cold storage was at the 2nd month of storage, what was correlated with the highest observed respiration activity at this time (data not shown). At 6% CO_2 plus 0,5% O_2 the lowest ATP production and the highest incidence of physiological disorders as flesh browning and cavities was observed (data not shown). The behaviour of ADP was directly inversed compaired with ATP, i.e., the highest ADP accumulation was observed at 6% CO_2 plus 0,5% O_2 and the lowest at cold storage.

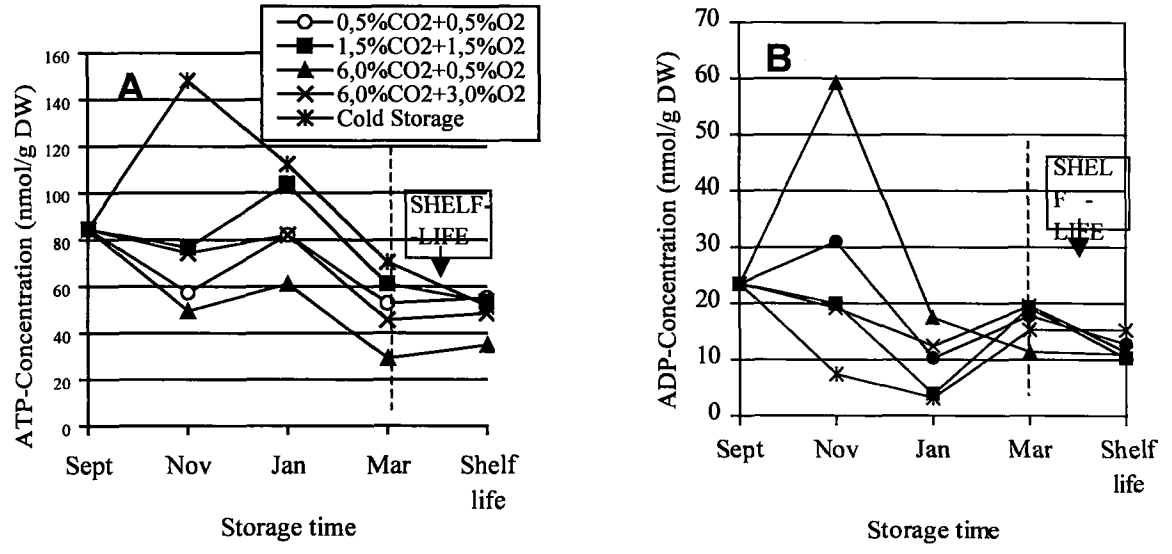

Fig.22: Changes in the concentration of ATP (**A**) and ADP (**B**) of Conference pears under various CA-conditions

Conclusions

The results of quality, mineral and biochemical analysis provide some interesting relationships to browning disorders Especially, decrease of vitamin C can possibly act as an indicator to predict browning disorders. Very interesting relations seem to exist also between antioxidative potential and energy metabolism on one hand and occurence of disorders on the other hand. Detoxifying processes need energy which seems not available under detrimental CA conditions. This seems to confirm that energy metabolism plays an important role in avoiding brown heart.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1998TO 31-05-1999

Task 6 Respiration and diffusion measurement: The respiration and diffusion measurement for sampling date 4 (end of storage after 6 months)couldn't carry out because of technical problems with the Micro GC. Due to bad separation of argon and oxygen peak with the used molsieve A column we had to change different parts of the instrument.

Task 7 Biochemical analysis: Developed methods for determination of lipoxygenase activity (LOX) was e not reproductible enough. We have to improve the protocols further and we have to solve problems with the freeze stored fruits due to increased temperature on the top of the freezer.

The determination of ethane was delayed due to problems with the installation of a photoionisation detector. This investigations will be possible next periode.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

The research activities for the next periode depend on the evaluation of the work which we have done up to the present. This evaluation will take place during the next meeting of all partners mid of June 1999 in Lleida, Spain.

According to our results we have a lot of informations about the effect of harvest time, postharvest treatments and storage conditions on the occurance of browning disorders but we don't know so much about the causes why late picking, delayed CA or high CO_2 concentrations effect these disorders. Therefore we will concentrate our work more on the physiological and biochemical part (task 7c). First results seem to confirm that lack of energy, mainly ATP, may play a fundmental part in browning disorders. More work we have to do on the ability of fruit tissue to react under stress situation by peroxide scavinging, detoxifing enzyms and antioxidative potential. Nevertheless, in order to obtain fruits of known history and growing conditions for additional investigations we have to continue with some tasks on the same level as before. Concerning the number of orchards used for our experiments, it might be possible, that one orchard (instead of two) is sufficient. But this depends on task 9 (modelling) how many data set are necessary in future.

From our point of view:

Following tasks can be stoppt:

- Task 4: Variation of crop load can be stopped
- Task 7b: Mineral analysis can be stopped

Following tasks can be reduced:

- Task 1: One orchard instead of two
- Task 5: Postharvest treatments
- Task 6: Respiration measurement
- Task 7a: Fruit quality evaluation

Following tasks can be continued on the same level:

Task 2:	Harvest dates
Task 4:	Climate registration
Task 6:	Ethylene measurement
Task 7c:	Ethanol, acetaldehyde measurement
	Vitamin C

Following tasks can be increased:

Task 3:	Storage under various O_2 concentrations
Task 6:	Diffusion measurements
Task 7c:	Biochemical analysis of: conductivity, antioxydative potential, malon dialdehyde, ethan, enzymatic antioxidants (Glutathion reductase, catalase, superoxid dismutase, ascorbat peroxidase , dehydroascorbate reductase)
	Energy metabolism (NAD(H), NADP(H), ATP, ADP, AMP)

E. DISSEMINATION

- Streif, J.: Erntetermine und Hinweise für die Lagerung und Vermarktung von Apfel und Birnen. In: Kernobstsortiment für die Obstregion Bodensee, 1998, 39-45.
- Streif, J.: Lagerung von Apfel und Birne unter besonderer Berücksichtigung von Braeburn Äpfeln und Conference Birnen. Oral presentation. Neustadt, 8/1/99
- Streif, J. Optimaler Erntetermin und Lagerungsbedingungen für Braeburn and Conference Früchte. Oral presentation. Bitzfeld-Öhringen, 8/3/.99
- Streif, J. Gasdiffusionsmessungen an Früchten. Poster at the annual meeting of the German Society for Quality Research (DGG), Freising-Weihenstephan, 22/3/99
- Streif, J. Fleischverbräunungen bei Elstar und Erklärungsmöglichkeiten mit Ergebnissen von Conference und Braeburn. Oral presentation, Oberkirch 31/3/99
- Rabus, C. und J. Streif: Effect of various preharvest treatments on the development of internal browning disorders in 'Braeburn' apples. 25. International Horticultural Congress, Brüssel, 2.-7.8.1998.
- Xuan, H. und J. Streif: Effects of pre- and postharvest treatments of 'Biofresh' on keepability of different apples varieties. 25. International Horticultural Congress, Brüssel, 2.-7.8.1998.