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FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

Type of contract:	Shared-cost research project		
Total cost:	1600,3 kECU	*EC contribution:*	1045 kECU (65,3%)
Participant no. 1 total cost:	608 kECU	*EC contribution to partner no.1:*	304 kECU (50 %)
Commencement date:	01-06-1997	*Duration:*	4 years
Completion date:	31-05-2001		
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Participant no.: 1 (ATO-DLO)

A. PARTNER INFORMATION

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- Ing. S.R. Robat	task 6
- Ing. A.C.R. van Schaik	tasks 2, 3, 7, 8
- Drs R.E. Schouten	task 9
- Drs. R.H. Veltman	task 7
- Ir. J.P.J. de Wild	task 6

Objectives

The main objective of the project is the optimisation of the quality of Conference pears, being the most important pear grown within Europe, and the reduction of losses during storage, by preventing the development of disorders resulting in Brown Heart in pears. The key element in the project is the development of technology which enables a rapid measurement and decision about post-harvest treatments and optimal storage conditions. The main advantage of such a technology is that it leads to an accurate advice based on characteristics of products of the same harvest, rather then doing research on products from one year to reveal advice on products harvested the next year (with the uncertainty that product physiology might have changed). Three pathways are most promising towards such an approach, and will be the main objectives of the project:

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

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

Actions in the project

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

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

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

Task 2: Harvest of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	1

Objectives

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

Material and methods

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

Results and Discussion

The pears from both locations were harvested at five different dates: September 3, 10, 17, 24 and 30 of 1998. Harvest three, September 16, was comparable to the harvest date generally advised and used in the Netherlands at these locations. Most pears were harvested at that date, because they were necessary for several experiments. Pears harvested at the other dates were used only for a comparison between harvest dates (especially gas exchange rates). Quality data of the pears directly after harvest are listed in table 1.

Task 3: Storage of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	1

Objectives

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

Material and methods

The standard pre-storage procedure can be described as follows: the cooling down procedure will be started as soon as possible after harvest, and after 48 hours the minimum temperature should be reached. From then on the temperature is constantly monitored. One week after the start of the storage the storage rooms are flushed with N_2 until a concentration of 4 kPa O_2 is reached. The rest of the O_2

in the storage room is removed by respiration of the pears, until 2 kPa O_2 is reached. From then on the gas concentrations are continuously monitored and controlled.
The pears were stored in boxes, placed in 600 liter containers with a water sealing, at -0.5 ^{0}C to -1 ^{0}C. Oxygen levels were kept at 2 kPa. Carbon dioxide was kept at two different conditions: low (<0.5 kPa), using a KOH scrubber, or high (5 kPa). The low CO2 conditions simulates storage conditions used in practice. The high carbon dioxide condition was selected to generate the disorder in every year.
In some experiments pears were kept in 60 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.

Results and Discussion

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

Table 1. Quality parameters of pears directly after harvest (Starch Index from 1 to 10)

Harvest	Weight (g)	Colour A (Minolta)	Colour B (Minolta)	Colour L (Minolta)	Firmness (kg/0.5 ^{2})	Sugars (refract)	Titratable acids (%)	Starch index
Grower 1: Ingen								
1	177	-14.49	31.40	52.63	7.39	10.5	0.15	2.68
2	234	-14.46	32.00	52.62	6.56	11.2	0.15	2.68
3	201	-14.58	33.17	52.90	6.44	11.2	0.14	4.56
4	222	-14.16	33.95	54.72	6.34	11.6	0.11	4.35
5	245	-13.89	35.93	54.24	6.14	12.0	0.14	6.92
Grower 2: Ommeren								
1	184	-13.73	31.74	54.20	7.47	10.3	0.13	2.96
2	211	-13.05	31.74	53.99	6.83	10.7	0.13	2.80
3	223	-12.84	32.75	54.98	6.45	11.2	0.10	4.32
4	243	-12.89	34.38	55.76	6.31	11.7	0.14	3.64
5	245	-13.08	36.13	57.94	6.28	11.8	0.11	7.08

