Quality improvement of pears by predictive and adaptive technology

A shared-cost project

2nd Meeting in Weingarten (Germany) organized by the Institut für Obstbau, Universität Hohenheim (UHOH), Ravensburg, Germany, 12 and 13 January 1998

EUROPEAR RESEARCH PROGRESS REPORT 1

To be discussed during
the 2nd meeting of the project group

organized by
Institut für Obstbau
Universität Höhenheim

At
Weingarten, Germany

EC-FAIR-CT96-1803

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Content

General objective of the project

The general objective of the project is to prevent the development of tissue disorders in pears resulting in so called Brown Heart. The key element in the project is the development of technology which enables a rapid measurement and decision about the post-harvest treatments and storage conditions. To check the physiological hypothesis given, 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. Research carried out thus far is strictly bound to one particular region. Therefore, the relationships found cannot simply be applied for other countries. Within this project, pears grown in a range of climate zones, including both climate zones with high and low risk on Brown Heart, are investigated. Six European groups are joined in this project, from five different countries: Belgium, Germany, Italy, the Netherlands and Spain. Because every year results in pears with different characteristics, the project is planned to be carried out in 4 successive years. 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.

Project participants

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

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Agenda of the 2nd meeting at Weingarten, Germany

12 January 1998

08.45	Welcome and outline of the 2nd meeting by the coordinator, Herman Peppelenbos
09.00	Presentation of progress and planning at UHOH (Josef Streif, Claudia Rabus)
09.30	Presentation of progress and planning at ATO-DLO (Herman Peppelenbos, Rob Veltman)
10.00	Presentation of progress and planning at IRTA (Christian Larrigaudierre)
10.30	Coffee/tea break
11.00	Presentation of progress and planning at IVTPA (Paola Eccher-Zerbini)
11.30	Presentation of progress and planning at FPO (Anton de Jager)
12.00	Presentation of progress and planning at VCBT (Bert Verlinden)
12.30	Lunch
14.00	Discussion on methodology: Orchard treatments and measurements
14.45	Discussion on methodology: Respiration measurements
15.30	Coffee/tea break
16.00	Discussion on methodology: Diffusion resistance
16.45	Critical assessment of research progress and achievements regarding the objectives and deliverables mentioned in the technical annex; Dissemination of research results; Miscellaneous.
17.30	Closure of the meeting
19.30	Diner

13 January 1998

09.00	Excursion to research facilities of UHOH
12.00	Lunch

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	10/11	1	2000	FPO	Wilhelminadorp (NL)
7	14/15	6	2000	?	?
8	10/11	1	2001	?	?
9	?**	7	2001	ATO	Wageningen (NL)

* The days are provisional

** Will be planned close before the 9th International Controlled Atmosphere Research Conference, to be held in the Netherlands.

Schedule of deliverables

Year	Month	Report	Access to deliverable
1996	October	Technical annex	*Public*
1997	June	*Start of the project*	
1998	January	Progress report 1	Confidential
1998	June	Progress report 2	Confidential
1998	August	*Annual report 1*	*Public*
1999	January	Progress report 4	Confidential
1999	June	Progress report 5	Confidential
1999	August	*Annual report 2*	*Public*
2000	January	Progress report 6	Confidential
2000	June	Progress report 7	Confidential
2000	August	*Annual report 3*	*Public*
2001	January	Progress report 8	Confidential
2001	July	Progress report 9	Confidential
2001	October	*Final report*	*Public*

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A. PARTNER INFORMATION

1. (Sub)Project title
Quality improvement of pears by predictive and adaptive technology

2. Project coordinator
Dr. H.W. Peppelenbos

3. Project participants
Ing. S.A. Robat
A.C.R. van Schaik
Drs. R.H. Veltman

B. INTRODUCTION

1. Description of the research topic or 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.

2. 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.

3. 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 (Perata and Alpi, 1991). 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 (Andreev *et al*, 1991; Zhang and Greenway, 1994). 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. The model of Peppelenbos *et al.* (1996) might function as a basis for the models that will be developed. The model uses ATP production rate as the main inhibitor of fermentative CO_2 production. This model enables to describe the relation between gas (O_2 and CO_2) concentrations and O_2 consumption and CO_2 and ATP production rates.

C1. OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

Objective

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 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. This time gas exchange and diffusion characteristics were measured directly after harvesting and after a period of cooling or ULO.

Material and Methods

Conference pears were harvested on various dates: September 3, 10, 17, 24 and October 1st 1997. September 17 is considered to be the optimum harvest date for long term storage. Pears were harvested at a grower located at Ingen (provence Gelderland). 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 18°C (to test the possibility of a rapid testing method). This comparison of harvest dates will be reported later. Gas exchange characteristics of the optimal harvest date were measured at 2 and at 18°C. This enables a comparison with the other harvest dates and with stored pears.

Gas exchange rates

Fresh weight and underwater weight (Baumann 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 figure 1). 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 3 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 head space directly and after a period of 6 hours. The GC used was a Chrompack CP 2002. The measured O_2, CO_2 and N_2 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.

Diffusion resistance

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. 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 after specific time intervals (seconds) the neon concentration was measured. The time intervals were selected based on the paper of Banks (1985), in order to compare two methods of analysis. One method calculates diffusion resistance based on an exponential change in concentration, and the second method is based on a linear change in concentration. For the 'exponential' method the final concentration in the second cuvette is necessary. For the 'linear' method many measurements are necessary directly after transferring the fruit. The exponential method results in a long period of measuring, which can be a disadvantage when a lot of repetitions have to be carried out. Also leaks of the cuvette will have a stronger influence on the results then the linear method. The linear method, however, can only be carried out when a quick gaschromatograph is available. Both methods were used in all the experiments described, and the difference in results will be analysed.

Delaying ULO
Pears from the optimal harvest date were put in ULO 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).

Ripening
Pears from the optimal harvest date were stored at ~~18~~ 10°C and ambient air for 21 days. Gas exchange characteristics were measured after ~~3 and 5~~ 2, 6 and 13 days. Firmness and ascorbate levels were measured as well (see part C2).

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

Gas exchange
Gas exchange rates are expressed in nmoles/kg.s, following the guidelines of Banks et al.(1995). Gas exchange rates were measured at 6 different O_2 concentrations (0, 0.5, 1, 2.5, 6 and 21 kPa). Although the differences between these gas concentrations used are not equal, the differences in respiration rates are comparable (Fig 3 and 4). In future experiments therefore comparable gas concentrations will be used. The percentage of explained variance is relatively high for the O_2 uptake measurements, but relatively low for the CO_2 production measurements (Table 1). One of the reasons for this lower number could be the influence of CO_2 on fermentation rates. In figures 3B and 3D, this influence can be observed: at 0% O_2 the CO_2 production (which can entirely be attributed to fermentation) is lower when the CO_2 is increased. If this influence is observed more often, it should be included in the CO_2 production model.

Diffusion resistance
For calculating the resistance for gas diffusion, two methods were compared. Values obtained by the 'exponential' or the 'linear' method do not show a good correlation (fig 8A). When instead of the internal gas volume as determined by the method of Baumann and Henze (1985), the internal gas volume estimated based on the end concentrations found in the cuvettes was used, a much better correlation was found (Fig 8B). After extensive analysis it appeared that the internal gas volume used has a strong influence on the final resistance value that was obtained. When the internal gas volume is

not exactly clear, this is a strong disadvantage of the exponential method. One explanation for the difference between the internal gas volumes obtained is the release of neon from the water phase of the pears. In the exponential procedure, where pears stay at least 4 hours in the cuvette, this release could contribute to the end concentration found, thereby suggesting a bigger internal volume. For the linear method the pears stay maximally 10 minutes in the cuvette. This difference in time period will also influence the role of leakages of the cuvette. Al these observations lead to the conclusion that the linear method is preferred for further research.
The good correlation between both methods (using the estimated internal volume) suggests that there is only one main resistance for gas diffusion. According to Banks (1985) the exponential function will lead to bad results with tissues having more than one diffusion barrier, such as potatoes.
To estimate internal concentrations of O2 and CO2, Grahams law can be used. A validation of this procedure by actually measuring internal gas concentrations remains necessary.

Delaying ULO
Directly after harvest the respiration rate (expressed as the maximum O2 uptake) is about 19 nmoles/kg.s. The fermentation rate (expressed as maximum fermentative CO2 production) is about 11 nmoles/kg.s. (see Table 1). When pears are put in ULO conditions directly after harvest, they show comparable gas exchange rates after 5 weeks of storage. Pears that were only cooled and stored in ambient air, show substantial higher respiration and fermentation rates (Table 1, Fig 4).
One of the hypotheses of the project is that energy metabolism is related to the onset of Brown heart. Therefore the gas exchange rates were used to calculate ATP production, assuming that all O2 uptake can be attributed to respiration, and all CO2 production exceeding the RQ for respiration (RQox, Table 1) can be attributed to ethanolic fermentation. Also a limit value is used, resembling ATP production necessary to cover maintenance needs. This limit value is currently estimated as the ATP production at standard ULO conditions. The results are presented in Fig 5. In this figure it is demonstrated that at 5 kPa of CO_2 the ATP production is equal to the limit value at about 6 kPa of O_2, much higher than the 2 kPa of O_2 used at 0.1 kPa of CO_2. Another interesting observation is that at 2 kPa of O_2 the pears that were cooled for 5 weeks show a substantial higher ATP production. This might explain the reduced risk for Brown heart, as found recently by partner 4.

Ripening
When pears ripen, respiration rates increase substantially (fig 8). Differences between individual pears are remarkably small (fig 8). When the O2 uptake rate at the start of the experiment (day 2) is compared with the experiment on delaying ULO, the influence of temperature becomes clear. Respiration measured at 2°C (delayed ULO) is fourfold lower than at 18°C (ripening).
Diffusion resistance remains almost equal during ripening. The observed variation can not be attributed to a change in time, but to differences between individual pears (compare pear nr. 27 and 28 in Fig 8.) or to differences between measurements (Fig 8, pear nr. 5). The combination of increased respiration and equal diffusion resistance will result in a larger difference between external and internal gas composition. In other words, during ripening the O_2 concentrations will be decrease and CO_2 concentrations will increase in the internal gas volume of pears.

Conclusions
Methodology for gas exchange and diffusion resistance measurements was tested, and the methodology to be used can now be advised. Large differences in resistance for gas diffusion are found between individual pears. Advice on safe storage conditions should include the extent of this variation. An experiment, using a postharvest technique to reduce the risk for Brown heart, seems to confirm the central hypothesis of the project that ATP production plays an important role.

C2. OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

Objective

In the first research episode we investigated the factors influencing browncore in *Conference* pears, like harvesting date, growing location and storage conditions. Another objective was to induce the aberration. In the second episode experiments on PPO activity lead to the decompartmentation hypothesis: decompartmentation of intracellular structures induce browning independent of PPO activity and polyphenol concentrations in pears. Decompartmentation can be initiated by free radicals. One of the main objectives was to investigate the putative relation between antioxidant levels and browning. Vitamin C can possibly act as an indicator to predict browning. Antioxidant need to be (re)generated: an energy consuming process. Regeneration of antioxidants is expected to be dependent of diffusion and respiration characteristics and external gas conditions.

Introduction

Core browning is a disorder in pears, and can frequently be observed in the Conference cultivar. The phenomenon is characterized by tissue breakdown, and it develops during CA-storage (van Schaik, 1990). Aberrations start in the core of the fruit and expand concentrically to the cortex tissue. In a later stage the pears form cavities in and nearby the core (Wang and Worthington, 1979; Hoehn *et al.*, 1996). It is not sure if those cavities (also known as core breakdown) are a consequence of the flesh browning. In some cases they are formed in healthy tissue. Preharvest factors, like growing location, picking time of the fruit, and the climate, can affect the susceptibility of pears for core browning. During storage, temperature, oxygen and carbon dioxide influence the initiation and development of core browning.
A fundamental step in core browning is the enzymatic oxidation of polyphenol substances to *o*-quinones (Mayer, 1987; Steffens *et al.*, 1993). This step is catalysed by the metallo-enzyme polyphenoloxidase (PPO). PPO-generated quinones are highly reactive molecules, which modify and crosslink with a variety of cellular substituents. Quinone reactions end in the formation of brown or black melanin polymers (Mathew and Parpia, 1971). There is a general agreement that PPO is a plastid enzyme (Hutcheson and Buchanan, 1980). It is located in the thylakoid membrane of chloroplasts and in vesicles, or other bodies, in non-green plastid types. However, in apples it was assessed that PPO was found both, in the chloroplasts and in the mitochondria (Mayer, 1987; Nicolas *et al.*, 1994).
At the subcellular level phenolics are compartmentalized in the vacuole. Yamaki concluded that 97% of the total phenolic content is accumulated in the apple vacuole, only three percent was located in the free space, and none in the cytoplasm (Yamaki, 1984). We hypothesize that browning is induced by decompartmentation of intracellular membrane structures. Due to leakage of internal membranes, PPO's can find their substrate, which results in browning of the tissue. Membrane damage can be caused by lipid peroxidation (Dahle *et al.*, 1962; Pryor *et al.*, 1976). During aging of the fruit more radicals are formed (Kumar and Knowles, 1996a). These radicals can be inactivated by radical scavengers like α-tocopherol (vitamin E), ascorbate (vitamin C) and glutathione (Winkler *et al.*, 1994). The regeneration of these scavengers is an energy-consuming process. In potatoes a correlation was found between aging, the formation of radicals and the energy production (Kumar and Knowles, 1996b). There is a strong indication that ascorbate is an important substance in the defence mechanism against free radicals (Bermond, 1990). Ascorbate has been demonstrated to reduce oxidized tocopherol directly (Chan, 1993). This can be an explanation for the synergistic effects of both antioxidants seen in peroxidase systems. Secondly, ascorbate is directly involved in the removal of alkoxyl (LO.) and peroxyl (LOO.) radicals (Chan, 1993). Subsequently the semidehydroascorbate molecule or ascorbate radical is enzymatically or non-enzymatically reduced, or broken down. Third, ascorbate is able to convert enzymatically formed *o*-quinones back to their precursor phenols (Nicolas *et al.*, 1994).
Our research focuses on the development of brown core and this paper reports that PPO-activity and the level of polyphenols are no indicators for the sensitivity to the aberration. Furthermore, the vitamin C content in pears stored under different gas mixtures might be indicative for the development of core

browning.

Material and methods

Material.
To compare fruits with a different sensitivity towards brown core, pears were picked from two locations, at three harvest dates. Pears were harvested in Strijensas (grower 1) and Zuid Beijerland (grower 2), both in the west of the Netherlands (province Zuid-Holland). From an historical perspective the pears from Strijensas are relatively sensitive to the development brown of core, whereas the pears from Zuid Beyerland are usually not. The pears from both locations were picked at three dates. The optimal (second) pick, predicted by Streif-index, was harvested on the 18th of September 1996. The other pears were picked on the 11th of September (pick one) and the 25th of September (pick three). In 1995 the pears were picked on the 6th, the 15th and the 26th of September respectively. The pears were stored in boxes, placed in 600 litre containers with a water sealing, at -1 ^{0}C and 2% oxygen (static system). Carbon dioxide was kept low (<0.5%), using a KOH scrubber. During monitoring pears were kept in 60 litre 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.

Polyphenoloxidase activities.
Parts of the cortex of the pear were frozen in liquid nitrogen and freeze-dried. The freeze-dried samples were grinded in a mortar. For the enzyme assays, the method of Gerritsen *et al.* (1994) was used. For measurement of laccase 0.5 mM Tropolone was added. Tropolone is a specific inhibitor of tyrosinase activity (Kahn and Andrawis, 1985).

Total phenolic content.
The method is based on the reaction between the Folin Ciocalteus reagent and substituted phenyl residues (Spies, 1957). Freeze-dried tissue was homogenized in water. Cell remainders were removed by centrifugation (15-30 min., 11000g). The homogenate (0.5 ml) was mingled with 0.5 ml 10% TCA to remove proteins. After vortexing and centrifugation (15-30 min., 11000g) 0.75 ml of the sample was transferred to a 3 ml cuvet and mixed with 1.5 ml 1.4 M Na_2CO_3 and 0.45 ml Folin-Ciocalteus reagent (Merck nr. 9001). After 30 minutes the samples were again centrifuged, to avoid precipitation. The extinction was measured in triplicate at 650 nm. Calibration was done with tyrosine standards.

Ascorbate measurements on HPLC.
Samples were taken from individual pears (peel, cortex and core). What was left of the fruit was longitudinally divided in four quarters. The core tissue plus the middle vein were removed. The cortex was cut in small pieces. The three fractions were immediately frozen in liquid nitrogen. The extraction was performed in the dark on ice. Frozen samples were crushed in a kitchen mixer. Ten ml methanol, 10 ml 9.5% oxalic acid and 70 ml Milli-Q was added to 10 gram homogenized tissue. This mixture was blended with an Ultra Turrax and filtered over a S&S 595 ½ filter, a 0.45 µm sterile filter and a Sep-pak C-18 column. To determine the amount of vitamin C (total ascorbate), the dehydroascorbate fraction was chemically reduced with homocystein, after neutralizing the solution with 0.5 M TRIS-buffer, pH 7.6. A Symmetry C-18 3.9 mm x 150 mm column (Waters) was used, with a particle size of 5 µm, in combination with a Sentry C-18 Guard column. The column temperature was 25 °C. Ascorbate was detected by a Waters 486 UV-VIS detector at 251 nm. Since ascorbate is light, temperature and pH

sensitive, a manual injection system was used with a 20 µl loop. Samples were injected immediately after isolation. The eluent consisted of 2.5 gram tetrabutylammoniumhydrosulfate (z.s. Merck 818858) and 55 ml methanol (p.a. Merck 6009) per litre Milli-Q. Before use, the eluent was filtered through a 0.45 µm Millipore filter (HVLP 04700). The flow rate was 1 ml/minute.

Firmness measurements.
During monitoring the firmness of the pears was determined with an Effe-gi penetrometer with an 11 mm ⌀ probe.

Results

No relation between occurrence of internal browning disorder and PPO activity.
Pears were stored in the static system under 0.5% and 3.0% CO_2. Tyrosinase and laccase activities were measured from individual pears. The latent tyrosinase activity was determined after activation with SDS. The PPO activities were related to different growing and storage parameters of the pears, like carbon dioxide during storage, harvesting date, growing location, and size of the pears. Of all correlations possible only two were evident (table 2 and 3).
A relation was found between laccase activity, harvesting date, and storage condition of the pears. Secondly, there is a relation between tyrosinase activity, harvesting date and growing location. Both relations can be explained by the fact that browning of the tissue lowers the activity of PPO. Pears of a later harvesting date and pears from grower 1 are more susceptible for browning. Pears stored under enhanced carbon dioxide concentrations also show a higher sensitivity towards browning. In table 2 and 2 it is shown that pears of a later harvesting date (3), pears from grower 1 and pears stored under 3% carbon dioxide show a lowered PPO activity. In other words it seems that a higher PPO activity does not induce browning. Browning lowers PPO activity.

Effects of gas conditions on PPO activity.
The previous results do not seem to explain a relation between PPO activity and gas conditions. In this experiment pears under bad conditions became brown. In another experiment (figure 9) tyrosinase activity was determined in pears under different conditions before browning occurred. Laccase, tyrosinase and total tyrosinase (latent plus active) activities were determined after 25 days storage. Only total tyrosinase activity seems to be affected by high carbon dioxide concentrations. Tyrosinase and laccase activities (data not shown) were not affected.

Vitamin C in pears
In a flow-through system we are able to simulate practical situations. With this system we are able to induce browning in pears at a high carbon dioxide concentration and, if necessary, a higher temperature. During storage in the flow-through system it is possible to do experiments that give more insight in the induction of the browning process. One of the parameters monitored is the vitamin C concentration of pears. During normal storage (2% oxygen, carbon dioxide <0.5%) the vitamin C content of pears decreases minimally in time (table 4).
Atmospheric conditions during storage play a key role. Decreased oxygen concentrations (0.3%) result in a 30% decrease of ascorbate in the cortex tissue and a 24% decrease in the core. Adding 10% carbon dioxide decreases the ascorbate content with 60% compared to standard conditions (figure 10).
Figure 12 shows the ascorbate levels in mixed samples: samples of five individual pears were put together. This method masks the individual variations in vitamin C levels of the pears. Another point of criticism is that figure 10 only shows a static measurement. In a second experiment vitamin C content in pears were monitored, and vitamin C levels were determined in individual fruits (figure 11).
Pears stored at normal conditions (2% O_2, control) show an increase in vitamin C content after 31 days from 3.60 ± 0.79 to 5.18 ± 0.70 mg vitamin C per 100 gram fresh weight (data not shown). After 50

days the vitamin C concentration decreases sharply to 2.58 ± 0.47 mg. This is attributed to a ripening effect and was not seen in pears stored under 10% CO_2, because CO_2 inhibits ripening. At normal conditions, penetrometer values drop from 11.40 ± 0.91 at t=0 to 5.3 ± 2.0 at t=31. Under high CO_2 conditions the firmness is nearly stable. There is a slight decrease to 10.7 ± 0.80 at t=31.

Ripening
Figure 12 shows a significant decrease in ascorbate during the last days of the experiment. At this time the pears are ripe and consumable. The decrease of ascorbate content is not as high as expected. In literature it is described that ascorbate level decreases sharply during ripening. An explanation can be that the pears used for this experiment have not been under CA conditions, the pears were stored cooled. During the experiment the ethylene emission was monitored. At 10, 14, 17 and 20 October the ethylene production was 46.08 ± 9.98, 273.44 ± 63.54, 431.33 ± 146.29 and 731.72 ± 109.31 ppb/min. respectively.

Artificial accumulation of ascorbic acid in pears
Ascorbic acid concentrations in pears decrease under enhanced carbon dioxide concentrations and lowered oxygen concentrations. The relation between browning and vitamin C content in pears, however is not clear.
We tried to increase the vitamin C content in pears artificially by vacuum infiltration. Two sterilized needles were stung into the belly of the pear. The pears were placed in a waterbath containing 1% w/w ascorbic acid. Control pears were placed in a bath containing only water. After infiltration the pears were stored at 2% oxygen and 10% CO_2. Indeed the pears infiltrated with the 1% w/w ascorbic acid solution contained more ascorbic acid for a couple of weeks. Over a longer period the differences between infiltrated and non-infiltrated pears were not evident. The little needle-holes in the peel induced rotting of the fruit flesh. Rotting tissue can be distinguished from enzymically browned tissue. Vitamin C vacuum infiltrated pears show less browning. Also water infiltrated pears show less disorders. Question is if the decreased incidence of browning can be attributed to vitamin C, or to an other factor, like differences in diffusion and respiration characteristics. Figure 13 shows the results of a pilot experiment but certain tendencies are clear. Only 20% of the pears that were Vitamin C infiltrated show -after 6 weeks storage- more or less browning. Without the vitamin C treatment about 70% of the pears show the disorder.