Task 6: Gas exchange and diffusion resistance

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	29
No. of man-month devoted already to the task:	10

Objectives

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

Material and Methods

Harvest dates

Conference pears were harvested on various dates: September 3, 10, 17, 24 and 30 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 kPa O_2 and 0 kPa CO_2. Pears from the optimal harvest date were stored at 2 kPa O_2 and 5 kPa 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 (Bauman and Henze, 1983) were measured. Samples from pears were taken to measure the specific weight of pear juice (used in the calculation of the porosity). Outer surface area of the pears was estimated using the length and the maximum circumference of the pear (see fig. 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 kPa O_2 with 0 and 5 kPa CO_2. Relative humidity was high (>95%) since the gas was led through water flasks. After 4 and 5 days of storage O_2 uptake and CO_2 production was determined. This was done by disconnecting the cuvettes from the flow throuh system, and sampling the headspace directly and after a period of 6 hours. The GC used was a Chrompack CP 2002. The measured O2, CO2 and N2 was corrected to 100 % to account for possible pressure variations inside the GC. Then the concentration values (in %) were multiplied with the actual pressure inside the cuvette (in kPa). Because the volume of the cuvette and the volume of the pears is known, the gas exchange rates can be calculated in nmoles/kg.s.

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 fuit 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 gas chromatograph 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 8°C and ambient air for 21 days. The temperature was selected in such a way that ripening was not too slow (2°C) and not to fast (18°C) in order to enable observations on changes in gas exchange. Gas exchange characteristics were measured after 2, 6, 9 and 13 days. Firmness and ascorbate levels were measured as well (see part C2).

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

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

Results and discussion

Harvest date
When gas exchange characteristics of pears harvested at different dates are compared, the most dramatic differences can be found between harvest 3 (17 September) and harvest 5 (30 September, harvest 4 was not measured). For instance Vm_{O2}, the maximum O_2 uptake rate, increases with harvest date, changing from 68.4, 79.3, 71.6 to 152 for harvest 1, 2, 3 and 5 respectively (average values table 2). VmfCO2, the maximum fermentative CO2 production rate, seems to decrease with harvest date: 81.0, 78.9, 74.5 to 68.1 for harvest 1, 2, 3 and 5 respectively (average values table 2). The resistance for gas diffusion varies between 823 $s.mm^{-1}$ (harvest 2) to 908 $s.mm^{-1}$ (harvest 3). All these variables separately do not give a clear view on changes of the fruit. Therefore it is interesting to combine them, in order to calculate internal gas concentrations. For this calculation gas exchange rates, diffusion resistance, fruit weight and fruit surface area are needed. Resistance values for diffusion of O_2 or CO_2 are calculated from neon values using Grahams law.
After doing this, the most interesting result is that internal CO_2 concentrations increase for harvest 5, when stored in ambient air (Figure 2a). This result is found when modelling results are used for the calculations, and also if the original data are used (Figure 2b). There is, however, a difference between between both figures: calculations based on the gas exchange models result in higher internal CO_2 concentrations. One explanation is that the data are found at O2 concentrations very close to anoxia, but never exactly 0 kPa. The model therefore estimates higher fermentation rates than actually found. The main conclusion of this experiment is that there was not a big difference in gas exchange characteristics between pears from different harvest dates.

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 3). 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 (Fig 3 and 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.

Storage period

Results of fitting the generated data to gas exchange models are found in table 4a (pears stored at 0 kPa CO_2) and table 4b (pears stored at 5 kPa CO_2). Figures 6a to 6d, based on the measurements after 86 days of storage, give a good representation of the modelling results.
First the influence of both O2 and CO2 on O2 uptake (fig 6a) and CO2 production (fig 6b) are shown. These results were found for pears that were previously stored at 2 kPa O2 and 5 kPa CO2. When these pears are compared with pears that were stored at 2 kPa O2 and 0 kPa CO2, it can be observed that there was no difference in metabolic response (Fig 6c, O2 uptake and fig 6d, CO2 production). This was not only observed after 86 days of storage but during the whole storage period. In figure 7b CO2 production at 2 kPa O2 in combination with 0 or 5 kPa CO2 is given. Here it becomes clear that the history of the pear (long term storage at 0 kPa or 5 kPa CO2) can no longer be detected in metabolic rates three days after transferring the pears to 0 kPa CO2. The conclusion is that adaptation in metabolic rates is very quick, and that storage at 5 kPa CO2 does not cause irreversible effects in metabolic rates.

Changes during the storage period in metabolic rates are quite small. In figure 8 it can be observed that the maximum O2 uptake rates (VmO2) and the maximum fermentative CO2 production rates (VmfCO2). Interestingly carbon dioxide does not seem to have a strong influence on metabolic rates directly after harvest (Km_{CO2} remains high, table 2). This is remarkably different from data obtained in the same period (Table 4a and 4b, 0 storage days). The difference between those tables is that gas exchange rates for the 'harvest date' experiment were measured at 18 ^oC, while gas exchange for the 'storage' experiment was obtained at 2 ^oC. Solubility in water seems to influence this contrasting result. Solubility for CO2 in the water phase is $7.1*10^{-4}$ $mol.l^{-1}$ at 2 ^oC and $4.1*10^{-4}$ $mol.l^{-1}$ at 18 ^oC. Unclear is whether the differences in diffusion resistance (table 4a and 4b, figure 10) are actual changes over time, or just fluctuations caused by variation between batches of pears. When gas exchange rates and diffusion resistances are used to calculate internal gas concentrations at conditions normally used during CA storage (2 kPa O_2 with 0 kPa CO_2), O_2 levels tend to decrease (shown as a bigger difference with external O_2). Internal CO2 levels, however, remain quite stable.

Ripening

When pears ripen, respiration rates increase substantially (fig 12B). Differences between individual pears are remarkably small. 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^oC (delayed ULO) is fourfold lower than at 18^oC (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 12A.) or to differences between measurements (Fig12A, 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.

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 (see fig 3 and 6). 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 (Tables 2 to 4). One of the reasons for this lower number could be the influence of CO_2 on fermentation rates. In figure 3b this influence can be observed: at 0 kPa O_2 the CO_2 production (which can entirely be attributed to fermentation) is lower when the CO_2 is increased. When modelling results for Vmf_{CO2} throughout the storage season are compared with maximum fermentative CO_2 production rates actually found, also a difference is noticed between pears measured at 0 or at 5 kPa (figure 8d). If the CO_2 influence on fermentation 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 12C). 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 12D). 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.

Conclusions
Methodology for gas exchange and diffusion resistance measurements was developed and can be used by all partners. Using the methods this year, 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.
A remarkable fast adaptation of pear metabolism to other gas atmospheres was noticed. Once CO_2 is reduced, metabolic rates are comparable to pears stored under low CO_2 for the whole period (graph 6b). Calculated internal gas concentrations show relative small differences caused by harvest date (figure 2) and storage period (figure 11). However, unclear at the moment is the direct relationship between CO2 concentrations and the onset of disorders (for instance the reduction in vitamin C content). Therefore the impact of small differences in CO_2 on pear tissue remains to be discovered.