Conclusions
There is no clear relation between PPO activity and sensitivity for internal browning. Browning lowers PPO activity. Following our hypothesis intracellular membranedamage is a key factor in the browning process. Vitamin C acts as a radical scavenger in cooperation with other antioxidants and is known to play a role in the protection of membranes against peroxidation. Vitamin C levels in pears depend on external gas conditions during storage. There is a strong indication that vitamin C infiltrated pears show a decreased susceptibility towards browning. Vitamin C levels, and possibly other antioxidants, can putatively act as indicators for browning.

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D. DESCRIPTION OF THE WORK PLANNED FOR THE SUBSEQUENT PART OF THE PROJECT (1-12-97 TO 1-6-98)

In the next time period the stored pears will be sampled at regular time intervals for various measurements. Changes in gas exchange rates and diffusion resistance during ULO storage will be monitored. The experiment where pears were monitored during ripening (at a high temperature) will be repeated to compare ripening behaviour between pears that were stored for a short and a long time period. Based on experiments the 'linear method' of analysis of diffusion resistance measurements is adviced. This specific method will be further optimized so it can be implemented by other partners in the next season. Data on meteorology and orchard factors will be received from the other partners and analysed for use in the neural network type of model. Results of this analysis will be used for advice on measurements in the coming growing season. 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.
The biochemical part of our tasks will focus on two subjects. First monitoring vitamin C during storage at different gas conditions, and the consequences of changing conditions during the storage period. 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 University Nijmegen.

E. DISSEMINATION

Paper

Veltman R.H., van Schaik A.C.R., 1997. Membrane damage in fruits possibly explains brownheart. Fruitteelt, 12: 12-13 (in Dutch).
Peppelenbos H.W., 1997. Modelling the influence of oxygen and carbon dioxide on the quality of fruits and vegetables. VoedingsMiddelenTechnologie, 30 (3): 30 (in Dutch).

Lecture

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.
Peppelenbos H.W., 1997. Gas exchange models and the prediction of disorders in CA storage. COST915 meeting Leuven, 3-6 June 1997.

Tables

Table 1. Overview of the statistical results of fitting the gas exchange models to the data, using Genstat nonlinear regression analysis (Fit = fitted value, se = standard error, R^2 = percentage of explained variance).

	Harvest		Cooling		ULO	
	Fit	std	Fit	std	Fit	std
R^2	74.8	2.8	92.1	2.1	94.3	1.35
Vm_{O2}	19.2	2.2	24.5	1.4	20.4	1.1
Km_{O2}	1.37	0.52	0.90	0.18	1.80	0.28
Km_{CO2}	16.9	8.4	14.4	3.8	10.9	2.1
R^2	50.5	2.0	74.9	2.0	77.4	1.6
Vmf_{CO2}	11.0	2.5	25.0	5.3	9.85	1.93
Kmf_{O2}	0.39	0.26	0.07	0.03	0.14	0.09
RQox	0.89	0.05	0.86	0.01	0.88	0.04

Table 2. Relation between laccase activity, carbon dioxide concentration during storage and harvesting date of pears. (a) And (b) show significant differences ($p<0.05$). Activities are shown in nkatal.

laccase (nkat.)		
	CO2 concentration	
harvest	0.5	3.0
1	9.5 (a)	8.9 (a)
2	5.4 (ab)	9.4 (a)
3	4.9 (b)	2.3 (b)

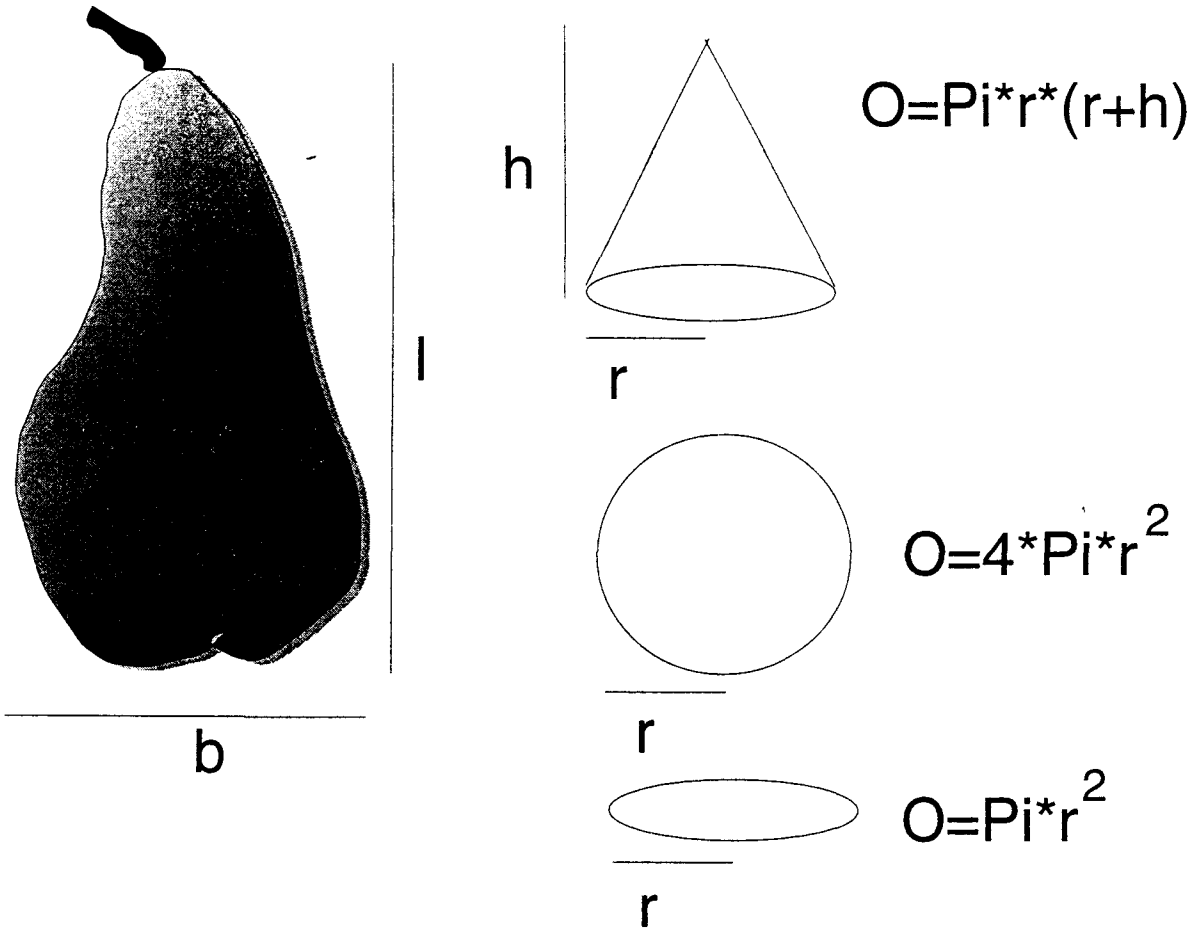
Table 3. Relation between tyrosinase activity, growing location and harvesting date. (a) And (b) show significant differences ($p<0.05$). Activities are shown in nkatal.

act. tyrosinase (nkat.)		
	Location	
harvest	grower 1	grower 2
1	27.9 (a)	14.8 (ab)
2	17.6 (ab)	14.7 (ab)
3	4.6 (b)	13.8 (ab)

Table 4. Vitamin C content of pears stored under normal conditions: 2% oxygen, ,0.5% carbon dioxide (-1 °C). Pears are from grower 1 (second pick). Average values are given with standard deviations. January: n=8; November n=15.

Vitamin C (mg/100 gr FW)		
	avg.	std.
14 january		
cortex	3.403	0.319
core	3.387	0.594
5 november		
cortex	3.285	0.103
core	3.364	0.101

Figures



Surface

$$= Pi*r*(r+h) + 0.5*4*Pi*r^2 - Pi*r^2$$

$$= Pi*0.5*b*(0.5*b+l-0.5*b) + 0.5*4*Pi*(0.5*b)^2 - Pi*(0.5*b)^2$$

$$= 0.5*Pi*b*l + Pi*(0.5*b)^2$$

Figure 1: Methodology for estimating pear surface

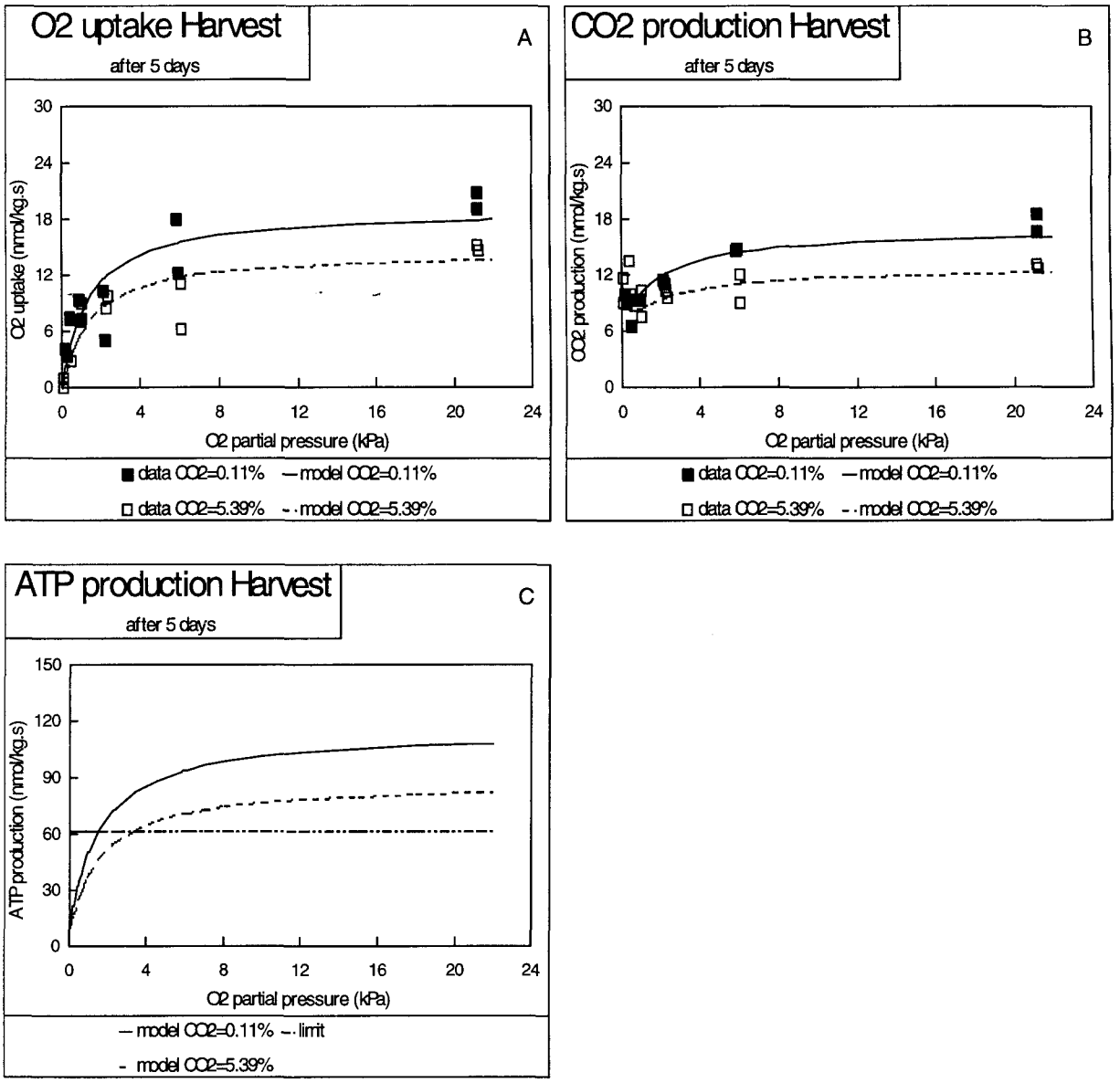


Figure 2.The O_2- uptake and CO_2- production of pears directly after harvest, figure A and B. The respiration is measured after 5 days storage at different oxygen and carbondioxide conditions. Figure C gives the ATP production which is calculated after 5 days storage at different oxygen and carbon dioxide conditions.

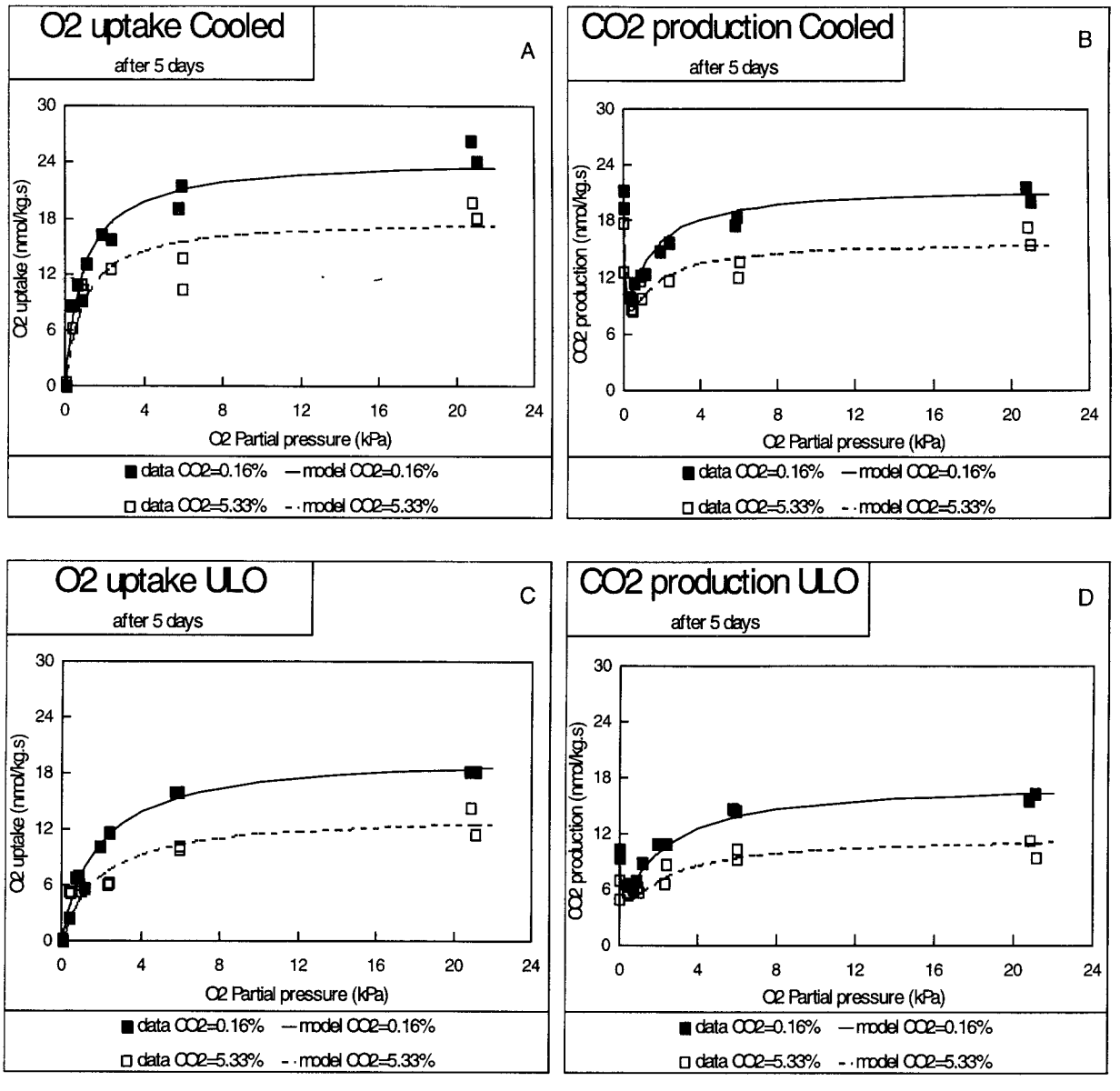


Figure 3. The O_2- uptake and CO_2- production of pears stored at normal air (cooled), figure A and B and at ULO, figure C and D, for 5 weeks. The respiration is measured after 5 days storage at different oxygen and carbon dioxide conditions.

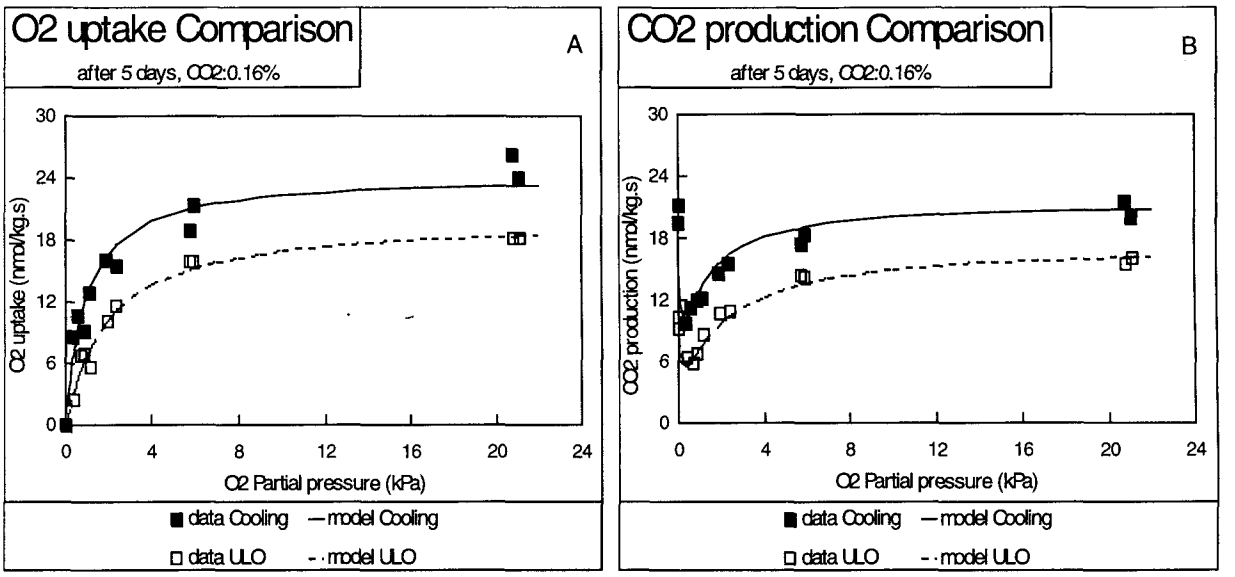


Figure 4. The comparison of the O_2- uptake (figure A) and CO_2- production (figure B) of pears stored at normal air (cooled) and ULO after 5 weeks storage. The respiration is measured after 5 days storage at different oxygen and carbon dioxide conditions.

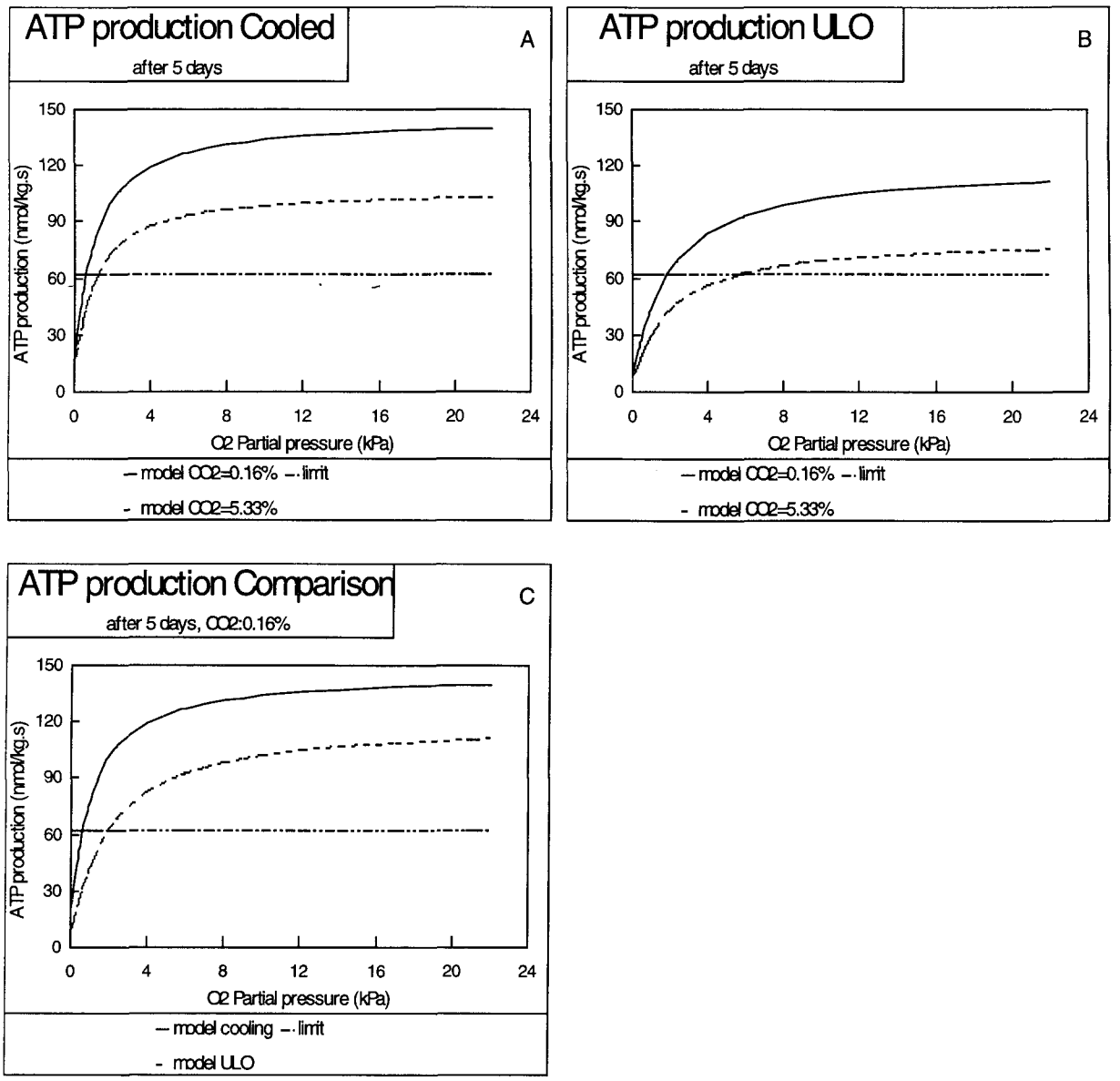


Figure 5. The comparison of the ATP production of pears stored at normal air (cooled) and ULO after 5 weeks storage. The ATP production is calculated after 5 days storage at different oxygen and carbon dioxide conditions.

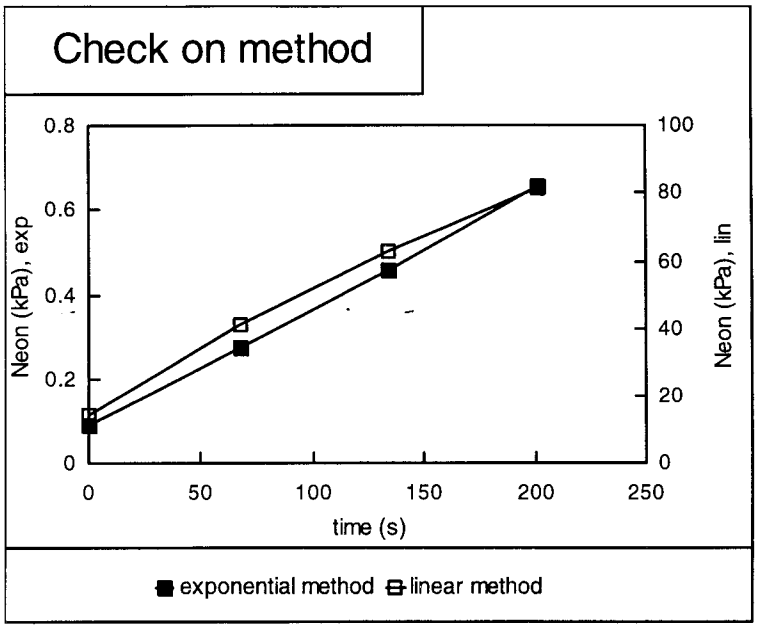


Figure 6. Check on method for calculating resistance, there is one sample of the experiment ripening used. The neon concentration after replacing the pears from flask 1 to flask 2 gives for both methods, exponential and linear, a straight line.

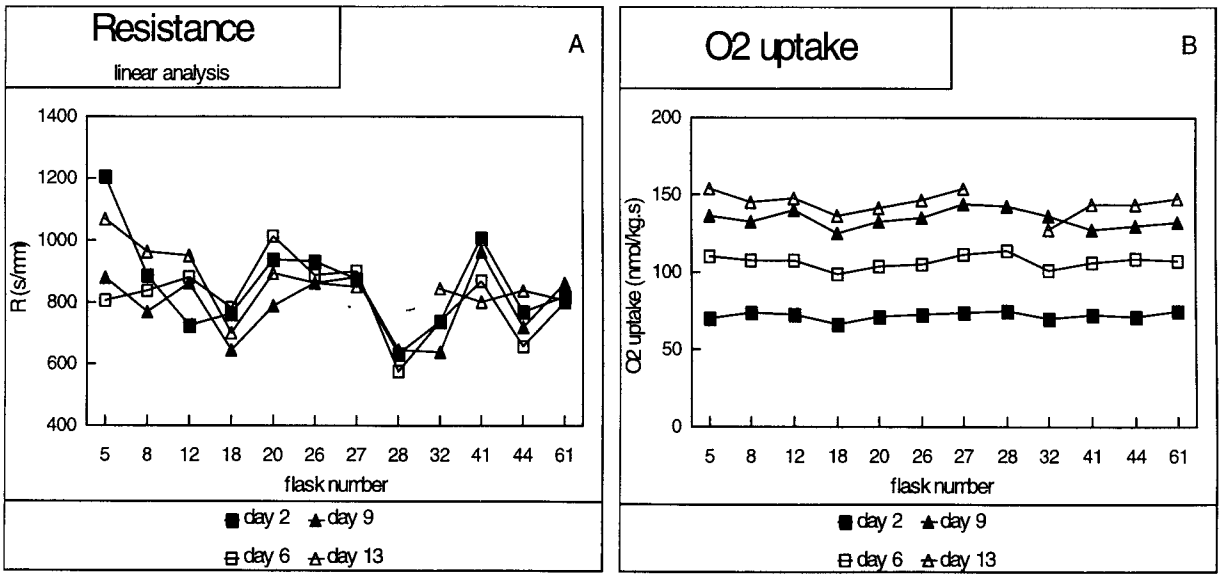


Figure 7. The reproducibility of the methods for calculating resistance and O_2 uptake. All the results (4 days) of the experiment ripening are used. Figure A gives the results of the resistance. Figure B gives the results of the O_2 uptake.

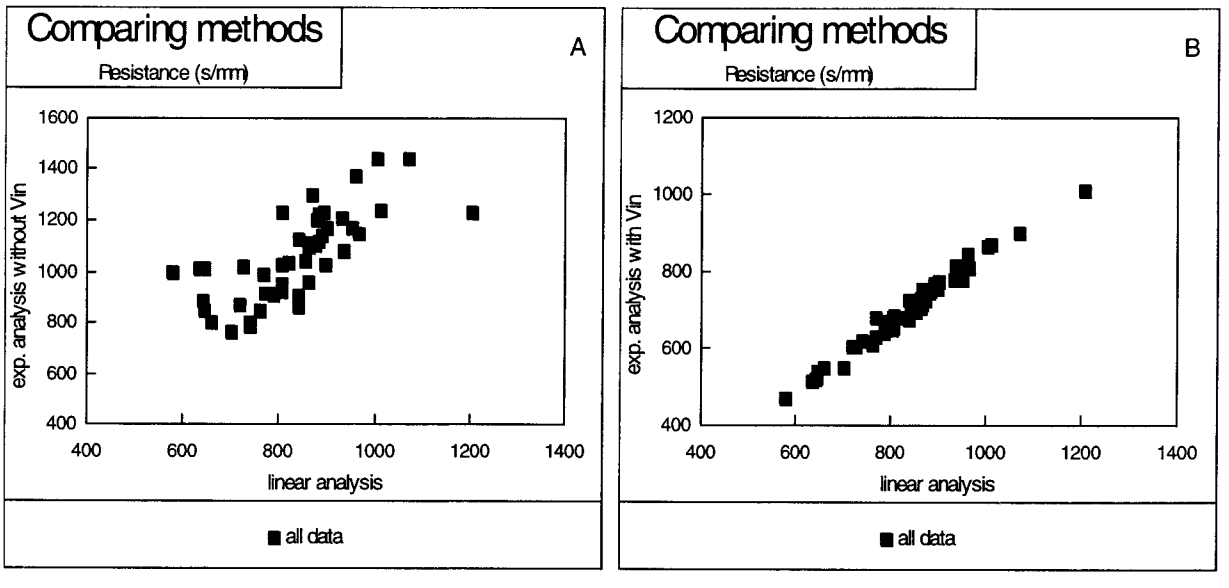


Figure 8. Comparing methods for calculating resistance. All the results (4 days) of the experiment ripening are used. Figure A gives the results of the exponential analysis without a correction for the intern volume, figure B gives the results of the exponential analysis with a correction for the intern volume. Both are plotted against the results of the linear analysis.

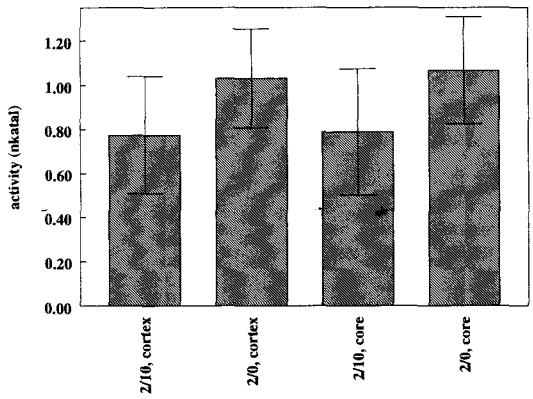
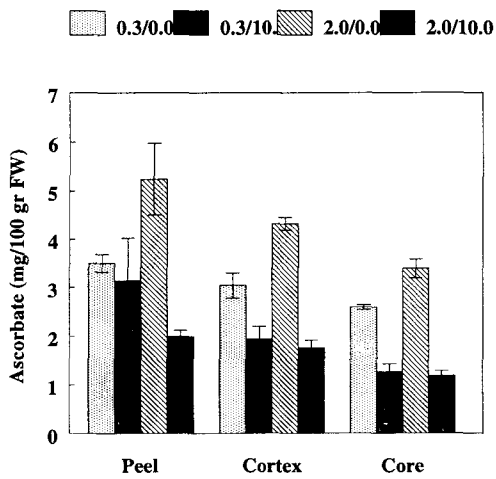


Figure 9. Total tyrosinase activity measured in core and cortex tissue of pears. Legends: 2/10: storage under 2% oxygen and 10% carbon dioxide; 2/0: storage under 2% oxygen. Measurements were done after 25 days. One bar is the mean of 20 measurements: two duplicate experiments with 10 pears. Standard errors of the mean were respectively 0.27, 0.22, 0.29 and 0.24.



Afbeelding 24

Figure. 10. Ascorbic acid content of pears after 60 days storage under different conditions in the flow through system (5 °C). Pears were from grower 1 and harvested at the optimal date (2). Values are the mean of a duplicate experiment of mixed samples of 5 pears (n=10) with standard errors. Gas conditions within a cluster: 0.3% oxygen, no carbon dioxide; 0.3% oxygen, 10% carbon dioxide, 2% oxygen, no carbon dioxide (standard condition); 2% oxygen, no carbon dioxide. Clusters represent the peel, cortex and core of the fruit respectively.

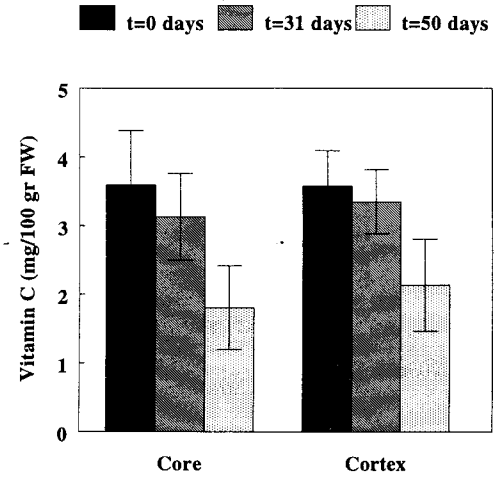


Fig. 11. Vitamin C after 31 and 50 days storage under 10% CO_2 and 2% O_2 in the flow through system (5 °C). Fruits were from grower 1 (optimal harvesting date). Bars are the mean of measurements on 5 individual pears. One bar is the average of two containers with identical gas conditions (n=10) ± SE. Bars within the clusters correspond with the different measurements during monitoring. The clusters represent the core and cortex fraction of the pear.

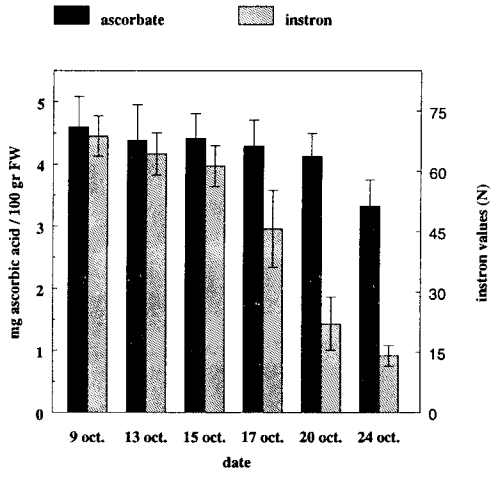


Figure 12. Pears were stored at -1 °C after harvest. At 9 October the ripening experiment started. During the experiment pears were stored at 9 °C. At different dates ascorbic acid and firmness (instron) were determined. For both purposes 20 pears were measured. Bars show the mean value with standard errors.

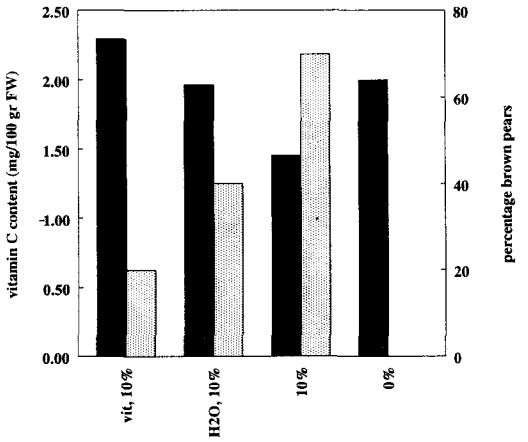


Figure 13. Pilot experiment. Pears were vacuum infiltrated with a 1% w/w vitamin C solution ('VitC') and water ('H_2O'). After treatment pears were stored at 2% oxygen and 10% carbon dioxide in the flow through system. Pears stored without carbon dioxide served as a blanc (0%). After 4 weeks in 10 pears (duplicate experiment of 5 pears) the vitamin C content of the core tissue was examined. Standard deviations of the vitamin measurements from left to right 0.513, 0.036, 0.503 and 0.428. After 6 weeks pears were judged on browning. From left to right n=62, 68, 20 and 20.

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A. PARTNER INFORMATION

1. (Sub)Project title:
-

2. Project coordinator
Dr. C. Larrigaudierre

3. Project participants

B. INTRODUCTION

Research topic or practical problem
Brown heart (BH) occurence in southern countries like Spain is lower than in Northern countries. Reasons for this difference remained unclear and will be searched comparing the results of both regions which regards the orchard practices, quality behaviour and biochemical differences in Conference pears.

Goal of the research and description of the approach
The main goal of the work performed during this first period concerned a study of various factors likely involved in B.H disorder. This study has been carried out at three levels estimating:
- the influence of orchard practices and particularly the influence of N_2 fertilization on B.H occurence.
- the relationship between the changes in quality parameters and B.H
- the changes in several biochemical pathways expected to be involved in the development of B.H in Conference pears.

C - OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

Objective

The work described in this report is a first approach of the problem and was carried out with the purpose to define the underlying mechanisms involved in B.H. Emphasis was given to the biochemical aspect and to complementaries studies which concerns the orchard practices (N_2 fertilization).
This approach has been carried out on a long-term storage basis (removal of chambers has been planned each 2 months) in order to see the general physiological changes. Once defined, study of the relevant factors will be completed on a short-term scale using micro-chambers devices.

Material and Methods

Material and orchard management
Conference pears were grown in 2 orchards (Gimenels and Albatarrech) near the experimental station. Pears from Gimenels were grown according the following conditions: variety: Conference, superficy: 1.2 ha, rootstock: BA-29, tree distances: 4 x 1.5 m, formation type: central axis, fertilization: N_2 - 70 U / ha.year or 140 U / ha.year. Pears were harvested mature at three different picking date, the 04/08, 12/08 nad 18/08/1997.

Storage conditions
After harvest the maturity indexes of the fruits were analyzed and the fruits placed in cold chambers (-0.5°C, 95% RH) as follows: air storage (21% O_2, <0.1% CO_2), CA type 1 (2% O_2, 0.7% CO_2) and CA type 2 (2% O_2, 5% CO_2).

Analysis of membrane peroxidation
Membrane peroxidation was established using standard methods like the analysis of electrolyte leakage (Lurie et al., 1987) and in the content of MDA (Rustérucci et al., 1996) or by an estimation of the ethane produced by thermical degradation as described by Degousée at al. (1995).

Enzymatic determinations
Parameters of fermentative metabolism were determined by gas chromatography for ethanol and acetaldehyde or spectrophotometrically for the analysis of the activities of Alcohol Deshydrogenase (ADH) and Pyruvate decarboxylase (PDC) according to the protocol of Nanos et al. (1992).
Polyphenol oxidase activity and intensity of browning were determined as described in the paper enclosed following the protocols of Sciancalepore and Logone (1984) and Mapson et al. (1963) respectively. Activities of enzymes involved in peroxide-scavenging was determined as follows: SOD activity was determined spectrophotometrically estimating the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) as described by Du and Bramlage (1994), Catalase activity was assayed measuring the rate of decrease of the absorbance of hydrogen peroxide at 240 nm (Beaumont et al., 1990), Ascorbate peroxidase (APX) and guaiacol peroxidase (POX) activities were separately and specifically assayed by a method derived from Amako et al., 1994.

Results and Discussion

Effect of N_2 fertilization on quality and B.H incidence
At harvest Conference pears fertilized with low dosis of N_2 (70 U / ha.year) showed lower value in all the main mineral compounds (N_2 , P, K, Mg) except Ca which was slightly higher (table 1). This changes in mineral composition lead to significative differences in the indexes currently used to predict the fruit conservability (table 2). This difference was related to an higher incidence in B.H showing that

the dosis of N_2 is a first factor involved in B.H and that Ca is likely the more relevant mineral element to be controlled to prevent this disorder.
As expected and in accordance with the results previously described above, the fruits fertilized with high N_2 levels were slightly more mature at harvest (less firmness, see Fig.1A; more sugars, Fig.1B but no significant changes in acidity, Fig.1C). After 2 months in CA storage the fruits were significantly more mature than at harvest but no significant difference between the two CA regime were observed for the two dosis of fertilization.
In conventional atmosphere (0.7 % CO_2), no damage were observed after two months of storage. In contrast, fruits stored at 5%.CO_2 already showed some damage (Fig.2). The intensity of the damage depends on the maturity stage at harvest and on the dosis of fertilization. The 140 U treated fruits developped more disorders (particularly senescent breakdown).

Relationship between browning, Polyphenol Oxidase enzyme and B.H in Conference pears
An important study has been carried out during years 1996 and 1997 to determine the relationship between B.H disorder and enzymatic browning - *see paper enclosed* -. Results indicated that enzymatic browning and PPO enzyme are not directly involved in the B.H disorder.

Harvest-maturity related changes in peroxide-scavenging enzyme and relation with B.H
Conference pears were harvested at three different picking date at a one week interval. Fruits were picked mature (table 3) in order to induce a maximum of disorders. Immediately after harvest, the activity of the enzymes involved in hydroperoxide metabolism was determined. Results can be resumed in the following table:

	Event	Metabolic consequence	Expected consequence on B.H
	decrease in SOD activity	less efficient scavenging of AOS	oxydative damage + B.H
Increasing marutity	decrease in CAT activity	accumulation of H_2O_2	membrane peroxidation + B.H
at harvest	increase in POX (slight)	consumption of H_2O_2 and phenolics	increase browning = B.H
	increase in APX	consumption in ascorbate	less antioxidant + B.H

Conclusion: decrease in natural peroxide-scavenging potential and increase in the sensibility of pears to B.H with increasing maturity.

Superoxide Dismutase (SOD) activity sharply decrease with increasing maturity indicating a lower capability to scavenge active oxygen species (AOS) in the more mature fruits (Fig.3). Concomitantly more mature fruits exhibited lower Catalase activity (Fig.3) but an increase in Peroxidase (POX) and Ascorbate Peroxidase activities (Fig.4). As a result of these metabolic changes, the more mature fruits showed less capability to prevent an oxidative damage and will be as generally decribed more sensitive to physiological disorders like B.H. As expected and in accordance with these results only the more mature fruits (H2 and H3) presented physiological disorders (particularly senescent breakdown) when stored 2 months at 5% CO_2 (Fig.5).

Effect of storage on membrane status
According to Degousée et al., there is a clear relationship between the anount of ethane produced by the

fruit (after thermical descomposition) and the level of hydroperoxy fatty acids. This method was used to determine the status of membrane peroxidation and it integrity. Stored fruits at various CA regimes already showed after 2 months of storage a peroxidative damage. Fruits stored in air were sharply damaged, more that fruits stored in CA at 0.7% CO_2 but less that the same fruits stored at 5% (Fig.6). This result shows that the CO_2 is likely involved in membrane peroxidation process, the higher the CO_2 during storage, the higher the damage.

Effect of storage on peroxide-scavenging enzymes

As shown in Fig.7, pears exhibited a sharp increase in SOD activity when stored in air. This increase was less important in CA but the activity remained always higher than at harvest. In contrast CAT activity decrease steadily during storage, particularly when the fruits were stored in CA. No important changes in POX activity were observed, inversely to APX activity which significantly increase in fruits stored at 5% CO_2 (Fig.8). This metabolic changes and its incidence on B.H are resumed in the following table:

	Event	Metabolic consequence	Expected consequence on B.H
Increase in CO_2 levels during storage	increase in SOD activity	scavenging of AOS increase in H_2O_2	opposite effect on B.H
	decrease in CAT activity	accumulation of H_2O_2	membrane peroxidation + B.H
	no changes in POX		no change in browning = B.H
	increase in APX activity	consumption in ascorbate	less antioxidant + B.H

Conclusion: decrease in antioxidant and enzymatic protective systems (against H_2O_2) in CA stored fruits Ù + B.H.

Differential responsiveness to storage as a function to maturity stage at harvest

Significant changes in SOD activity were observed between the different maturity stages (Fig.9). The more immature fruits (H1) showed no changes in SOD activity when stored at increasing level of CO_2. In contrast, the more mature fruits (H2 and H3) showed an intermediate behaviour and a sharp increase in activity respectively. In accordance with Foyer et al. (1997), we can consider that an increase in SOD activity may represent a double phenomenon i.e: - an enhanced capability to scavenge AOS - the reflection of an oxidative stress (SOD is inductive by AOS and an increase in activity may reflect an oxidative damage). According to these results, more mature fruits will likely generate more AOS and as a consequence be exposed to an higher oxydative stress.

Changes in CAT activity was the inverse to those of SOD activity (Fig.10). Activity remained low and constant in the mature fruits (H3) but sharply decrease for the immature fruits (H1) when stored in AC.

Except for immature fruits which showed less POX activity when stored in 5% CO_2 (Fig.11), activity remain constant for the other maturity stages. In contrast, CA storage and particularly the storage at 5% CO_2 induced an increase in APX activity for the three different maturity stages (Fig.12).

This diferential behaviour in functionof the maturity stage at harvest is resumed in the following table:

	H1	H2	H3
SOD	no change	increase: +	increase: ++
CAT	decrease: --	decrease: -	no change
POX	decrease	no change	no change
APX	increase: +	increase: +	increase: +

Conclusions

- N_2 fertilization and particularly Ca content seem to be an important factor to prevent storage disorder like B.H.
- Enzymatic browning and PPO enzyme are not directly correlated with B.H and likely are not basicaly involved in the occurence of this disorder.
- We observed significant differences in the capability of Conference pear to prevent oxidative damage as a function of maturity at harvest. Mature fruits have less natural antioxidant and less peroxide-scavenging potential than the immature fruits. This difference is correlated with an higher incidence in B.H in mature fruits.
- The storage and particularly CA storage at 5% CO_2 caused a significant decrease in the capability of the fruit to metabolize H_2O_2. This peroxide will accumulate in the fruit and likely caused membrane peroxidation and as a consequence B.H damage.
- During storage, the initial differences previously described for the fruits harvested at different picking date disappear and we observed an harmonization of the various metabolic activities. After 2 months of CA storage at 5% CO_2, fruits of all the maturity stages presented an important alteration of its capabilities to prevent peroxidative damage.