Tables and Figures

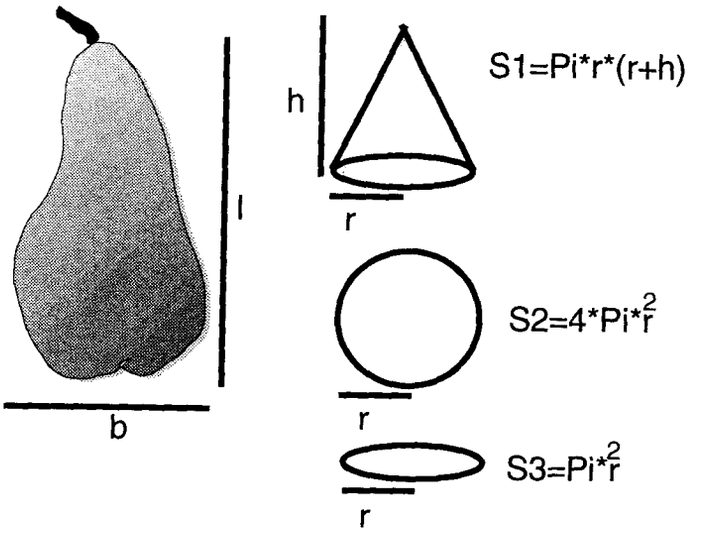
Tables 2 to 4 give an overview for task 6 of the statistical results of fitting gas exchange models to the data obtained, using Genstat nonlinear regression analysis. Statistical parameters: Fit = fitted value, se = standard error, R^2 = percentage of explained variance, ** = could not be estimated. Model parameters: Vm_{O2} = maximum O_2 uptake rate (nmol/kg.s), Km_{O2} = Michaelis-Menten constant for the influence of O2 on O2 uptake (kPa), KmCO2 = Michaelis-Menten constant for the influence of CO2 on O_2 uptake (kPa), Vmf_{CO2} = maximum fermentative CO_2 production rate (nmol/kg.s), Kmf_{O2} = Michaelis-Menten constant for the influence of O_2 on fermentative CO_2 production (kPa), RQox = RQ for oxidative processes, Resist = resistance for diffusion of neon (s/mm).

Table 2. Results of the 'harvest date' experiment (day3 and day 4 = measured after 3 or 4 days under altered gas conditions).

	Harvest date							
	3 September		10 September		17 September		30 September	
	day 3	day 4	day 3	Day 4	day 3	day 4	day 3	day 4
R^2	95.0	95.3	94.5	94.7	95.5	94.3	96.8	96.1
Vm_{O2}	72.9	63.9	79.4	79.1	78.2	64.9	126	178
Km_{O2}	1.67	1.60	2.54	2.54	2.50	2.26	2.45	3.96
Km_{CO2}	51.9	128	164	**	**	**	107	14.7
R^2	74.1	80.9	93.9	86.7	90.4	93.9	90.0	93.3
Vmf_{CO2}	82.5	79.5	81.1	76.6	80.3	68.6	91.0	45.2
Kmf_{O2}	0.193	0.190	0.131	0.200	0.136	0.123	0.136	0.226
RQox	0.808	0.837	0.828	0.859	0.788	0.785	0.636	0.666
Resist	858		823		908		887	

Table 3. Results of the 'delayed ULO' experiment.

	Harvest	Cooling	ULO
R^2	74.8	92.1	94.3
Vm_{O2}	19.2	24.5	20.4
Km_{O2}	1.37	0.90	1.80
Km_{CO2}	16.9	14.4	10.9
R^2	50.5	74.9	77.4
Vmf_{CO2}	11.0	25.0	9.85
Kmf_{O2}	0.39	0.07	0.14
RQox	0.89	0.86	0.88



Calulation of pear surface

$= S1 + 0.5*S2 - S3$

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

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

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

Figure 1: Methodology for estimating pear surface

Table 4a. Results of the 'storage' experiment. Pears were stored in 2 kPa O_2 with 0.1 kPa CO_2.

	Storage period (days)					
	0	42	86	126	177	233
R^2	86.2	83.8	91.6	82.6	89.0	70.5
Vm_{O2}	20.8	18.1	21.7	20.8	19.2	15.0
Km_{O2}	0.90	0.83	0.68	1.20	0.35	0.69
Km_{CO2}	11.6	11.9	8.95	11.4	10.0	20.0
R^2	80.1	25.1	78.0	**	82.1	34.0
Vmf_{CO2}	27.0	12.4	11.6	18.6	19.3	25.3
Kmf_{O2}	0.049	0.250	0.099	0.373	0.062	0.024
RQox	0.885	0.855	0.864	0.842	0.898	0.848
Resist	929	827	**	873	**	1011

Table 4b. Results of the 'storage' experiment. Pears were stored in 2 kPa O_2 with 5.0 kPa CO_2.