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D. DESCRIPTION OF THE WORK PLANNED FOR THE SUBSEQUENT PART OF THE PROJECT (1-12-97 TO 1-6-98)

The work planned for the subsequent part of the year will concern the following aspects:

- the work which regards biochemical studies (membrane peroxidation and peroxide-scavenging enzymes) will be completed with the results obtained from 1 or 2 others storage removals.
- the relationship with fermentative metabolism (ethanol, acetaldehyde, ADH and PDC) will be studied.
- changes of these metabolic activities in fruits altered by B.H and senescent breakdown at various

degree will be examined.
- the effect of a delay time before cooling and of the prerefrigeration will be studied.
- B.H occurence and changes in quality parameters will be compared for two different orchards.

E. DISSEMINATION

Results which concerns the relationship between browning, PPO and B.H incidence are submitted for publication in the Journal of Science of Food and Agriculture.

Tables and Figures

	70 U	140 U
% N_2 (dw)	0.29 +/- 0.020	0.33 +/- 0.029
P (%)	0.063 +/- 0.005	0.066 +/- 0.005
Ca (%)	0.046 +/- 0.005	0.040 +/- 0.001
K (%)	0.74 +/- 0.043	0.79 +/- 0.054
Mg (%)	0.036 +/- 0.005	0.040 +/- 0.001
Cu (ppm)	3.31 +/- 0.49	3.37 +/- 0.47
Mn (ppm)	2.64 +/- 0.19	2.51 +/- 0.14
Zn (ppm)	3.56+/- 0.65	4.50 +/- 0.59
Fe (ppm)	5.52 +/- 1.39	5.06 +/- 0.31
B (ppm)	9.67 +/- 0.49	10.84 +/- 1.69

Table 1: Effect of the fertilization at a low dosis in N_2 (70U) or a high dosis (140U) on the mineral content of the fruits at harvest.

Storage indexes

(TASC Ltd., POMANJOU)
S.I 1 = ((Mg + (K/P)xN)) / Ca

The higher S.I 1, the shorter the conservation

(GORSEM)
S.I 2 = K / Ca

The lower S.I 2, the longer the conservation

	70 U	140 U
S.I 1	76.1	99.8
S.I 2	16.1	19.8
Alterations (%)	0.6	3.2

Table 2: Effect of the fertilization at a low dosis in N_2 (70U) or a high dosis (140U) on the storability and % of alterations.

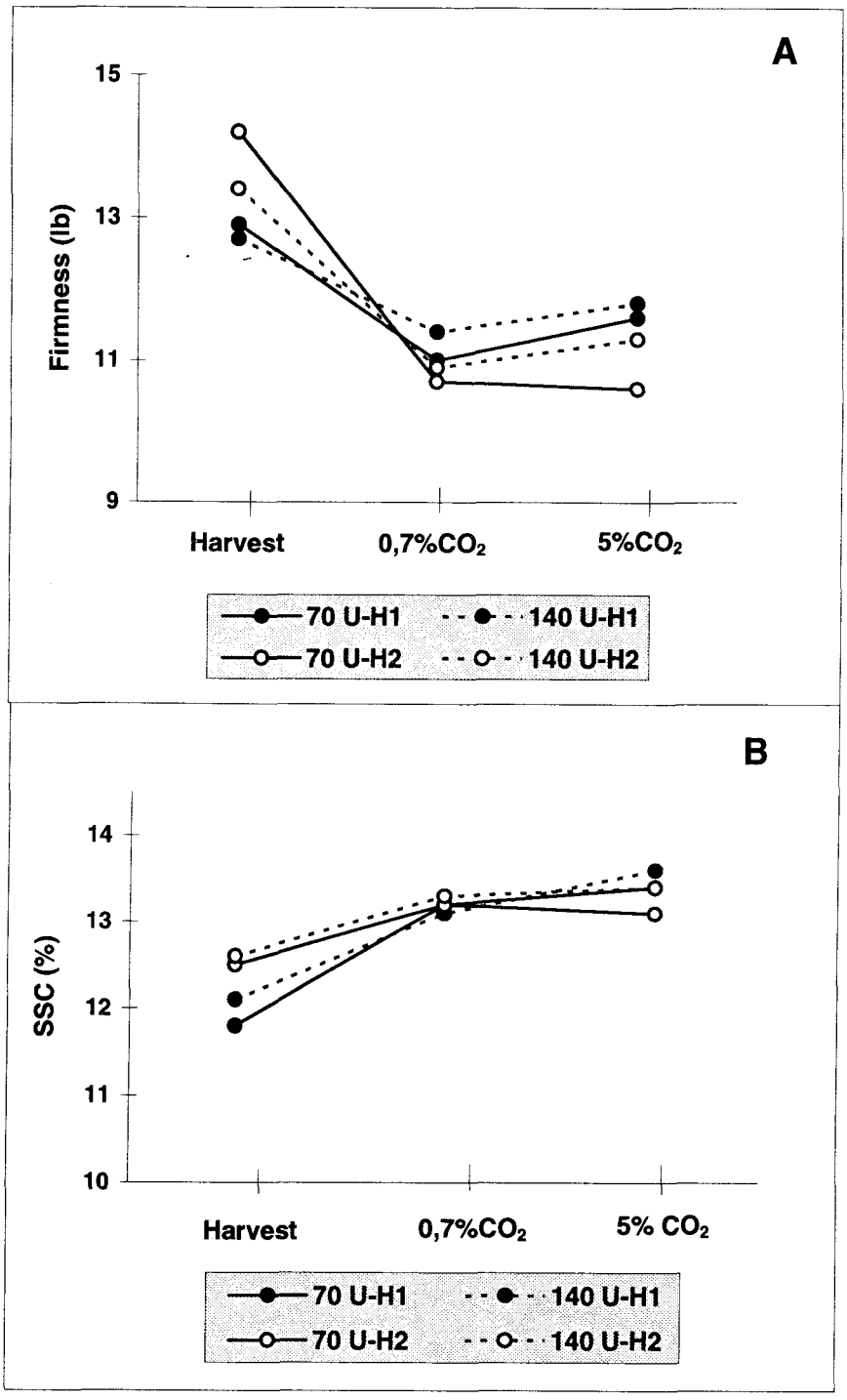

Figure 1A and 1B: Changes in firmness (A) and sugar content (B) at harvest and after 2 months in CA at 0.7 and 5% CO_2 in 70U and 140U-fertilized pears.

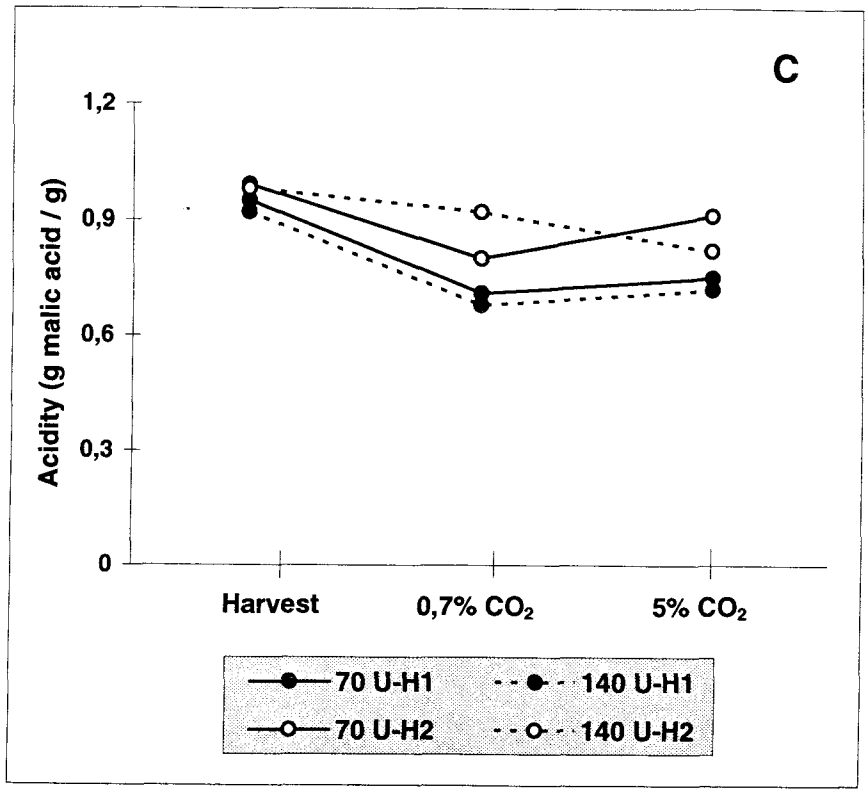

Figure 1C: Changes in acidity at harvest and after 2 months in CA at 0.7 and 5% CO_2 in 70U and 140U-fertilized pears.

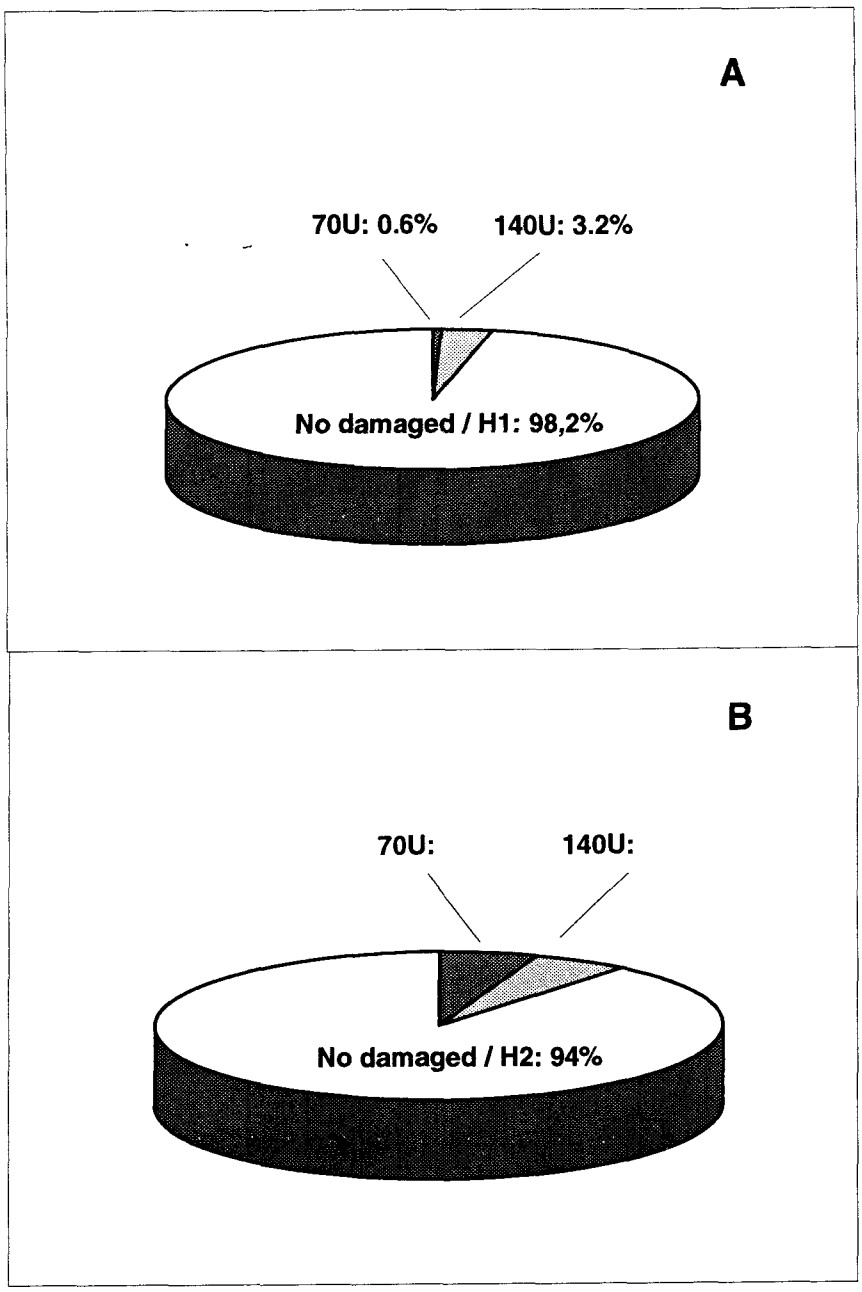

Figure 2: Changes in the % of alterations in pears stored 2 months in CA at 5% CO_2 and fertilized with different dosis in N_2. A: Harvest 1 (12/08); B: Harvest 2 (18/08).

	HARVEST 1	HARVEST 2	HARVEST 3
Color (a value)	-18.24 +/- 0.98	-18.45 +/- 0.97	-18.63 +/- 0.80
Firmness (lb)	12.8 +/- 0.9	12.9 +/- 1.0	14.2 +/- 1.3
Sugars (%)	11.1 +/- 0.5	11.8 +/- 0.4	12.5 +/- 0.3
Acidity (g malic acid /l)	1.32 +/- 0.15	0.99 +/- 0.1	0.95 +/- 0.12

Table 3: Changes in various maturity indexes at harvest in fonction of the picking date. Harvest 1, 2 and 3 were picked the 04, 12 and 18/08 respectively.

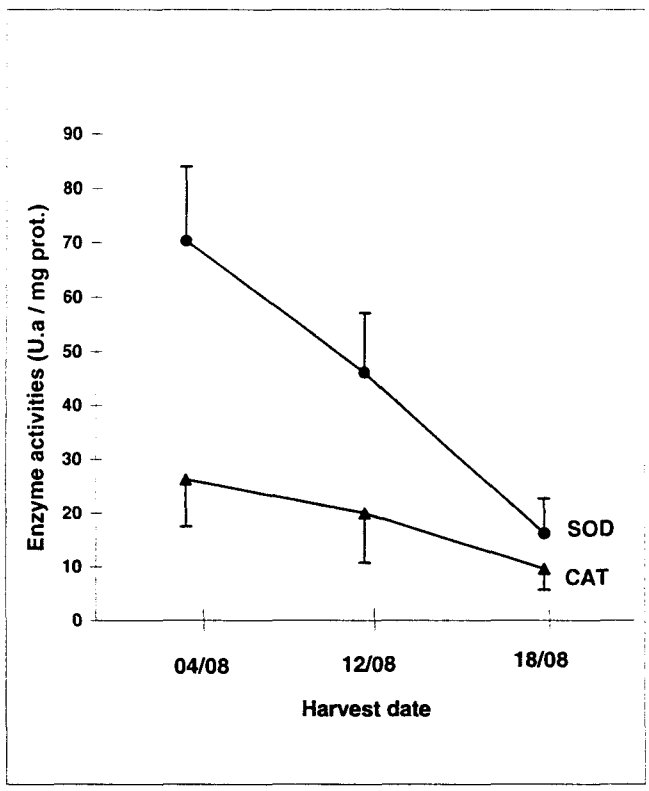

Figure 3: Effect of harvest date on the activities of SOD and CAT enzymes.

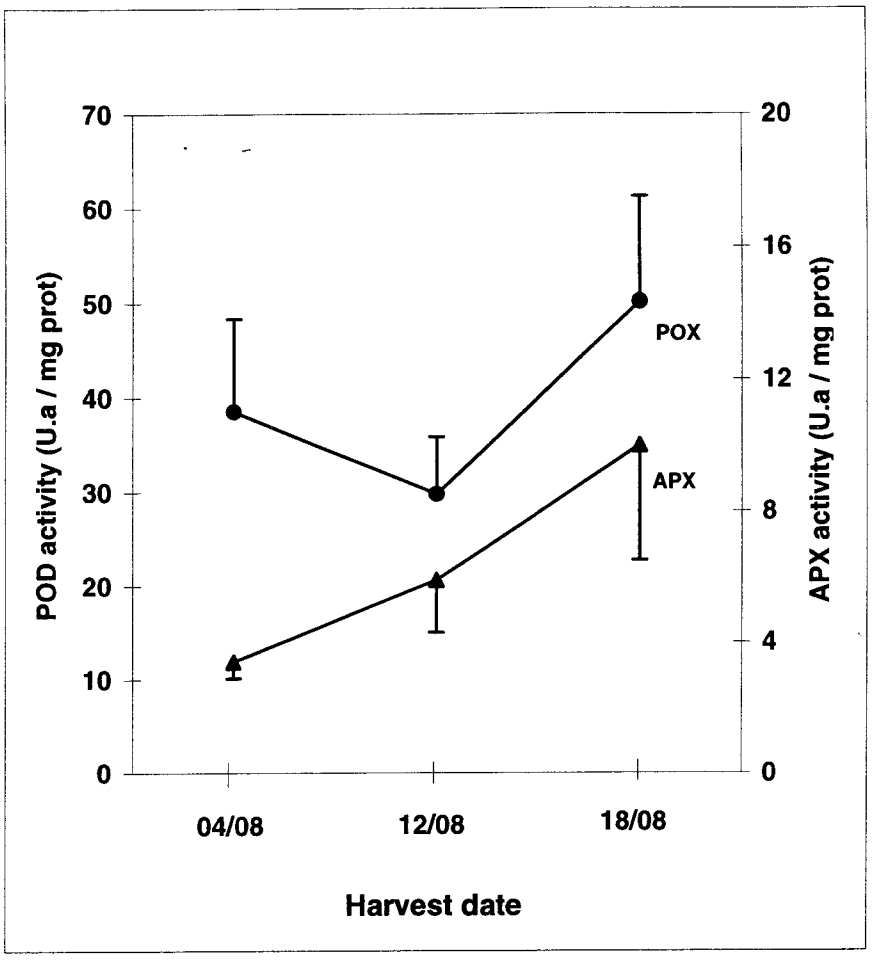

Figure 4: Effect of harvest date on the activities of POX and APX enzymes.

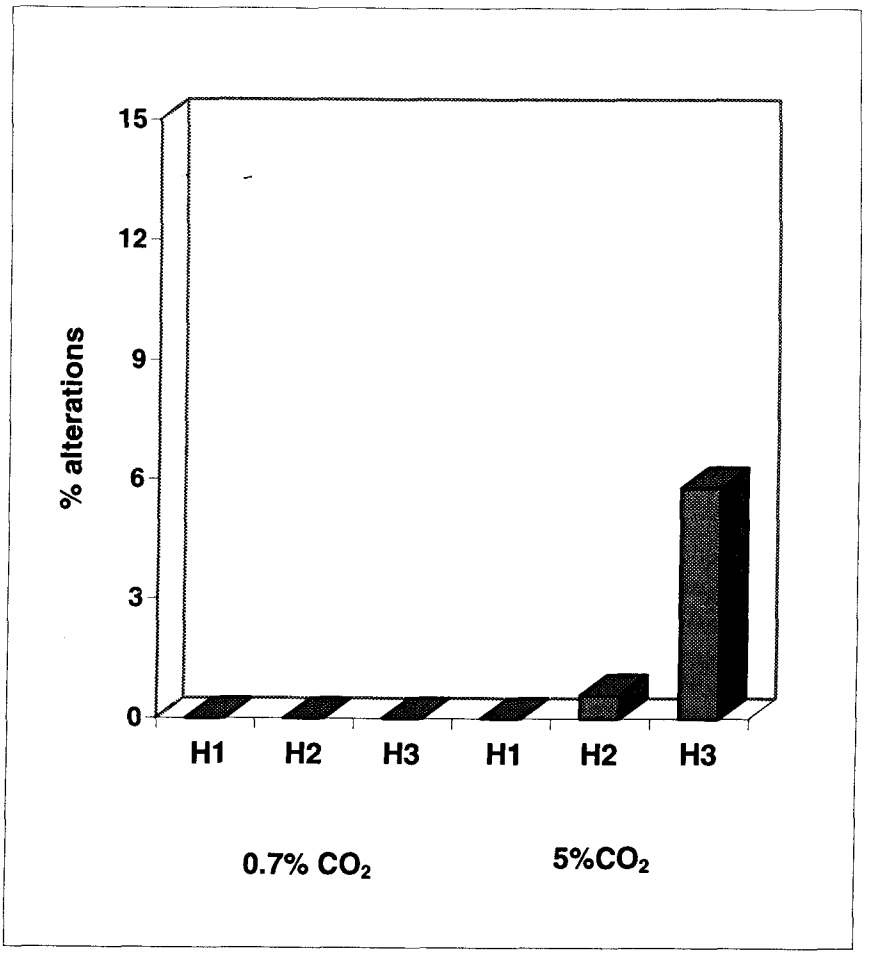

Figure 5: P Harvest-maturity related changes in physiological disorders (% alterations) in Conference stored in CA at 0.7 an 5 % CO_2.

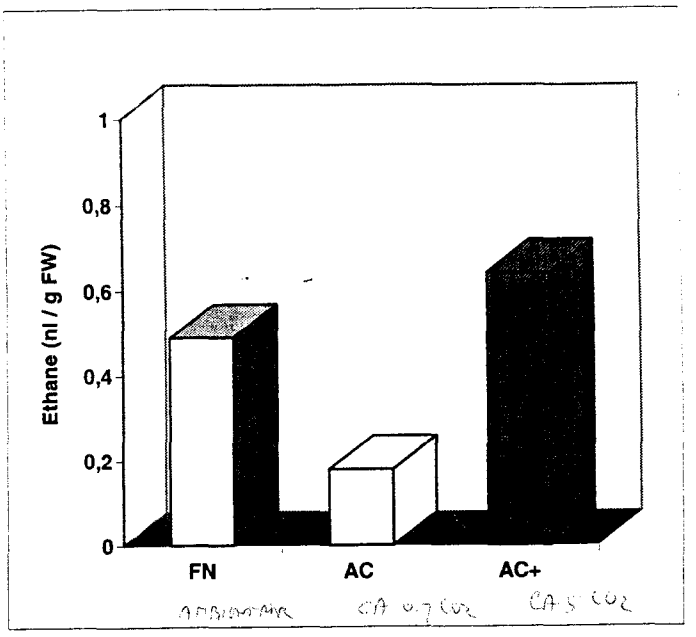

Figure 6: Effect of storage conditions on the peroxi dative status of membrane (ethane production).

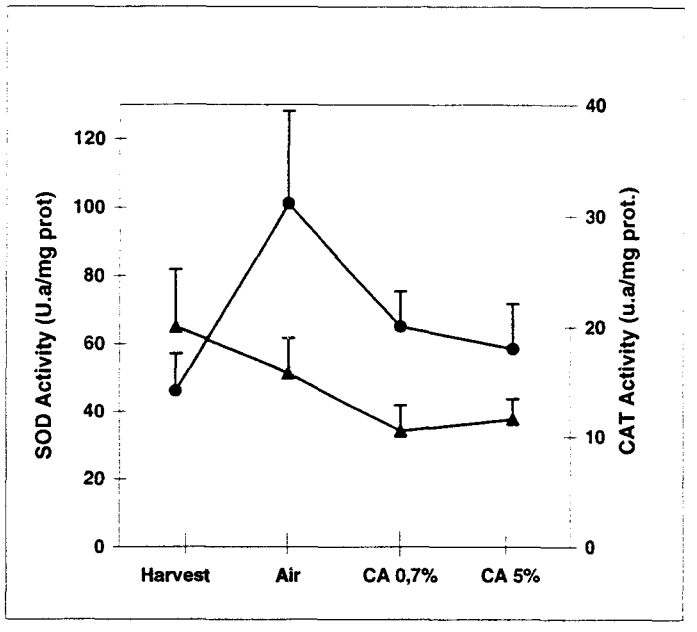

Figure 7: Changes in Superoxide Dismutase (SOD) and Catalase (CAT) activities in fonction of CO_2 concentration. Fruits were stored 2 months in CA.

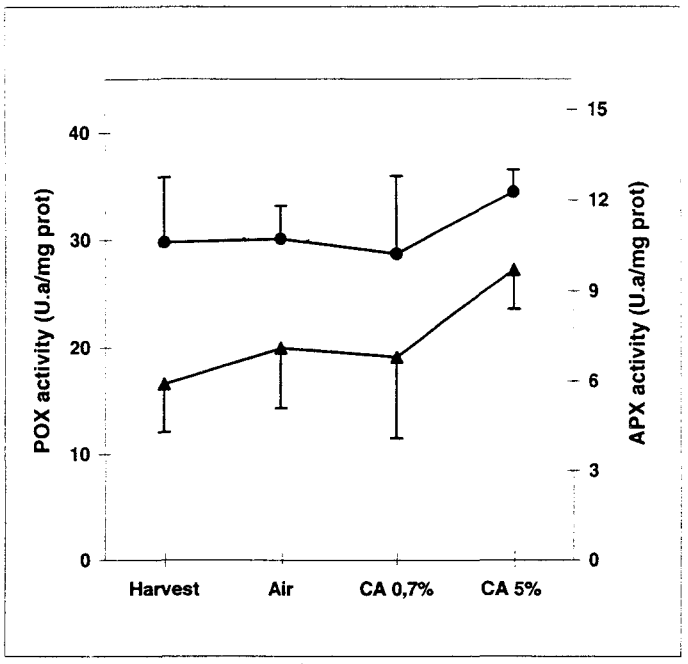

Figure 8: Changes in Peroxidase (POX) and Ascor bate Peroxidase (APX) activities in fonction of CO_2 concentration. Fruits were stored 2 months in CA.

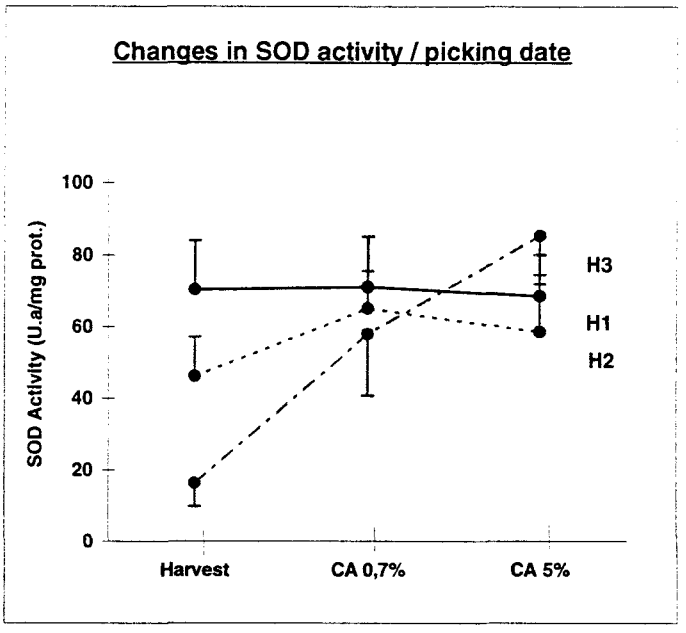

Figure 9: Harvest maturity related changes in SOD activity after 2 months of storage at different CO_2 concentration. H1: harvest 1; H2: harvest 2 ; H3: harvest 3.

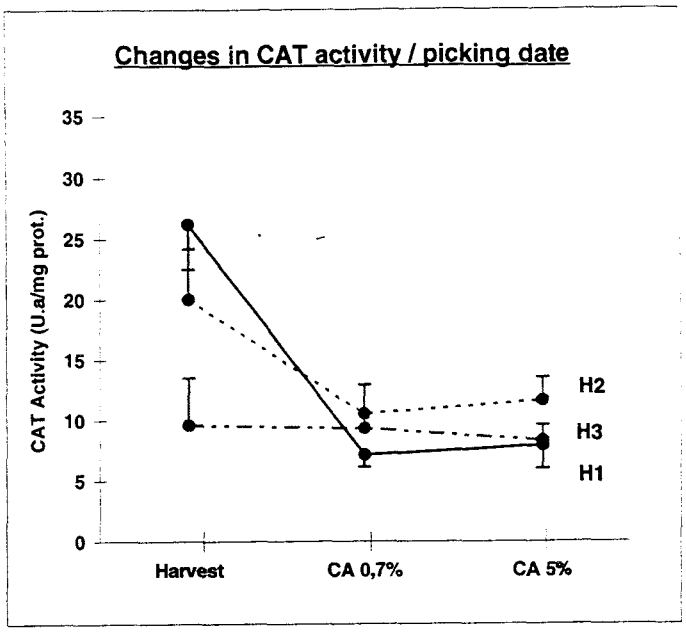

Figure 11: Harvest maturity related changes in POX activity after 2 months of storage at different CO_2 concentration. H1: harvest 1; H2: harvest 2 ; H3: harvest 3.

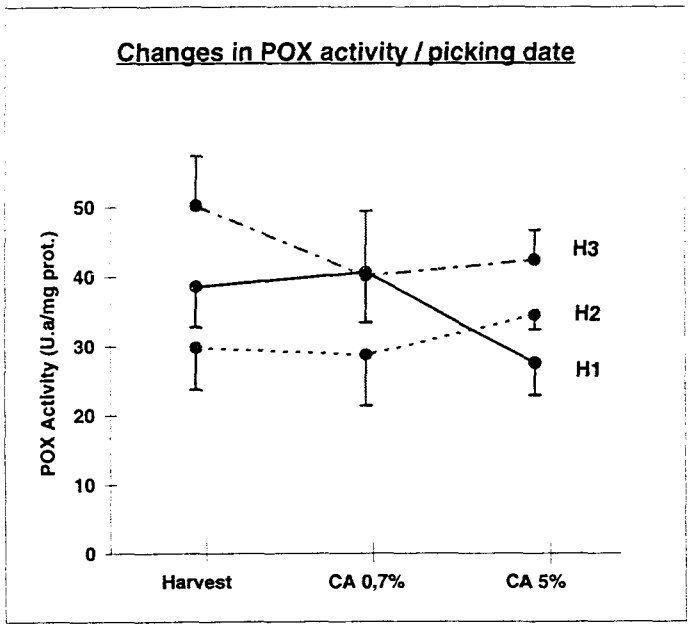

Figure 10: Harvest maturity related changes in CAT activity after 2 months of storage at different CO_2 concentration. H1: harvest 1; H2: harvest 2 ; H3: harvest 3.

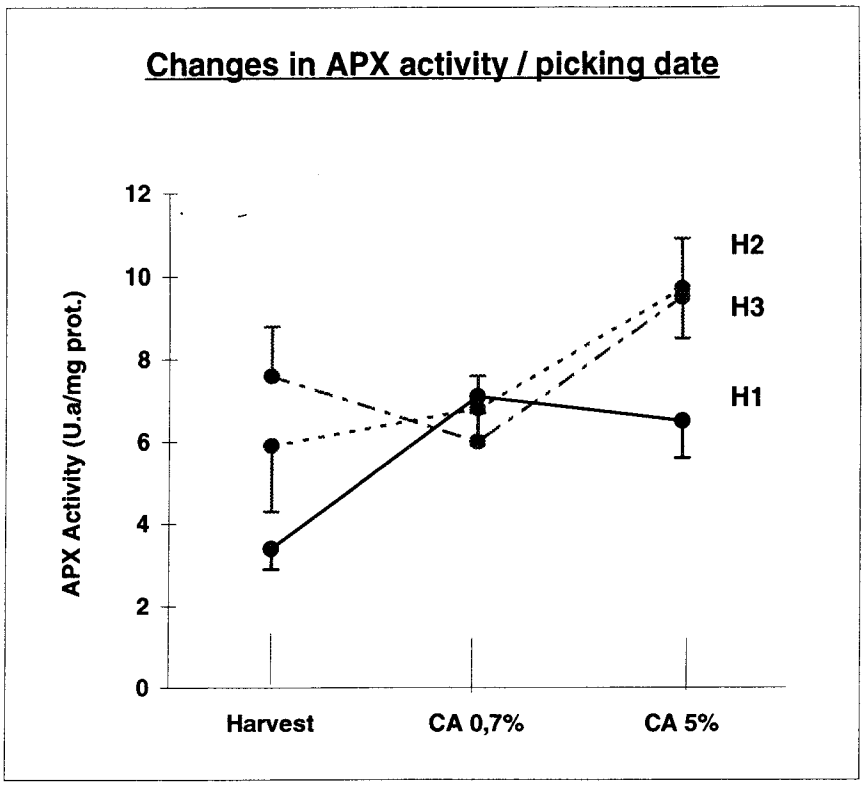

Figure 12: Harvest maturity related changes in APX activity after 2 months of storage at different CO_2 concentration. H1: harvest 1; H2: harvest 2 ; H3: harvest 3.

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A. PARTNER INFORMATION

1. (Sub)Project title:
Reduction and prediction of Brown Heart in pears

2. Project coordinator:
Dr. P. Eccher Zerbini

3. Project participants
Dr. A. Rizzolo
M. Grassi

B. INTRODUCTION

1. Description of the research topic or practical problem
Conference pears can be stored for several months in controlled atmosphere storage with excellent quality, but in some occasions they are susceptible to brown heart disorder and to cavities, which develop internally without any external sign of the disorder. The pears then are not suitable for consumption. The causes of the disorder are not known. The occurrence is influenced by weather factors, orchard factors, picking date and storage conditions. There is a different susceptibility to brown heart between pears grown in northern and southern European countries, perhaps due to different climatic and/or agronomical factors, which can affect pear growth and development, resulting in different metabolic rates.

2. Goal of the research
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. This has to be achieved by development of technology which enables a rapid measurement and decision about optimal storage conditions.

3. Short description of the approach
Research work at IVTPA is focused on the relationships between orchard factors (fruit maturity, mineral content) and storage disorders. The work programme includes: a. recording meteorological variables and agronomical features in selected orchards, b. picking fruit at different dates, c. storing in experimental CA containers, d. analysis of fruit at harvest to evaluate fruit maturity, e. analysis of fruit after storage to evaluate quality by common physical and chemical analysis, f. sensory analysis to evaluate eating quality during post-storage ripening, g. control of the occurrence of storage disorders, h. respiration measurements at harvest and during storage; the system for gas mixture and gas exchange, i. measurement have to be set up in the course of the project, j. the predictive models built with the data of all partners will be validated by comparison with storage results in commercial packinghouses.

C. OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

Objective

The main objectives of the work in this period were to collect data according to the different tasks attributed to this participant where methodology was already set, and to develop methodology in tasks new to the experience of the participants. The first objective was to collect data regarding climatic conditions and orchard factors, to harvest fruit in different dates, to analyze fruits at harvest, and to store them in standardized CA conditions. The second objective regarded the development of equipment and methodology for gas exchange analysis, diffusion resistance measurements, and lactic acid analysis.

Material and Methods

Orchards. Two orchards were selected in a pear growing region (Campogalliano, Modena province):

- Orchard 1 (Malaguti): planting year=1989. 18-years-old trees grafted on MA, planting distances 3.5 x 1.5 m, training system=palmetta. Orchard treatments for scab and 'brusone'; no growth regulators treatment. Full bloom occurred on 1 April. Severe frost (several degrees below zero) occurred some days after bloom (18 April), with fruitlets already set. Fruitlets were further damaged on 18 June by hail without rain. At harvest the production was on the whole tree, with deformed fruit (cylindrical, banana-shaped, etc.) especially in the lower part.
- Orchard 2 (Rinaldi): planting year=1991. 6-years-old trees grafted on BA 29, planting distances 4 x 2 m, training system=palmetta. Orchard treatments for scab, 'brusone' and Psylla. Full bloom occurred on 1 April. Severe frost (several degrees below zero) occurred some days after bloom (on 18 April), with fruitlets already set. At harvest practically there were no fruits below the height of 2 m (internal browning, no seeds), but the yield was abundant above 2 m.

Trees. In each orchard two rows of average vigour trees were selected and considered as blocks. Each block was divided into 3 groups of 9-12 trees; each group of trees in each block was dedicated to one harvest time. On each tree the size of the canopy was measured (height=h, width=dl anf thickness=dq), and the K index (=yield capacity) was calculated according to the Winter method: K= [(dl + dq) /2] x h. On each tree the number of fruits visible on one side of the canopy was photographed and counted. The Winter method for assessing the number of fruits beared by the trees perhaps needs some adjustment, especially this year due to spring frost and irregular fruit set on the canopy.

Meteorological data. Data were obtained from two stations. One station (belonging to Consorzio Fitosanitario Obbligatorio Modena) was in orchard 2: this station recorded temperature (T) (min, max, mean), relative humidity (RH) (max, at 14, at 20, mean), rain, evapotranspiration (method Blaney and Criddle), vapour pressure deficit calculated as a function of T and RH. The other station, belonging to the Geophysical Observatory of the Engineering Sciences Dept. of the University of Modena (Osservatorio Geofisico del Dipartimento di Scienze dell'Ingegneria, Università di Modena) is at Marzaglia, in a location at 3 km from the orchards, and the variables recorded are: temperature at a height of 10 cm, 2 m and 10 m, RH at 2 m, atmospheric pressure, direction and speed of wind at 2 and 10 m, maximum speed of wind in the day, rain, and global radiation (daily integral of energy flux in the 0.3-3000 nm range with a solarimeter Kipp & Zonen). Data from both stations were obtained free of charge for scientific use, provided that the institutions are mentioned when publishing the results.

Sampling for preharvest mineral analysis. On July 17 some fruitlets were sampled for mineral analysis. Two fruitlets / tree were sampled from 6 trees in each group, obtaining a total of 144 fruitlets (2 fruits x 6 trees x 3 groups x 2 blocks x 2 orchards). The fruitlets were freezed for subsequent preparation and analysis.

Harvest. In each orchard pears were harvested on 11, 19 and 25 August 1997. Harvesting of pears followed a protocol in order to obtain a sufficient number of fruits for quality analysis (120 fruits) and for controlled atmosphere storage (12 boxes) from as many trees as possible, and to obtain several groups of fruits similar within a group (i.e. of same size and colour, and from the same tree) for gas exchange measurements in different oxygen concentrations (about 300 fruits). Pears were individually numbered in order to recognize orchard, harvest, block and tree. The fruits were transported to IVTPA for analysis and storage.

Storage. Pears were stored in controlled atmosphere containers with 2 % oxygen and 0.7 or 5 % CO_2, in a room at 0/-1 C. The temperature in the containers was about 0.5 C higher than that in the room. It was not possible to lower further the temperature due to condensation and ice formation in the tubes for gas control of containers.

Quality analysis of fruit. On 20 fruits per orchard and harvest the following measurements were made on the day after harvest: background colour of the skin (Minolta Chromameter II) on the largest and greenest part of the fruit; largest diameter; height; weight; fruit density; firmness with 8 mm plunger (Effegi penetrometer); starch hydrolysis (%); soluble solids; juice density; relative intercellular space volume was calculated; a part of each fruit was frozen for mineral analysis. The fruits subjected to respiration measurements after completion of the latter were weighted and fruit density was measured; then they were cut and examined for internal browning and cavities, and the number of seeds was recorded; then the pulp was divided into three parts: one for lactic acid analysis, one for juice density, and one for ethanol and acetaldehyde measurement; the latter was immediately homogenized. All the portions were frozen and kept at -20 C.

Respiration measurements. This kind of research is new for our laboratory, so we had to solve various problems, regarding equipment and methods: type of jar to use, time of jar flushing with the selected gas mixtures, gas sampling, time to leave the fruit in the jar in order to have a reasonable (0.1-0.2 %) increase of CO_2, calibration and use of new GC and data station, time of equilibration of fruit in a new atmosphere before the beginning of respiration measurements, time and concentration for diffusion measurements. All the experiments regarding gas exchange measurements and diffusion were carried out at -1 C (storage temperature).

Equipment for gas exchange measurements.

Jars.It was necessary to look for containers of a size suitable for one pear, with a gas-tight cover, with access for introduction of gas mixtures, with gas tight access for gas sampling. Due to the very short time available before pear harvest, and to cost considerations, after some tentative solutions a cost effective equipment was designed. Glass jars (1700 ml volume) with twist-off caps (100 mm diameter) were adopted as containers for single pears. On each cap three holes were made (painted against rust); for the two holes used for inlet and outlet of gas mixtures automatic gas-tight fittings (Metalwork Italia) were inserted (4 mm diameter). In the third hole a custom made septum holder was inserted for gas sampling. The inlet port was fitted with a tube reaching the bottom of the jar. In the gas outlet, a two-way piece was inserted: one way was for the gas outflow, while on the other way a gas sampling rubber balloon was fitted, designed to compensate for pressure differences due to atmospheric pressure changes and to gas sampling during the experiment. Gas tightness of the containers was checked by leaving them closed for 72 hours after inflating the balloons, and controlling air bubbles underwater. The fittings on the caps had excellent tightness; some leaks can occur if the caps are not properly twisted on when closing the jars.

Gas mixtures. Four gas mixtures (air, N_2, 2 % O_2 + 0.7 % CO_2, 2 % O_2 + 5 % CO_2) were used for respiration measurements. The mixtures had been prepared in cylinders; the cylinders were kept in the storage room, i.e. at -1 C.

GC analysis. Gas samples 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 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, using He at 55 ml/min as carrier gas, and the following temperatures: TCD 33 C, oven 33 C. Data were stored and processed using a DaniChrom data station. At the beginning the system was calibrated using the calibration mixture especially manufactured for the column, containing 15.01% CO_2, 6.98 % O_2, 66.65 % N_2 and other gases to 100 %. A one point calibration was performed, forcing the calibration line through the origin.

Gas sampling. Different type of syringes were tested: "gas-tight syringes" at -1 C were no more gas-tight because the plunger shrinked more than the glass body due to the low temperature. The best tightness was achieved with disposable PE syringes. During preliminary experiments and respiration measurements at harvest, a PE syringe with a removable side hole domed needle was used; gas was sampled after filling and emptying the syringe ten times in order to mix the gas in the jar; after sampling the needle was removed and the syringe was closed with a rubber stopper and transferred to the GC laboratory.

Method for respiration measurement at harvest. Two fruits for each block, orchard and harvest were used for each of the measurement atmosphere. Each pear was put in a jar, the jar cap was tightly closed, and flushed at 110 l/h for 10 min (see below) through the fittings with one of four gas mixtures (air, N_2, 2 O_2 + 0.7 CO_2, 2O_2 + 5 CO_2). Then the outlet was closed, the balloon was slightly inflated, and the inlet was closed. The first sampling (10 ml, two replicate GC analysis) was made immediately after closing, and the second after a period of 16-24 h (see below). The jars were kept at -1 C.

Calculation of gas exchange rates. The total amount (%) of O_2, CO_2 and N_2 were corrected to 100% in one step. In order to express the amount of gas in mM/(kg*h), we had to correct the percentage for the actual pressure. As we had not a direct measurement of pressure in the jars, but the jars were fitted with balloons to keep the internal pressure balanced with the external one, we assumed that inside the jars the pressure was the same as outside, so we used the atmospherical pressure data recorded not far from the laboratory by the Meteorological Observatory of Brera. The data were reported as % gas consumed or produced, as mM/(kg*h) with the atmospheric pressure correction, and as mM/(kg*h) without the latter correction (i.e. considering standard pressure conditions).

Preliminary experiment to assess flushing time and flow. Nitrogen was flushed at 20, 60 and 110 l/h in jars, and gas was sampled after 2, 4, 6, 8 and 10 minutes and analyzed. The oxygen decreased more slowly than theorically calculated. The safest combination was 110 l/h for 10 min.

Verification of time of respiration. The day after the first harvest two fruits, one kept at -1 C, the other kept at room temperature (25-27 C), were put in jars and flushed with nitrogen, in order to have the worst conditions for respiration. Gas was sampled and analyzed immediately after closing, after 1.5 h and after 16 h. The next day another experiment was planned with 4 fruits kept at -1 C, in 4 jars, each one flushed with one of the 4 atmospheres. Gas was sampled and analyzed at closing and after 1, 2, 3, 4, 5 h, as given in the protocol.
After examining the results of respiration measurements carried out at harvest, some more testing was carried out in order to clarify why oxygen increased and CO_2 decreased in some jars, and why oxygen was found in nitrogen jars. After verifying the absence of oxygen in cylinders and of leaks in the jars, we carried out *ad hoc* trials to test the following items: calibration of GC gas analysis, stratification of CO_2 in jars, permeability of rubber balloons, diffusion of gases between fruit and surrounding atmosphere. A new procedure of gas sampling and of injection in the GC loop was also devised.

Calibration of GC gas analysis: calibration of GC was checked and redone with four-five levels for each gas component to correctly quantify the low amounts of CO_2 and O_2, as well as the high amounts of nitrogen. In fact we found a quite different response factor between gas directly sampled at the outlet of the cylinder and the one sampled from jars, perhaps these differences are due to different gas pressures. Furthermore in the case of gases directly sampled at the outlet of cylinder, we found different response factors between cylinders kept at 20 C and those kept at -1 C. Taking into account all these differences, we decided to recalibrate gas analysis using 10 empty respiration jars (replications) kept at -1 C and flushed with the following atmospheres: air, nitrogen, 2% O_2 + 0.7 % CO_2, 2% O_2 + 5% CO_2, and.0.1 % O_2 + 0.1% CO_2 . After having calibrated the gas analysis system, weekly we checked the performance of the instrument by injecting at the beginning of the group of analysis the following gas mixtures: 2% O_2 + 0.7 % CO_2, 2% O_2 + 5% CO_2, and 0.1 % O_2 + 0.1% CO_2, always sampled from newly prepared calibration jars.

Stratification of CO_2 in jars: in some jars with different atmospheres, gas was sampled after fruit respiration at three different heights: at top as usual, in the middle with a longer needle, and at bottom from the inlet port. Extra fruits were put in other jars with the atmosphere 2 % O_2 +5% CO_2 and gas was sampled at top and bottom of jar after 24 and 48 hours without mixing.