	Storage period (days)					
	0	42	86	126	177	233
R^2	86.2	77.7	91.1	78.9	88.0	79.5
Vm_{O2}	20.8	15.5	22.4	16.3	17.0	17.9
Km_{O2}	0.90	0.62	0.75	0.54	0.42	0.80
Km_{CO2}	11.6	16.2	10.0	21.2	11.2	9.8
R^2	80.1	24.8	85.9	**	84.9	33.1
Vmf_{CO2}	27.0	16.1	16.1	19.9	15.1	15.8
Kmf_{O2}	0.049	0.072	0.034	0.072	0.049	0.168
RQox	0.885	0.925	0.860	0.94	0.882	0.712
Resist	929	851	**	789	**	940

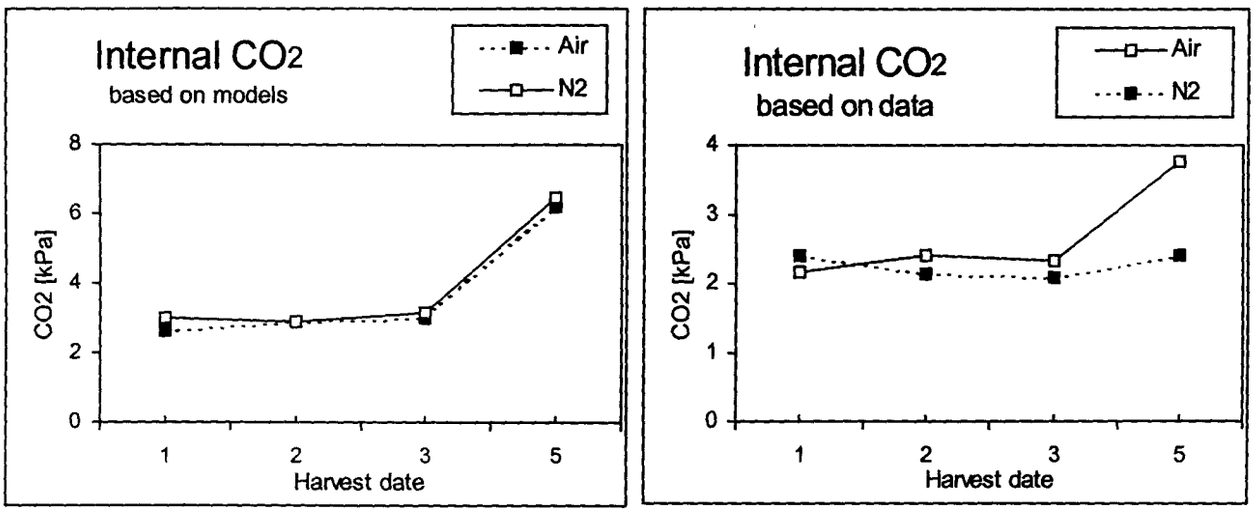


Figure 2. Calculated internal CO_2-pressures (kPa) per harvest date. A: based on model parameters (Vm_{O2} and Vmf_{CO2}). B: based on maximum O_2 uptake and fermentative CO_2 production rates data.