Permeability of rubber balloons: the permeability of balloons was tested by inflating two balloons with the atmosphere $2O_2$+5 CO_2 and putting them inside a tightly closed jar with air as the surrounding atmosphere. Two more ballooons were inflated with air and put in another jar with N_2 as the surrounding atmosphere. Both jars were not fitted with the external balloon, and were kept at -1 C. The surrounding atmospheres were sampled after 0, 4 and 22 hours.

Diffusion of gases between fruit and surrounding atmosphere. To quantify the time necessary to equilibrate external and internal atmospheres, two trials were set up. The first one reproduced the situation of the respiration measurements at harvest: a) *from air to atmospheres* : extra pears which had been kept in air at -1 C for 2 months were put into jars with the 4 atmospheres and gas was analyzed after 5, 24, 30, 48 hours; then the same atmosphere was renewed, and gas was analyzed after 24, 30 and 48 hours. The second trial reproduced the situation of respiration measurements of stored fruits: b) *from storage atmosphere to air and N_2*: pears, which had been equilibrated for 48 hours either in 2%O_2+5%CO_2 or 2%O_2 + 0.7% CO_2 (storage atmospheres), were put in jars with air or N_2 and gas composition was analyzed after 6, 24 and 48 hours.

New procedure for gas sampling and injection in the GC loop. After the evidence of insufficient gas tightness of rubber balloons, they were no more used. After each gas sampling from the jar, an equal quantity of gas was sampled from the corresponding cylinder and injected into the jar, in order to avoid depression inside the jar. Before gas sampling the PE syringe was 'washed' 2 times and filled with pure nitrogen. The needle was inserted on the syringe, and nitrogen was expelled from the syringe only immediately before inserting the needle in the jar septum for gas sampling. Immediately after sampling, the hole of the needle was covered with a silicone rubber septum, or, if the needle was taken away from the syringe, the latter was immediately closed with the finger, which proved to be the most rapid and gas-tight type of closure. After transport to the GC laboratory, the syringe was slightly put in pressure by acting on the plunger before opening and injecting into the GC. It was of paramount importance to keep the time while the syringe was open very short, and to keep a slight overpressure inside the syringe when opening or closing, in order to avoid the entrance of oxygen.

Ethanol and acetaldehyde measurements

Sample preparation: 9-11 g of fruit pulp is sliced, homogenized, and put into a 25 ml vial. The vial is tightly closed with a silicone-teflon rubber septum. Samples are immediately frozen and kept at - 20 C

until GC analysis. Before injection, each vial is kept at room temperature for 30 min, then it is placed in a heat chamber at 60 C for 1 hour. Immediately after that an aliquot (200-500 µl) of the headspace is sampled and injected into the GC using a pre-heated gas-tight syringe. The headspace compounds, detected by FID, are separated on a Carbowax 20M column (30 m x 1.5 mm I.D., 0.25 µm film thickness), using helium at 0.6 bar as carrier gas, with the following temperature program:. 40 C for 5 min, from 40 C to 150 C at 30 C/min, then held for 5 min. Calibration was performed using 200-500 µl headspace of a 10 ml water solution containing known amounts of ethanol and acetaldehyde, which was treated exactly in the same way as the samples.Under these conditions the retention times are: acetaldehyde: 1.3 min and ethanol: 3 min. The amounts of acetaldehyde and ethanol present in the headspace are expressed as µg/100g of fruit pulp.

Lactic acid analysis.
Sample preparation: 10 g of thawing fruit pulp cut into small pieces is homogenized in a 100 ml centrifuge tube in the presence of 25 ml of water (HPLC degree); then the suspension is centrifuged at 3000 g for 15 min and the extract is transferred into a 100 ml volumetric flask, after filtration through glass wool; the procedure is followed through once more and the second extract is combined with the first; the volume is brought to the mark with water. Before HPLC analysis, the extract is filtered through a 0.22 µm Nylon 66 membrane. Using the extracts from extra pears which had been kept in nitrogen atmosphere for 2 months at -1C, to separate lactic acid from the other compounds present in the extract we tested two columns: Polysphere OA HY (300 x 6.5 mm, Merck) and Inertsil C8 (250 x 4.5 mm, GL Science). The analytical conditions of the HPLC were:

- Polysphere OA HY column: mobile phase: 0.01 or 0.0025 N H_2SO_4; flow rate: 0.6 ml/min, column temperature: 65 C; detection : UV 210 nm; sample amount: 100 µl.
- Inertsil C8: mobile phase: 0.2 M H_3PO_4 ; flow rate: 0.6 ml/min, column temperature: 20 C (ambient); detection: UV 210 nm; sample amount: 100 µl.

Using the Polysphere column, lactic acid coeluted with another compound, present in the extract in a large amount. By changing mobile phase and/or temperature of analysis there was not any improvement in the resolution. By changing the stationary phase, i.e. using Inertsil C8, lactic acid was resolved from interferring peaks and it was possible to detect it even in the low amount present in the test extracts. After having calculated the response factor using Inertsil C8 column, now we are analysing the extracts from pears at harvest.

Results and discussion

Yield capacity. The mean number of fruit produced by trees and yield capacity are reported in Table 1. The different planting distance influences the K value. The number of fruits is clearly affected by the early frost.
Quality of fruits. Analysis at harvest are reported in Table 2. Fruit size and starch hydrolysis increased with harvest time, while firmness decreased. RISV seems to be inversely related to the number of seeds, in fact the fruits of Malaguti orchard had no seeds.
Ethanol and acetaldehyde content. Data for ethanol and acetaldehyde content at harvest are reported in Table 3. Ethanol content is relatively high in fruits subjected to respiration measurement in nitrogen atmosphere.
Verification of time of respiration. The fruit kept at -1 C did not produce enough CO_2 after 1.5 h, but it produced about 0.4 % after 16 h (Table 4). In air the percent CO_2 did not reach 0.1 % after 5 hours, as well as in nitrogen, while in the two mixtures with 2 % O_2, the CO_2 percentage was very variable (Table 5). Based on these results, it was decided to wait for 16 - 24 hours before doing respiration measurements.
Respiration measurements at harvest. In some jars strange results were found, as O_2 increased and CO_2 decreased; in some cases O_2 was found in N_2 jars (Tables 6, 7, 8). After examining the results of

respiration measurements carried out at harvest, some more testing was carried out in order to clarify these unexpected results. Possible causes could be: impurities in nitrogen cylinders, leaks in the jars, procedure of gas sampling and of injection in the GC loop, stratification of CO_2 in jars, diffusion of gases between fruit and surrounding atmosphere, improper calibration of GC gas analysis and permeability of rubber balloons. After verifying the absence of oxygen in cylinders and of leaks in the jars, ad hoc trials were carried out to test the other hypotheses (see Materials and Methods section).
Stratification of CO_2 in jars. No differences were found between different heights of sampling in both trials, so stratification was excluded as a main cause of CO_2 decrease. Differences were found between analyses after 24 and 48 hours during the stratification tests, confirming that diffusion was occurring. At the end of stratification experiments, some baloons looked less inflated than at the beginning. The jars were put underwater to check for leaks, but no leaks were found.
Permeability of rubber balloons. Already after 4 hours the air filled balloons were visibly collapsed, and oxygen was present in high amount in the surrounding nitrogen atmosphere. The 2% O_2+5%CO_2 mixture filled balloons were apparently well inflated, but CO_2 level increased and O_2 decreased already after 4 hours. These results indicate that this type of gas sampling rubber balloons is very permeable both to CO_2 and to O_2 at -1 C. After these results the balloons were no longer used.
Diffusion of gases between fruit and surrounding atmosphere. The results of the first trial (from air to atmospheres) are reported in Table 9. The only atmosphere where diffusion was evident was 2%O_2+5%CO_2: there was a decrease of CO_2 percentage till 30 hours and only after 48 hours CO_2 began to increase again, even if it did not reach the initial value. By renewing the atmosphere the CO_2 did not decrease any more; on the average CO_2 increased 0.1 % in 24 hours. In air (+ 0.2 % in 12 hours) and in $2O_2$+ $0.7CO_2$ (+ 0.2 % in 24 hours) CO_2 increased from the beginning of the trial. In nitrogen CO_2 was not detectable after 5 hours, but then it increased (+0.2% in 12 hours).
Results of second trial (from storage atmosphere to air and N_2) are reported in Table 10. For both storage atmospheres the per cent CO_2 increased more quickly in the first 24 hours than in the second 24 hours. In N_2 CO_2 increased more than in air. The diffusion phase can be considered concluded after 48 hour of equilibration; however it is not sure that a steady-state is reached, so respiration should be verified after 24 and again after 48 hours, always after renewal of atmosphere.

Conclusions
It is necessary to wait for the end of the storage period and of chemical analysis to reach some conclusion about the quality of fruit. The main preliminary results here reported give some indication for improvement of respiration measurement methodology. Jars proved to be effective in maintaining gas-tightness, but rubber balloons cannot be used for pressure equilibration, so actual pressure should be measured in the jar, or pressure should be otherwise compensated for. Diffusion must be taken into account together with respiration measurements. The time for respiration measurement at -1 C should be not less than 12-24 hours, after equilibration in the measuring atmosphere.

D. DESCRIPTION OF THE WORK PLANNED FOR THE SUBSEQUENT PART OF THE PROJECT (1-12-97 TO 1-6-98)

In the second part of the year it is planned to evaluate pear quality after storage, by analyzing several quality indexes (soluble solids, firmness, acidity), and by examining the incidence of browning and cavities. Mineral analysis will be performed on the material already collected. Data on gas exchange will be processed and analyzed, and examined to see if methodology needs to be further refined. Data on tree load, quality evaluation, mineral analysis and chemical analysis will be processed.

E. DISSEMINATION

Not yet

Tables

Table 1. Mean values of number of fruits produced, of potential production capacity (K) and of trunk section of pear trees.

		orchard	block				
		M		Total M	R		Total R
harvest	Data	1	2		1	2	
1	n.fruit	65,56	49,00	62,55	73,30	65,22	69,47
	K=(dl+dq)*h/2	39802,78	43388,89	41595,83	48735,00	50231,94	49444,0
	trunk section	164,04	174,05	169,04	63,47	67,08	65,18
2	n.fruit	33,67	24,89	29,28	41,89	69,73	57,20
	K=(dl+dq)*h/2	35401,39	35479,17	35440,28	53230,00	51202,78	52269,7
	trunk section	165,43	208,90	187,17	70,94	76,16	73,41
3	n.fruit	21,11	34,88	27,59	42,40	45,73	44,14
	K=(dl+dq)*h/2	34751,39	35185,94	34955,88	49315,00	47762,50	48538,7
	trunk section	157,88	199,39	177,42	56,94	66,60	61,77

Table 2. Means for qualitative variables at harvest (20 replications).

orchard	harvest date	fruit weight (g)	fruit volume (g)	fruit density (g/cm^3)	juice density (g/cm^3)	RISV* (%)	fruit diameter (mm)	fruit height (mm)
Rinaldi	12-aug	219.08	218.62	1.002	1.068	6.131	70	98
Rinaldi	20-aug	231.44	230.16	1.006	1.057	4.824	73	102
Rinaldi	27-aug	273.50	272.75	1.003	1.051	4.546	76	163
Rinaldi	*mean*	*241.34*	*240.51*	*1.004*	*1.059*	*5.167*	*73*	*121*
Malaguti	12-aug	183.45	183.52	1.000	1.056	5.364	65	101
Malaguti	20-aug	214.37	214.33	1.000	1.072	6.665	68	155
Malaguti	27-aug	233.24	234.33	0.996	1.061	6.114	71	109
Malaguti	*mean*	*210.35*	*210.73*	*0.999*	*1.063*	*6.048*	*68*	*122*

orchard	harvest date	L*	a*	b*	hue (rad)	chroma	firmness (kg)	starch hyd.. (%)	soluble sol. (° Bx)
Rinaldi	12-aug	57.70	-16.14	36.74	1.983	40.13	6.44	4.75	12.9
Rinaldi	20-aug	60.62	-16.26	36.00	1.993	39.51	6.43	9.75	13.4
Rinaldi	27-aug	60.49	-15.95	36.16	1.985	39.53	6.18	16.50	12.4
Rinaldi	*mean*	*59.60*	*-16.12*	*36.30*	*1.987*	*39.72*	*6.35*	*10.33*	*12.9*
Malaguti	12-aug	56.00	-16.02	34.87	2.000	38.37	6.74	3.75	11.8
Malaguti	20-aug	55.86	-14.07	33.20	1.970	36.06	6.80	10.00	11.9
Malaguti	27-aug	59.30	-15.84	34.76	1.997	38.20	6.29	11.50	12.0
Malaguti	*mean*	*57.05*	*-15.31*	*34.28*	*1.989*	*37.54*	*6.61*	*8.42*	*11.9*

* : Relative Intercellular Space Volume = (1-(fruit density/juice density))*100

Table 3. Mean acetaldehyde and ethanol content in fruits at harvest after respiration measurements (2 fruits x 2 blocks replications).

orchard	harvest date	gas mixture	acetaldehyde (mg/100 g f.w.)	ethanol (mg/100 g f.w.)	RISV* (%)
	12-aug	2% O_2 ; 5% CO_2	0,323	0,168	4,476
		air	0,273	0,158	4,423
		N_2	0,399	1,049	3,782
		2% O_2 ; 0.7 CO_2	0,341	0,203	5,432
Malaguti	20-aug	2% O_2 ; 5% CO_2	0,190	0,071	3,974
		air	0,209	0,076	3,858
		N_2	0,284	0,911	4,535
		2% O_2 ; 0.7 CO_2	0,183	0,082	4,099
	27-aug	2% O_2 ; 5% CO_2	0,182	0,073	6,001
		air	0,169	0,087	6,037
		N_2	0,238	0,744	5,501
		2% O_2 ; 0.7 CO_2	0,215	0,077	5,424
		N_2 (5)	0,339	0,727	4,875
	12-aug	2% O_2 ; 5% CO_2	0,442	0,185	6,314
		air	0,244	0,094	5,292
		N_2	0,346	0,262	5,095
		2% O_2 ; 0.7 CO_2	0,238	0,098	4,691
Rinaldi	20-aug	2% O_2 ; 5% CO_2	0,166	0,042	4,533
		air	0,244	0,076	4,863
		N_2	0,267	0,576	4,732
		2% O_2 ; 0.7 CO_2	0,182	0,040	5,000
	27-aug	2% O_2 ; 5% CO_2	0,341	0,205	4,343
		air	0,341	0,232	4,962
		N_2	0,259	0,717	5,343
		2% O_2 ; 0.7 CO_2	0,266	0,164	4,610
		N_2 (5)	0,154	0,408	4,558

* : Relative Intercellular Space Volume = (1-(fruit density/juice density))*100
(5) new nitrogen cylinder

Table 4. Assessment of respiration time: percent CO_2 produced and O_2 consumed after different time of respiration in nitrogen atmosphere; fruits: first harvest kept at different temperature.

	fruit kept at room temperature		fruit kept at -1 C	
time (h)	%CO_2	%O_2	%CO_2	%O_2
0	-	0.436	-	0.362
1.5	0.156	0.386	0.073	0.306
16	0.595	4.049	0.394	1.520

Table 5. Assessment of respiration time: percent CO_2 produced and O_2 consumed after different time of respiration in different atmospheres; fruits: first harvest kept at -1 C.

	Atmosphere							
	2% O_2 +5% CO_2		air		nitrogen		2% O_2 +0.7% CO_2	
time (h)	%CO_2	%O_2	%CO_2	%O_2	%CO_2	%O_2	%CO_2	%O_2
0	4.936	1.962	-	21.892	-	0.364	0.696	2.226
1	4.802	2.338	-	21.995	-	0.570	0.700	2.278
2	4.668	2.270	-	21.857	0.077	0.582	0.708	2.363
3	4.537	2.345	0.075	21.995	0.081	0.750	0.722	2.070
4	4.584	2.118	0.088	21.991	0.090	0.234	0.732	1.989
5	4.572	2.117	0.090	21.752				

Table 6. Respiration measurements at harvest: Δ % CO_2 / h and Δ % O_2 / h at three harvests for pears coming from two orchards. Averages of blocks.

	ORCHARD 1 (Malaguti)								ORCHARD 2 (Rinaldi)							
	Δ % CO_2 / h				Δ % O_2 / h				Δ % CO_2 / h				Δ % O_2 / h			
Harvest	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	-0.77	0.29	0.33	0.13	0.12	-0.27	0.21	1.25	-0.94	0.29	0.24	0.10	-0.08	-0.44	0.29	-0.02
2	-0.62	0.41	0.43	0.34	-0.11	-0.38	0.15	-0.36	-0.78	0.49	0.52	0.31	0.94	-0.52	-0.01	-0.34
3	-0.62	0.43	0.49	0.33	-0.15	0.18	0,20	0.12	-0.54	0.53	0.51	0.34	-0.05	-0.29	0.07	0.18
3			0.47				0.06				0.45				0.22	

Atmospheres: A: 2% O_2 + 5% CO_2; B: air; C: nitrogen; D: 2% O_2 + 0.7% CO_2.

Table 7. Respiration measurements at harvest: Δ mM CO_2 / (kg*h) and Δ mM O_2 / (kg*h) at three harvests for pears from 2 orchards. Averages of blocks. Corrected for atmospheric pressure of the day of analysis.

	ORCHARD 1 (Malaguti)								ORCHARD 2 (Rinaldi)							
	Δ mM CO_2 / (kg *h)				Δ mM O_2 / (kg * h)				Δ mM CO_2 /(kg * h)				Δ mM O_2 / (kg *h)			
Harvest	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	-0.18	0.07	0.09	0.03	-0.005	0.011	-0.008	-0.051	-0.15	0.05	0.04	0.02	0.003	0.016	-0.01	0.0005
2	-0.09	0.06	0.06	0.04	0.003	0.01	-0.004	0.01	-0.09	0.05	0.05	0.03	-0.03	0.013	-0.0002	0.009
3	-0.07	0.05	0.05	0.03	0.004	-0.005	-0.005	-0.003	-0.06	0.06	0.05	0.03	0.001	0.007	-0.002	-0.004
3			0.06				-0.002				0.06				-0.006	

Atmospheres: A: 2% O_2 + 5% CO_2; B: air; C: nitrogen; D: 2% O_2 + 0.7% CO_2.

Table 8. Respiration measurements at harvest: Δ mM CO_2 / (kg *h) and Δ mM O_2 / (kg * h) at three harvests for pears coming from two orchards. Averages of blocks. Values without correction with atmospheric pressure of the day of gas analysis.

	ORCHARD 1 (Malaguti)								ORCHARD 2 (Rinaldi)							
	Δ mM CO_2 /(kg *h)				Δ mM O_2 / (kg *h)				Δ mM CO_2 / (kg *h)				Δ mM O_2 / (kg *h)			
Harvest	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	-0.18	0.07	0.09	0.03	0.03	-0.066	0.055	0.324	-0.15	0.054	0.045	0.019	-0.009	-0.,81	0.056	-0.002
2	-0.10	0.06	0.06	0.05	-0.011	-0.057	0.023	-0.046	-0.09	0.051	0.05	0.034	0.107	-0.051	0.003	-0.034
3	-0.07	0.05	0.05	0.03	-0.017	0.013	0.024	0.012	-0.06	0.057	0.05	0.035	-0.006	-0.029	0.007	0.011
3			0.07				0.013				0.062				0.027	

Atmospheres: A: 2% O_2 + 5% CO_2; B: air; C: nitrogen; D: 2% O_2 + 0.7% CO_2.

Table 9. Diffusion from air to atmospheres (situation as at harvest): difference of % CO_2 in 24 h.

	Δ % CO_2 / 24 h				Δ mM CO_2 / 24 h				Δ mM CO_2 / kg * 24 h			
	diffusion		respiration		diffusion		respiration		diffusion		respiration	
	24h	48h	+24h	+48h	24h	48h	+24h	+48h	24h	48h	+24h	+48h
air	0.49	0.48	0.53	0.39	0.33	0.32	0.35	0.26	1.64	1.60	1.75	1.28
N_2	0.40	0.30	0.32	0.17	0.27	0.20	0.22	0.12	1.71	1.27	1.39	0.75
2+0.7	0.21	0.23	0.25	0.19	0.15	0.16	0.17	0.13	0.99	1.08	1.14	0.86
2+5	-0.53	0.19	0.11		-0.36	0.13	0.08		-2.12	0.78	0.46	

Table 10. Diffusion from storage atmospheres to air and nitrogen (situation as after storage) : difference of % CO_2 in 24 h.

		Δ % CO_2 / 24 h		Δ mM CO_2 / 24 h		Δ mM CO_2 / kg * 24 h	
storage	diffusion	24h	48 h	24h	48h	24h	48h
2+5	air	1.06	0.40	0.70	0.26	3.16	1.19
2+5	N2	1.20	0.64	0.80	0.43	3.80	2.03
2+0.7	air	0.27	0.08	0.18	0.05	1.00	0.30
2+0.7	N2	0.63	0.78	0.42	0.52	2.22	2.75

Participant 4: Fruitteeltpraktijkonderzoek
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A. PARTNER INFORMATION

1. (Sub)Project title:
Reduction and prediction of Brown Heart in pears

2. Project coordinator:
Dr. A. de Jager

3. Project participants
Dr. A. de Jager
B.Sc. F.P.M.M. Roelofs
C. Westerweele

B. INTRODUCTION

1. Description of the research topic or practical problem
The practical problem under investigation is the so-called brown heart disorder in pears, especially Conference pears. Large economic losses may occur rather unexpectedly and the incidence of the disorder appears to vary between orchards and years and to depend on ripening stage. A better understanding of the way in which these conditions affect the sensitivity to brown heart is needed in order to predict and to minimize the incidence of the disorder.

2. Goal of the research
The goal of the research can be summarized in three points
1. prediction of the risk of brown heart
2. minimizing the sensitivity to brown heart and
3. defining safe storage conditions for sensitive lots of fruit
The Fruit Research Station is involved in all aspects but with special emphasis on variation between orchards, the effect of nutrition, the effect of degree of bearing and of position of the fruit in the tree and on the scenarios of reaching final storage conditions after picking.

3. Short description of the approach
Orchards are selected on the basis of known incidence of brown heart in the past to represent a serie from insensitive to very sensitive. In some orchards nutrition treatments are carried out by frequent spraying. Fruits are picked from all orchards at several times (ripening factor) from different positions of the tree (position factor). Different scenario's are compared for imposing the final storage conditions on the fruit. Fruit quality (weight, air filled porosity, ground color, firmness, sugar- and acid concentration, starch-iodine coloration pattern, and nutrient concentration) was measured at harvest and after storage and subsequent shelf life to test the consequence of different scenario's for fruit quality. Directly following harvest, fruit of some piking dates were subjected to different types of stress in order to find a quick test for sensitivity for brown heart.

C. OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

Objective
The aim of the present experiments is fourfold:

1. Influence on the sensitivity to brown heart of orchard factors
   - nutrient concentration in the fruit
   - degree of bearing (variation both natural and by thinning)
   - position of the fruit in de tree
2. Influence of storage conditions
3. Influence of post harvest treatments and scenario's
4. Development of a rapid sensitivity test by post harvest stress

Furthermore data are collected on climate and on orchard factors.

Material and Methods

Influence on the sensitivity to brown heart of orchard factors
In three orchards nitrogen, potassium and calcium have been sprayed 11 times to 15 trees from June 17 to August 12. the trees were selected for having normal and equal degree of bearing. Spraying was until start of dripping of the fluid. The solution contained 10 gr potassium sulfate (K) or 1.7 gr Calcium chloride (Ca) or 1.9 gr Ureum per liter (N).
In three orchards trees were selected in which 1/3 or 2/3 of the fruits were removed manually to be compared with trees of normal degree of bearing. In one of these orchards trees were selected that had already naturally 1/3 or 2/3 of the normal bearing by reduced number of flowers and/or reduced fruit set.
In three orchards trees of comparable normal bearing were subdivided into 5 zones i.e. 'top', 'high', 'inner', 'north+east' and 'south+west'. From September 1 to 29, each week at least 3 trees per treatment were empty-picked in all experiments. After picking, a sample was used for direct quality measurements and one box, composed of all replications (containing 16-17 kg) was cooled to -0.5°C. After 3 weeks these boxes were transferred to the final storage conditions i.e. -0.5°C, 2% O_2 and 0.5% CO_2.

Influence of storage conditions
In 11 orchards, each week from September 3 to October 1, complete trees, were empty-picked (at least three trees per replication). Following picking a sample was used for direct quality measurements and two boxes (16-17 kg each) were composed of all fruits per treatment and cooled to -0.5°C. After three weeks one box was placed at -0.5°C, 2% O_2 and 0.5% CO_2 and the other at -0.5°C, 2% O_2 and 5.0% CO_2.

Influence of post harvest treatments and scenario's
In 11 orchards, each week from September 17 to October 1, complete trees, were empty-picked (at least three trees per replication). Following picking a sample was used for direct quality measurements and five boxes (16-17 kg each) were composed of all fruits per treatment and cooled to -0.5°C. After 0, 2, 7, 21 and 50 one box was transferred to -0.5°C, 2% O_2 and 0.5% CO_2

Development of a rapid sensitivity test by post harvest stress
From the fruit under points 2 and 3 six boxes were composed at the at the 2nd and the 4th picking date (September 10 and 24) and placed at 18°C. After 2, 5 and 9 days two of these boxes from each of the 11 orchards were removed. One box was placed at 4°C, 2 % O_2 and 1% CO_2 and the other at -0.5°C, 2% O_2 and 5% CO_2. Fruits were inspected for brown heart on September 29 and October 13 (see table 1 and 2). These results will be connected to the incidence of brown heart after storage in order to detect any

possibility of a quick test for sensitivity to brown heart. From all experiments a fruit sample was collected on August 19 for an assessment of ripeness stage and of quality characteristics. In this sample, and in the sample of September 18 (pick 3) an analysis of nutrient concentration was done.
Following picking the temperature condition within the boxes was monitored in a number of cases by including small temperature loggers. Meteorological data pertinent to the different orchards during the whole growing season are available. Orchard characteristics (age, tree density, root stock, soil type etc.) have been collected in all experiments.

Results and discussion
All fruit is at the moment still in storage. Apart from the results of the stress test , at this moment only data are available concerning orchard characteristics and quality characteristics at harvest.

Stress test
A good and reliable stress test should indicate the risk of brown heart in the relative short period of cooling preceding establishment of the gas conditions. Considering the influence of ripening stage it may be expected that differences in sensitivity between the 11 orchards are visible in the late pick 4 and not yet in the early pick 2 (2 weeks earlier). Those orchards that show no problems in fruit of both picking dates might be suitable for CA-storage. The results of the test after 9 days at 18°C (table 1) show that this type of test is not suitable since fruit from all orchards show brown heart. The test during 5 days at 18°C followed by 14 days in CA at 4°C is more promising (table 2) since there are clear differences between the orchards. On the other hand the differences do not concur with differences in incidence of brown heart in the past (Roelofs et. al, 1997). The final judgment will be based, of course, on the comparison with the incidence of brown heart after long CA-storage of fruit from the same origin as used in the stress test.

Conclusions
It is possible to induce symptoms of brown heart and other disorders in Conference pears within a few days of treatment. Whether the severity of these sympoms have any predictive value remains to be seen. This can only be judged after observing the result of long CA-storage.

D. DESCRIPTION OF THE WORK PLANNED FOR THE SUBSEQUENT PART OF THE PROJECT (1-12-97 to 1-6-98)

Fruit from the experiments one to three remains in storage until April. After closing the storage period fruit quality, loss of weight, incidence of external disorders and of internal disorders will be characterized. For internal disorders (brown heart) all fruits will be inspected. For characterization of fruit quality 25 fruits will be used. Loss of fruit weight will be characterized per box of fruit.

E. DISSEMINATION

Roelofs, F.P.M.M., A. de Jager and C. Westerweele, 1997. Research on the possibilities to reduce disorders in Conference pears during CA storage. Intern tussentijds verslag FPO-Wilhelminadorp (in Dutch).

Tables

Table 1. Percentage healthy fruits, %fruits with internal browning, and %fruits with internal browning and hollow patches, for each picking date after different types of stress treatment (duration of whole treatment is 19 days; data are mean of 7 orchards)

pick	days at 18 °C	storage condition	good	brown	br.+hollow
2	2	4 °C, 2% O_2, 0.% CO_2	100.0	0.0	0.0
	5	4 °C, 2% O_2, 0.5% CO_2	99.6	0.0	0.1
	9	4 °C, 2% O_2, 0.5% CO_2	53.9	18.3	13.9
	2	4 °C, 2% O_2, 5.0% CO_2	99.9	0.1	0.0
	5	4 °C, 2% O_2, 5.0% CO_2	96.0	5.3	0.0
	9	4 °C, 2% O_2, 5.0% CO_2	11.0	9.1	40.2
4	2	4 °C, 2% O_2, 0.5% CO_2	100.0	0.0	0.0
	5	4 °C, 2% O_2, 0.5% CO_2	88.2	11.0	0.3
	9	4 °C, 2% O_2, 0.5% CO_2	55.8	16.2	13.8
	2	4 °C, 2% O_2, 5.0% CO_2	96.9	3.7	0.0
	5	4 °C, 2% O_2, 5.0% CO_2	99.2	0.4	0.1
	9	4 °C, 2% O_2, 5.0% CO_2	26.1	17.9	27.7

Table 2. Percentage healthy fruits, %fruits with internal browning, and %fruits with internal browning and hollow patches, for individual orchards following 5 days at 18 °C followed by 14 dagen at 4 °C, 2% O_2 en 5.0% CO_2.

orchard code	%good	%internal browning	%browning+hollow
12	100.0	0.0	0.0
33	90.7	8.0	1.3
77	72.7	25.4	1.9
78	86.1	13.9	0.0
79	96.2	2.7	0.0
80	86.5	13.5	0.0
81	85.3	13.3	1.5

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A. PARTNER INFORMATION

1. (Sub)Project title
Quality improvement of pears by predictive and adaptive technology

2. Project coordinator
Dr. J. Sreif

3. Project participants
C. Rabus

B. INTRODUCTION

1. Description of the research topic or practical problem
Brown heart in Conference pears is the browning of the flesh, especially in the core region and the subsequent development of cavities. Affected pears are not suitable for consumption, and it is not possible for the consumer to distinguish between healthy and affected pears from the external outlook, only. The occurence of this disorder is influenced by weather factors, orchard factors, picking date, post-harvest treatments and storage conditions.

2. Goal of the research
The goal of the research is to prevent the development of tissue disorders during and after CA storage. Methods should be developed which enables a rapid decision about the post-harvest treatments and storage conditions for 'Conference' pears. This can be achieved by development of predictive models using orchard and weather characteristics or physiological parameters like respiration rate, fermentation rate, gas diffusion. An other goal is the testing and implementation of post-harvest treatments, which improve the storability of the pears.

3. Short description of the approach
For establishing a prognosis for the exspected storage behaviour of pears, data are needed on climate and orchard conditions during the growing season and gas exchange rates, diffusion resistance and pear quality throughout the storage season. Because every year results in pears with different characteristics, the project will be carried out in 4 successive years and in 5 countries under different site conditions.

C. OVERVIEW OF THE WORK PERFORMED FROM 1-6-1997 TO 1-12-1998

1. Objective

The objective in the first project phase was to provide and to collect data during the growing of 'Conference' pears in two different orchards under the specific ecological conditiones in the Lake of Constance area, South-West Germany (tasks 1, 2, 4).
After different harvest dates (task 2) the pears were stored under various CA conditions (task 3, 5). The change of fruit quality and incidence of core browning (task 7a), mineral composizion (task 7c), biochemical changes (task 7b) as well as gas exchange characteristics (task 6) were evaluated in order to find physiological explanationsand practical decisions to prevent core browning disorders.

2. Material and Methods

The first progress report gives an overview of the work performed in the periode 1-6-97 to 1-12-97.
In the beginning of the project we had to select two different orchards which were suitable for the different tasks. Because of heavy spring frost during April '97 we found only one orchard with enough crop load at the Experimental Station Bavendorf. This orchard was treated two times during the frost periode (April, 17th and 24th) with phytohormons (Berelex ,GA_3 +Promalin GA3+Bencyladenine) in order to improve fruit set. The fruits from this orchard were all parthenocarpic developed without any seeds.
The second orchard for our experiments we found in a fruit farm 25 km far away near Salem/Überlingen.. This orchard is located in a frost protected place and the trees were completely untreated with phytohormons. Nevertheless, only 25-35 % of pears had fully developed seeds.
The site conditiones, planting system and special treatments of these two orchard are as follows:

Orchard conditions and cultivation

Orchard I:

Experimental Station, Bavendorf, Quartier 17

Geographical location:

47° 46' northern latitude, 9° 33' eastern of Greenwich, 485-490 m NN, inclination to east

Orchard characteristics:

Tree age: 18 years, rootstock: quince A, planting system: 4x2 m, area: 0,4 ha

Special treatments:

Spray of growth regulators for improved fruit set (17-4-97 and 24-4-97)
Hand thinning for normal crop load (23, 24, 27 CW)
Samples for mineral analysis: 23-7-97 and at picking dates:, 24 fruits (8 trees, 3 replicates)
Picking dates: 03-9-97: first pick, 10-9-97: second pick, 17-9-97: third pick

Climate characteristics:

	temperature °C			rel. humidity	rainfall	radiation	sunshine
	T. ⌀	T. min	T. max	%	mm/m²	W/m²	h
Mai	13.2	-0.4	29.7	67	54	160,3	270
June	15.7	4.9	30.1	74	136	141,8	187
July	16.7	6.9	29.0	76	107	137,7	202
August	19.0	6.0	31.0	73	73	142,2	192
⌀ or Σ	16,15	4.35	29.98	72.5	370	582.0	851

Orchard II:

Fruit farm Hans Knäple, Salem-Mittelstenweiler

Geographical location:

47° 45' northern latitude, 9° 21' east of Greenwich, 450 m NN, inclination to south-west

Orchard characteristics:

tree age: 31 years, rootstock:seedling, planting system: 4x6m, area: 0,15 ha

Special treatments:

The same as in orchard I, but not sprayed with growth regulators

Climate characteristics: (data from a comparable site, 2 km far away)

	temperature °C			rel. humidity	rainfall	radiation	sunshine
	T. ⌀	T. min	T. max	%	mm/m²	kW/m²	h
Mai	13.6	-2.3	31.7	70	32	133.8	
June	16.3	5.0	33.3	78	134	118.1	
July	17.1	5.3	31.0	82	104	103.1	
August	19.4	6.0	35.8	79	49	107.9	
⌀ or Σ	16,6	3.5	32.95	77.25	319	462.9	

Experimental design

Various experiments were started to test the influence of:

- picking dates in combinations with storage conditions (experiment. A: Tasks 2, 3, 4, 6, 7a, 7b, 7c,)
- cropload in combinations with storage conditions (experiment B: tasks1, 3, 6, 7a, 7b, ,)
- shifting CA conditions during the storage periode (experiment C: tasks3, 5, 6)

on various fruit quality parameters, physiological changes and incidence of core browning and cavities. The experimental design, the time of sampling and the number of trees and fruits used for the different analysis during the storage periode are shown in Fifure 1, 2, and 3 in the annex.

Estimation of crop load

To determine crop load of pear trees and to be able to vary crop load the estimation of the number or the weight of fruits per tree is necessary. The preliminary suggestion to use a method described by Winter (1977) for estimation the crop load of apples was not practical for our purpose.. Therefore we decided to count the fruits and to determine the average weight of the fruits at harvest and to calculate the 'specific yield' or 'specific fruit number' as described by Lafer (1996). For the 'specific yield' of a tree the relationship between the crop weight of the pears is related to the area of the trunk in 30 cm height above ground. In our case we regulate the crop load for:

high crop: 0,25 kg pears /cm²
normal crop: 0,18 kg pears /cm²
low crop: 0,12 kg pears /cm²

Picking date assessment

Samples of Conference pears for estimation the optimum picking date were taken several times before the beginning of harvest in order to get informations about the changes in firmness, starch conversion and soluble solid content. Special interest was given to firmness value which reached in former experiments values between 6-6.5 kg/0.5 cm² at optimum picking date. From the observed parameters ripening index was calculated, using the formula: F/(R*S) (Streif 1996) and compared with advised index values reported by Johnson and Luton (1996).

Storage devices

Pear fruits are stored in an experimental CA storage equipment consisting of 15 gas tight containers with 540 l volume (≅120 kg of fruits). The temperature of containers is regulated by the ambient temperature of the cool room. After closing the containers, the various atmospheres were established within few hours by flushing with N_2. The adopted gas composition in the different chambers is constantly controlled and regulated by a computerized system connected to an infrared CO_2 and

paramagnetic O_2 analyzers.

Respiration measurements
Respiration measurements are done by headspace GC anlysis of CO_2 and O_2 at four different times from the beginning to the end of the storage periode of the pears. The instrumental device (Micro GC, Chrompack) and the measuring method are similar to that described by Peppelenbos and van't Leven (1996). The average temperature of storage the pears for respiration measurement is 2 °C, the volume of the flasks to measure the headspace gases is 3150 ml, and the time between the first and second measurement for headspace measurement is 4 hours. The experimentory setup for respiration measurement is:

Temp. storage	% O_2	% CO_2	nr. of fruits	rep.	weight	days of
2 °C	0, 1, 2, 21	0,7, 5	2	2	305±12	3, 4, 5

Ethylene production:
Ethylene production of the pears is measured from the same fruits and at the same time when respiration measurement is done. A 10 ml gas sample is taken from the head space volume and ethylene is analysed by FID GC on a alumina column at 100 °C.

Ground colour of peel:
The measurement of colour changes during storage period is made by Minolta Chromameter CR 300. Only the ground colour is measured by CIE Lab colour measuring system on the greenest spot and on the widest part of the pear.

Flesh firmness:
The firmness of the fruit tissue is determined with an Effeggi penetrometer equipped with a 0.5 cm^2 probe. The measurements are done on two opposite sides and on the widest part of the pear. The peel is removed before.

Soluble solids content and acidity of fruit juice:
Individuel fruits are juice extracted by a centrifugal extractor and few drops of juice are used for refractometric determination of soluble solids. From the same juice the titratable acidity is measured using 0.1 N NaOH titrated to pH 8.1.

Mineral content of K, Ca, Mg, P:
Parts of fruits (24 fruits/sample) were homogenized, freeze-dried, ashed, and brought with hydrochloric acid containing 0.1 % lanthanum to a 50 ml volume. Potassium, calcium and magnesium are determined with a flame spectrofotometer in the absorption mode. Ca standards contained average amounts of the other constituents. Determination of phosphorus is done by a molybdenum blue colorimetric procedure, using average amounts of the other constituents added to the standards

Lypoxygenase activity (LOX):
Lypoxygenase activity is measured by a slighly modified polarografically method described by Feys et al. (1980). Fruit material without skin is homogenized together with 0.25 M phosphate buffer (pH 7,0), 1% Triton X-100 and 0.1 M $Na_2S_2O_5$ for 2 minutes and adjusted to pH 7 with few drops of 5 N NaOH. The homogenate is centrifuged at 15000 U/min for 15 min. The supernatant is used as enzyme extract. All steps are performed below 4 °C. Substrate solution is prepared with 0,25 M Na-P-buffer (pH 7.0) containing 6,64 mM linoleic acid (or oleic acid as control). LOX activity is measured with a Clark O_2 electrode.

1-aminocyclopropane-1-carboxylic acid (ACC):
For ACC determination in the fruit tissue the methode of Lizada and Yang (1979) will be used. Fruit material was frozen in liquid N_2, freeze-dried and stored together with drying material in a freezer at 25 °C till analyzed.

Ascorbic and dehydroascorbic acid:
Ascorbic acid and dehydroascorbic acid is separately determined in the inner and outer parts of the fruit cortex without peel by a fluorometric technique described in AOAC (1975) with small modifications. The ascorbic acid (AA) and dehydro-ascorbic acid (DHA)contents were separately determined by subtracting the measured DHA from total vitamin C, which was obtained after oxidation of AA-DHA in the presence of activated charcoal powder (Norrit).

Ethanol and acetaldehyde determination (method used at IVTPA, Milan):
For sample preparation fruit pulp is sliced, homogenized, and placed (20 g) in a 25 ml vial with a tightly closed silicon-teflon rubber septum. Samples are immediately frozen and kept at -20°C. For analysis a vial is taken out from the freezer and kept at room temperature for 30 min. Then it is placed in a heat chamber at 60 °C for 1 hour and an aliquot of the headspace volume is sampled and injected into the GC. GC conditions: column: Carbowax 20 M, 30m, 1.5 mmI.D., FID detector, temp. program: 40 °C 5 min, 30°C/min, 150 °C 5 min, retention times: acetaldehyde 1,3 min, ethanol 3 min.
The system is calibrated with the headspace of a water solution of 10 ml containing known amounts of ethanol and acetaldehyde, treated exactly in the same way as the samples.

Judgement of browning and cavities disorders:
After 10 days of shelf-life at 18 °C and 60 % of relative humidity the incidence of flesh browning and cavities were judged according a picture card with 5 stages (0-4) of damages. The cards were supplied by Frans Roelofs, Wilhelminadorp.

Results and Discussion
This report will give preliminary results of various rinning experiments. At the moment, only data of the first and second sampling dates for the experiment A (see Figure 1) and experiment C (Figure 3) are available. These are the data collected at harvest (beginning of storage period) and the data after 2 months of storage. Because of limited storage capacity the number of sampling dates have to restricted for experiment B (Figure 2) to the beginning and the end of storage period. The data are summarized in the annex in Tables 1 - 7.

Mineral composition
The mineral content of the pears was analyzed end of July, during the growth of the fruits, and directly after the various harvest dates of both orchards. These data are shown in Table 1.The mineral composition of pears from different crop loads of orchard I is shown in Table 2.
It's clearly seen that the mineral content in pears is as lower as later the fruits were taken for analysis. Nevertheless it's possible to get a realistic impression of the mineral relationship at harvest in September when the fruits were taken two months earlier in July.
At each samling dates, both orchards differ distinctly in potassium and phosphorus content whereas calcium is nearly the same. Orchard I has a higher K content, therefore the K:Ca ratio is somewhat higher and possibly unfavorable for storage life.
Table 2 show the mineral content as influenced by crop load. Differences in mineral composition between the treatments are very small, and not explicable by the crop load. Only the dry weight of fruits give a good connection to the tree load.

Quality evaluation
The results of quality changes in Conference pears monitored at harvest and during the first 2 months of CA storage are shown in Table 3. Because of limeted space of our storage facilities fruits from orchard II only can be sampled each second month during the 6 months storage periode. But the harvest data are available for both orchards. The different harvests were performed at the same dates in both orchards. Comparing the ripening and quality parameters, orchard II seems more ripe than orchard I, which can be seen in lower firmness and higher starch conversion values. The ripening index, calculated according Streif (1996), give the same results. Changes of other quality parmeters as influenced by storage conditions are not detectable after the first two months of storage.

Visual inspection for incidence of disorders
After 2 months of CA-storage, including a shelf life periode of 10 days at 18 °C and 60 % relative humidity, the incidence of disorders mainly core browning and cavities were monitored (see Table 4). The number of available fruits for internal inspection was somewhat different, but remarkable high. Fore core browning and cavities an index was calculated considering the intensity of the disorders. In general the development of disorders was relativ low after two month of storage. Fore all three used CA concentrations can be stated that the incidence of disorders is increased with later harvest dates, and the disorders are aggravated by higher CO_2 concentration in the CA atmosphere. Nevertheless, the CA concentration 2 % CO_2 + 4 % O_2 with higher CO_2- and also higher O_2-concentrations (advice from Wim Schmitz, Holland) shows similar good results as 0.7 CO_2 + 2 % O_2.

Vitamin C and ascorbic acid content and Lypoxygenase-activity
Antioxidative substances like vitamin C may play an importent 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 and 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 from the CA treatment 5% CO_2 + 2 % O_2 in general and from the late harvest date in special. This is also true for the activity of Lypoxygenase (LOX) which seems much higher in fruits from the late picking date (see Tab. 5, also).

Measuring gas exchange, diffusion rate and ethylene ptoduction
Because of technical problems and restricted placees for respiration measurement under CA conditions in the beginning, we could start with respiration measurement few weeks after harvest and only with fruits from the last picking date and from CA treatments 0.7 CO_2 + 2 % O_2 and 5% CO_2 + 2 % O_2. Because of the same reasons we had to postpone the planned diffussion measurements, too. At present only first results of respiration measurement are available. A summary of this results are given in Table 6. At the moment it is difficult to see clear connections between respiration intensity and the incidence of browning disorders. In general, it can be stated that fruits from unfavorable CA conditions (5% CO_2 + 2 % O_2) produce higher amounts of CO_2 whereas O_2 consuption seems not to be influenced.
The ethylene production was generally higher in air and in CA concentrations with higher O_2 concentrations (table 7).

Ethanol, acetaldehyde, polyphenol oxidases, peroxydases, 1-aminocyclopropane-1- carboxylic acid
Fruit samples for analysis of these various substances are collected and held under appropriate conditions. The analysis will be done as soon as possible.

Conclusions
At present time it is to early to give conclusions of the obtained results, because we just have got the first, preliminary data. First of all we have to continue with the planned storage experiments.

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D. DESCRIPTION OF THE WORK PLANNED FOR THE SUBSEQUENT PART OF THE PROJECT (1-12-97 TO 1-6-98)

For the coming half year period the running storage experiments will be performed till mid of March 1998. During this time a lot of measurements have to be done and new methode developed. Besides the running analysis various enzymes, involved in oxidation processes, and ACC, ethanol and acetaldehyde will be analysed. Diffussion measurement will be performed, also. After that the relations between different analytical data and the incidence of browning disorders will be evaluated and the second report have to been written at the end of the first year.

E. DISSEMINATION

Not yet

Tables

Tab. 1 Mineral content of Conference pears from 2 different orchards, and different sampling dates 6 weeks before harvest and at harvest

Orchard I: Harvest dates							
sampling date	Set	dry wt. %	K mg/g dry wt	Ca mg/g dry wt	Mg mg/g dry wt	P mg/g dry wt	K : Ca
23-07-97	1	20.5	11.2	1.6	0.7	1.4	7.1
pre harvest	2	19.7	11.0	1.2	0.7	1.4	8.9
	3	18.6	12.1	1.5	0.8	1.6	7.9
	mean	19.6	11.4	1.4	0.7	1.4	8.0
	SD	1.0	0.6	0.2	0.0	0.1	0.9
03-09-97	1	16.5	8.5	0.6	0.4	0.7	13.7
harvest date I	2	16.3	8.3	0.6	0.4	1.1	13.6
	3	17.7	8.5	0.6	0.4	0.9	13.7
	mean	16.8	8.4	0.6	0.4	0.9	13.6
	SD	0.7	0.1	0.0	0.0	0.2	0.1
10-09-97	1	16.6	7.4	0.6	0.4	0.8	13.5
harvst date II	2	16.5	7.3	0.5	0.4	0.9	14.9
	3	16.7	7.6	0.5	0.4	0.8	15.6
	mean	16.6	7.5	0.5	0.4	0.8	14.7
	SD	0.1	0.2	0.0	0.0	0.1	1.1
17-09-97	1	16.3	7.7	0.4	0.4	0.8	18.0
harvst date III	2	16.6	7.8	0.5	0.4	0.8	16.3
	3	16.7	8.2	0.4	0.4	0.8	19.0
	mean	16.5	7.9	0.4	0.4	0.8	17.8
	SD	0.2	0.2	0.0	0.0	0.0	1.4

Orchard II: Harvest dates							
sampling date	Set nr.	dry wt. %	K mg/g dry wt	Ca mg/g dry wt	Mg mg/g dry wt	P mg/g dry wt	K : Ca
23-07-97	1	17.8	10.7	1.3	0.6	1.2	8.4
	2	17.2	9.3	1.1	0.6	1.0	8.9
	3	18.1	9.4	1.3	0.6	1.1	7.5
	mean	17.7	9.8	1.2	0.6	1.1	8.2
	SD	0.4	0.8	0.1	0.0	0.1	0.7
03-09-97	1	16.0	7.1	0.5	0.4	0.9	13.2
harvest date I	2	16.1	7.5	0.6	0.4	0.8	13.4
	3	16.3	7.1	0.6	0.4	0.8	12.9
	mean	16.1	7.2	0.6	0.4	0.8	13.2
	SD	0.1	0.2	0.0	0.0	0.0	0.2
10-09-97	1	16.0	5.9	0.5	0.4	0.8	11.0
harvst date II	2	16.4	6.3	0.6	0.4	0.7	10.0
	3	16.5	6.2	0.6	0.4	0.7	11.3
	mean	16.3	6.1	0.6	0.4	0.7	10.7
	SD	0.3	0.2	0.0	0.0	0.0	0.7
17-09-97	1	16.9	6.8	0.5	0.4	0.7	15.0
harvst date III	2	16.4	6.4	0.4	0.4	0.7	15.1
	3	16.5	6.8	0.5	0.4	0.8	14.4
	mean	16.6	6.6	0.4	0.4	0.7	14.8
	SD	0.3	0.2	0.0	0.0	0.1	0.4

Tab. 2	Mineral content of Conference pears from different crop loads and sampling dates 6 weeks before harvest and at harvest						
Orchard I: Crop load							
sampling date	Set	dry wt. %	K mg/g dry wt	Ca mg/g dry wt	Mg mg/g dry wt	P mg/g dry wt	K : Ca
23-07-97	1	18.8	11.3	1.4	0.7	1.4	8.1
preharvest	2	18.7	12.3	1.4	0.8	1.6	8.7
high crop	3	19.1	11.5	1.3	0.8	1.5	8.7
	mean	18.9	11.7	1.4	0.8	1.5	8.5
	SD	0.2	0.5	0.0	0.0	0.1	0.3
03-09-97	1	20.5	11.2	1.6	0.7	1.4	7.1
preharvest	2	19.7	11.0	1.2	0.7	1.4	8.9
middle crop	3	18.6	12.1	1.5	0.8	1.5	7.9
	mean	19.6	11.4	1.4	0.7	1.4	7.9
	SD	1.0	0.6	0.2	0.0	0.1	0.9
10-09-97	1	20.3	11.2	1.6	0.7	1.4	7.1
pre harvest	2	20.1	12.3	1.5	0.7	1.4	8.5
low crop	3	20.3	12.4	1.6	0.7	1.4	8.0
	mean	20.3	11.9	1.5	0.7	1.4	7.8
	SD	0.1	0.7	0.1	0.0	0.0	0.7
09-09-97	1	16.3	7.7	0.5	0.4	0.9	15.0
harvest date II	2	16.2	7.5	0.5	0.4	0.8	14.1
high crop	3	16.2	7.3	0.6	0.4	0.8	13.0
	mean	16.2	7.5	0.5	0.4	0.8	14.0
	SD	0.0	0.2	0.0	0.0	0.0	1.0
09-09-97	1	17.0	7.7	0.6	0.4	0.9	13.8
harvest date II	2	17.2	7.5	0.6	0.4	0.9	12.2
middle crop	3	17.7	7.4	0.6	0.4	0.8	12.6
	mean	17.3	7.6	0.6	0.4	0.8	12.8
	SD	0.3	0.2	0.0	0.0	0.0	0.9
09-09-97	1	17.5	7.7	0.5	0.4	0.8	14.6
harvest date II	2	17.8	7.2	0.5	0.4	0.8	13.5
low crop	3	17.8	7.5	0.5	0.4	0.8	13.8
	mean	17.7	7.4	0.5	0.4	0.8	14.0
	SD	0.2	0.3	0.0	0.0	0.0	0.6

Tab. 3 Fruit quality and ripening parameters of Conference pears from two orchards at harvest (orchard I and orchard II) and after 2 months of CA storage (0rchard II)

Orchard I

Average of 6 fruits x 3 replicates

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 (03-Sept.)	begin	58.94	7.14	11.44	3.84	3.11	-16.63	0.20
	II (10-Sept.)	begin	59.28	6.70	11.99	3.18	3.28	-16.45	0.17
	III (17-Sept.)	begin	63.61	6.33	12.95	3.01	3.61	-17.03	0.14
			Standard deviation						
1	I (03-Sept.)	begin	5.82	0.69	0.53	0.48	0.83	0.76	0.08
	II (10-Sept.)	begin	5.06	0.62	0.50	0.41	1.08	1.31	0.04
	III (17-Sept.)	begin	3.27	0.35	0.61	0.55	0.92	1.17	0.06

Orchard II

Average of 6 fruits x 3 replicates

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 (03-Sept.)	begin	58.44	6.69	11.52	3.10	3.56	-16.20	0.16
	II (10-Sept.)	begin	61.67	6.26	12.20	2.86	3.89	-15.02	0.13
	III (17-Sept.)	begin	64.50	5.76	12.52	2.93	4.00	-16.60	0.12
2		0,7+2	59.89	5.83	12.66	2.51		-14.54	
	I (03-Sept.)	5+2	59.50	6.11	12.71	2.13		-16.30	
		2+4	61.22	5.75	13.01	2.18		-16.05	
		0,7+2	60.11	5.79	13.46	2.23		-14.77	
	II (10-Sept.)	5+2	60.72	5.56	13.19	2.12		-13.98	
		2+4	59.50	5.69	12.88	2.33		-14.85	
		0,7+2	62.67	5.37	13.23	1.98		-14.72	
	III (17-Sept.)	5+2	62.06	5.46	12.98	1.59		-15.15	
		2+4	62.67	5.55	13.35	2.07		-15.35	
			Standard deviation						
1	I (03-Sept.)	begin	4.74	0.71	0.48	0.33	0.92	1.04	0.03
	II (10-Sept.)	begin	4.28	0.67	0.72	0.36	0.76	2.06	0.04
	III (17-Sept.)	begin	3.42	0.41	0.72	0.34	0.77	1.20	0.03
2		0,7+2	4.38	0.37	0.60	0.37		2.13	
	I (03-Sept.)	5+2	3.22	0.54	0.57	0.30		0.97	
		2+4	3.00	0.42	0.61	0.34		1.48	
		0,7+2	4.04	0.58	0.69	0.35		1.41	
	II (10-Sept.)	5+2	4.34	0.58	0.71	0.44		3.19	
		2+4	5.97	0.58	1.00	0.42		1.22	
		0,7+2	5.58	0.76	0.96	0.37		1.62	
	III (17-Sept.)	5+2	5.59	0.52	0.88	0.27		1.36	
		2+4	3.73	0.48	0.54	0.29		1.23	

Tab. 4 Visual inspection of Conference pears for incidence of disorders and diseases after 2 month of CA storage and 10 days of shelflife at 18 °C

Orchard II

sampling date	storage cond. %CO2+%O2	harvest date	Set nr.	total nr. of fruits	healthy fruits %	fungus infected %	core brown. index	cavities index	
			1	118	97.5	2.50	0.00	0.00	
		I	2	113	97.3	2.70	0.00	0.00	
			3	119	98.3	1.70	0.00	0.00	
			mean	117	97.70	2.30	0.00	0.00	
			1	90	94.4	5.60	0.00	0.00	
	0,7+2	II	2	100	95.0	5.00	0.00	0.00	
			3	105	99.0	1.00	0.00	0.00	
			mean	98	96.13	3.87	0.00	0.00	
			1	72	98.6	0.00	0.17	0.00	
		III	2	78	93.6	5.10	0.00	0.32	
			3	74	98.6	1.40	0.00	0.00	
			mean	75	96.93	2.17	0.06	0.11	
			total mean	97	96.92	2.78	0.02	0.04	
			1	118	95.8	2.50	0.00	0.42	
		I	2	116	98.3	1.70	0.00	0.00	
			3	119	95.8	2.50	0.00	0.42	
			mean	118	96.63	2.23	0.00	0.28	
after			1	110	91.8	7.30	0.00	0.23	s
2 months	5+2	II	2	106	90.6	5.70	1.42	0.71	
of CA-			3	104	93.3	2.90	0.48	0.24	
storage			mean	107	91.90	5.30	0.63	0.39	
			1	65	78.5	10.80	3.85	2.31	
		III	2	82	72.0	1.20	7.62	5.37	
			3	89	69.7	9.00	5.62	7.58	
			mean	79	73.40	7.00	5.70	5.09	
			total mean	101	87.31	4.84	2.11	1.92	
			1	119	98.3	1.70	0.00	0.00	
		I	2	114	99.1	0.90	0.00	0.00	
			3	119	100	0.00	0.00	0.00	
			mean	117	98.70	0.87	0.00	0.00	
			1	114	99.1	0.90	0.00	0.00	
	2+4	II	2	103	95.1	4.90	0.00	0.00	
			3	107	98.1	1.90	0.00	0.00	
			mean	108	97.43	2.57	0.00	0.00	
			1	76	94.7	3.90	0.00	0,16	
		III	2	93	96.8	3.20	0.00	0.00	
			3	83	94.0	4.80	0.00	0.60	
			mean	84	95.17	3.97	0.00	0.30	
			total mean	103	97.10	2.47	0.00	0.10	

Tab. 5	Vitamin C and ascorbate content in the inner and outer part of fruit cortex (without peel and core) and the activity of Lypoxygenase (LOX)						
Orchard I							
			Average of 3 replicates				
sampling	stor.cond.	harvest	**vitamin C**	mg/100g FS	**ascorbate**	mg /100gFS	
date	%CO2+%O2	date	inner	outer	inner	outer	
1	begin	I	4.40	4.83	1.20	1.34	
at harvest		II	4.35	4.06	1.04	1.68	
		III	4.98	4.42	1.60	0.88	
		mean	4.58	4.44	1.28	1.30	
Orchard II							
			Average of 3 replicates				
sampling	stor.cond.	harvest	**vitamin C**	mg/100g FS	**ascorbate**	mg /100gFS	**LOX activity**
date	%CO2+%O2	date	inner	outer	inner	outer	umol O2/min/100g FS
	begin	I	5.45	5.24	2.13	1.51	
1		II	5.13	5.60	2.36	1.37	
at harvest		III	3.99	4.59	1.13	0.88	
		mean	4.86	5.14	1.87	1.25	
	0,7+2	I	3.42	3.52	1.53	1.10	10.09
2		II	2.94	3.55	1.53	1.45	13.67
		III	2.83	2.89	1.23	1.67	14.58
after		mean	3.06	3.32	1.43	1.41	12.78
2 months	5+2	I	2.18	3.01	0.22	1.38	11.13
of		II	2.13	3.28	0.59	1.34	14.71
storage		III	1.75	3.13	0.38	1.16	18.29
		mean	2.02	3.14	0.40	1.29	14.71
	2+4	I	2.72	3.13	0.88	1.44	3.65
		II	3.34	2.91	1.33	1.57	5.01
		III	2.62	2.85	1.05	1.12	8.66
		mean	2.89	2.96	1.09	1.38	5.77
			Standard deviation				
1	begin	I					
		II					
		III					
	0,7+2	I	0.27	0.10	0.16	0.31	2.55
		II	0.16	0.35	0.09	0.21	5.11
		III	0.29	0.07	0.12	0.30	1.47
2	5+2	I					2.71
		II					3.61
		III					4.32
	2+4	I					0.30
		II					0.79
		III					3.99

Tab. 6	Respiration of Conference pears kept under varibus gas concentrations				
	after harvest date III and after 2 months of CA storage				
	Harvest III	after harvest III, not stored before			
	atmosphere during	CO2-production days 3-5		O2-consumption days 3-5	
	respiration meas.	ml CO2 /kg x h		ml O2 /kg x h	
	% CO2 +% O2	mean	SD	mean	SD
	0 + 21 (air)	14.58	3.52	24.13	1.63
	0.8 + 8	8.86	1.86	18.20	2.24
	0.8 + 4	6.37	1.19	11.50	2.17
	0.8 + 2	3.55	0.87	5.10	1.66
	0,8 + 0	5.42	0.57	0.28	0.08
	5 + 8	6.32	1.38	21.14	3.78
	5 + 4	2.41	0.18	10.48	1.78
	5 + 2	0.86	0.54	5.60	1.57
	5 + 0	4.82	0.57	0.40	0.05
	Harvest III	after 2 months of storage - measurement at 2 °C			
	atmosphere during	CO2-production days 3-5		O2-consumption days 3-5	
storage conditions	respiration meas.	ml CO2 /kg x h		ml O2 /kg x h	
% CO2 +% O2	% CO2 +% O2	mean	SD	mean	SD
0.7+2	0 + 21 (air)	13.62	2.01	14.10	3.22
0.7+2	0.7 + 2	8.83	2.93	6.09	1.28
0.7+2	0.7 + 1	16.58	2.98	4.10	0.52
0.7+2	5 + 2	5.87	0.20	4.25	1.35
0.7+2	5 + 1	24.18	11.26	5.22	0.31
0.7+2	0 + 0 (N2)	2.33	0.91	1.09	3.69
5 + 2	0 + 21 (air)	15.98	0.35	11.98	4.21
5 + 2	0.7 + 2	13.33	0.38	6.52	1.13
5 + 2	0.7 + 1	21.17	9.46	3.05	0.68
5 + 2	5 + 2	33.84	4.26	4.90	0.35
5 + 2	5 + 1	42.76	7.67	-2.76	2.16
5 + 2	0 + 0 (N2)	3.38	0.95	1.36	0.58

Tab. 7 Ethylene production of Conference pears kept under various gas concentrations immediately after harvest and after 2 months of CA storage

	Harvest III	not stored before	
	atmosphere during respiration meas.	ethylene-production days 3-5 ulC2H2 /kg x h	
	% CO2 +% O2	mean	SD
	0 + 21 (air)	1.38	0.40
	0.8 + 8	0.16	0.00
	0.8 + 4	0.09	0.02
	0.8 + 2	0.05	0.03
	0,8 + 0	0.02	0.01
	5 + 8	0.10	0.02
	5 + 4	0.05	0.03
	5 + 2	0.02	0.01
	5 + 0	0.01	0.00

	Harvest III	after 2 months of storage	
	atmosphere during	ethylene-production days 3-5	
storage conditions	respiration meas.	ulC2H2 /kg x h	
% CO2 +% O2	% CO2 +% O2	mean	SD
0.7+2	0 + 21 (air)	24.18	1.90
0.7+2	0.7 + 2	1.20	0.10
0.7+2	0.7 + 1	0.76	0.02
0.7+2	5 + 2	1.05	0.01
0.7+2	5 + 1	0.77	0.05
0.7+2	0 + 0 (N2)	0.07	0.03
5 + 2	0 + 21 (air)	25.49	1.30
5 + 2	0.7 + 2	1.44	0.49
5 + 2	0.7 + 1	0.88	0.16
5 + 2	5 + 2	1.63	0.56
5 + 2	5 + 1	0.72	0.03
5 + 2	0 + 0 (N2)	0.03	0.02

Figures

Figure 1: Experimental design of experiment A: The influences of picking dates and storage conditions on various quality and physiological parameters

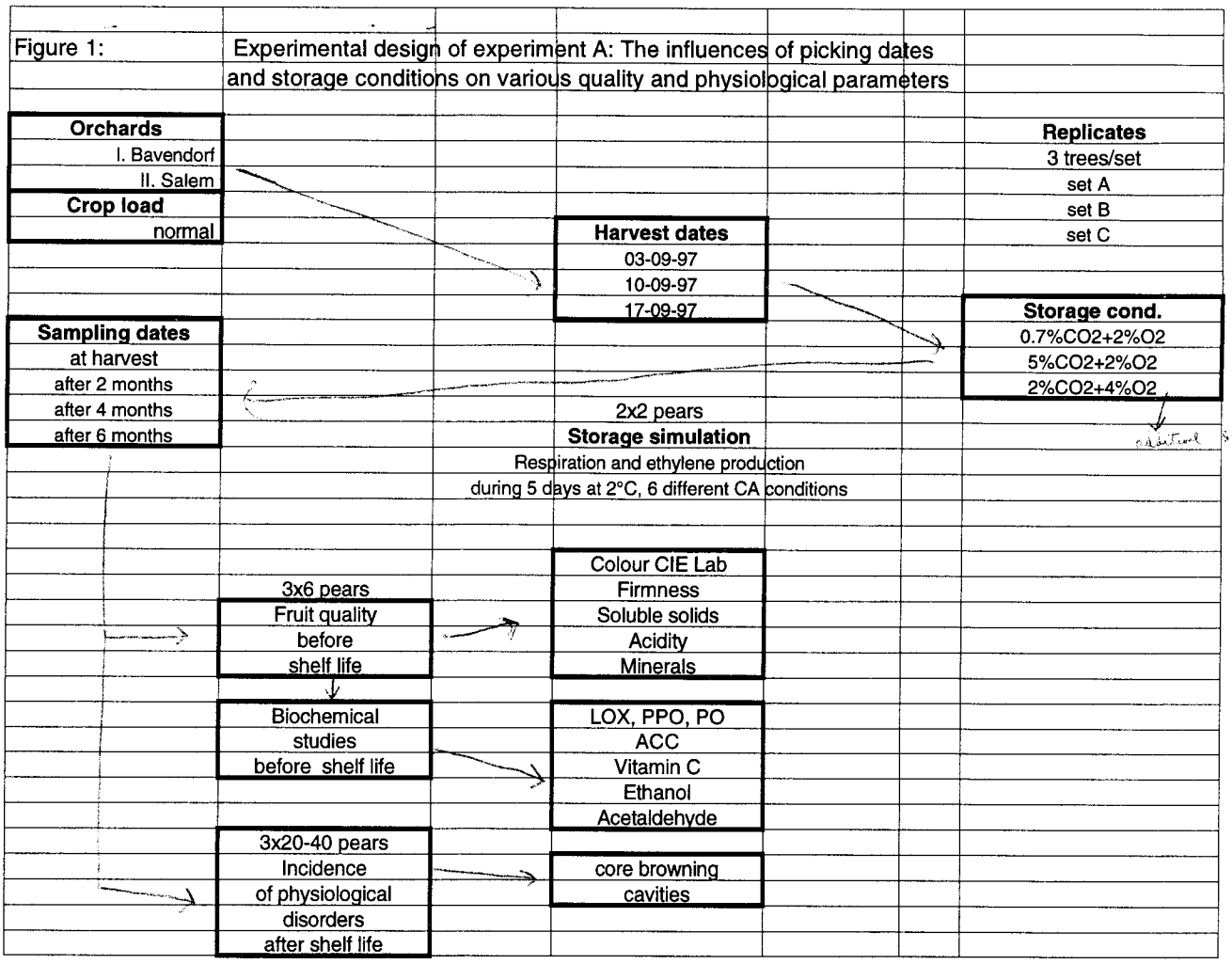

Figure 2: Experimental design of experiment B: The influences of crop load and storage conditions on various quality and physiological parameters

Orchards			**Replicates**
Bavendorf			set A
Harvest			set B
10-09-97			set C
	Crop load		
	high		
	normal		
	low		**Storage cond.**
Sampling dates			0.7%CO2+2%O2
at harvest			5%CO2+2%O2
after 6 months			2%CO2+4%O2
3x6 pears	Colour CIE Lab		
Fruit quality	Firmness		
before	Soluble solids		
shelf life	Acidity		
3x30 pears			
Incidence	core browning		
of physiol.	cavities		
disorders			

Figure 3: Experimental design of experiment C: The influences of shifting CA-concentrations on incidence of browning disorders

Orchards				
Bavendorf			**Storage cond.**	
Crop load			1.5->4.5%CO2/2%O2	
high			4.5->1.5%CO2/2%O2	
Harvest			3%CO2/2%O2	
10-09-97				
Sampling dates				
at harvest				
after 1 month		1x30 pears		
after 2 months		Incidence		core browning
after 3 months		of physiological		cavities
after 4 months		disorders		
after 5 months				
after 6 months				