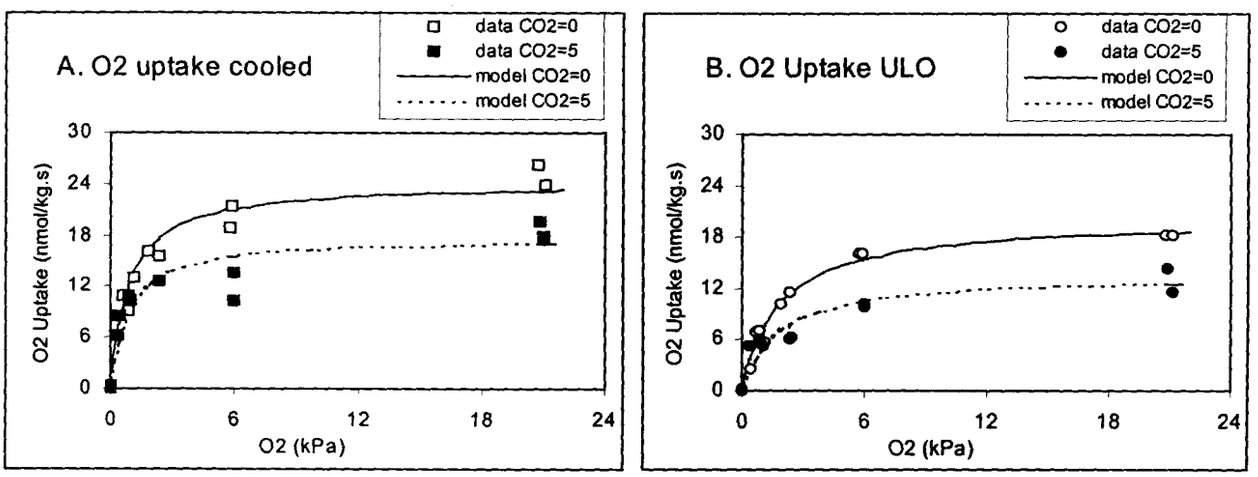


Figure 3. The O_2- uptake (nmoles/kg.s) of pears stored for 5 weeks in ambient air,'Cooled', (figure A and B) and at ULO (figure C and D). Gas exchange was measured 5 days after subjecting the pears to the various oxygen and carbon dioxide conditions.

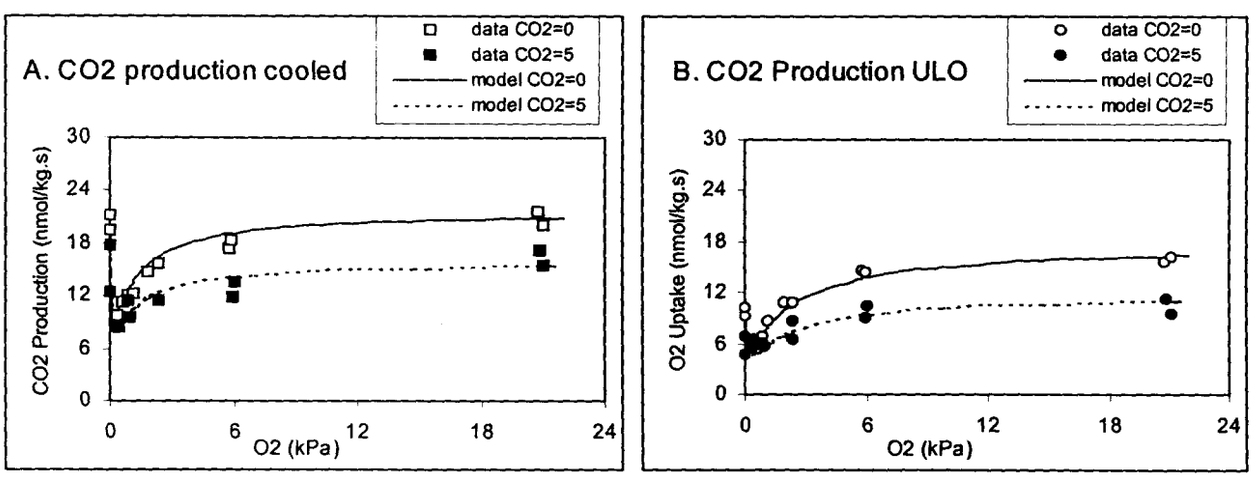


Figure 4. The CO_2- production (nmoles/kg.s) of pears stored for 5 weeks in ambient air,'Cooled', (figure A and B) and at ULO (figure C and D). Gas exchange was measured 5 days after subjecting the pears to the various oxygen and carbon dioxide conditions.

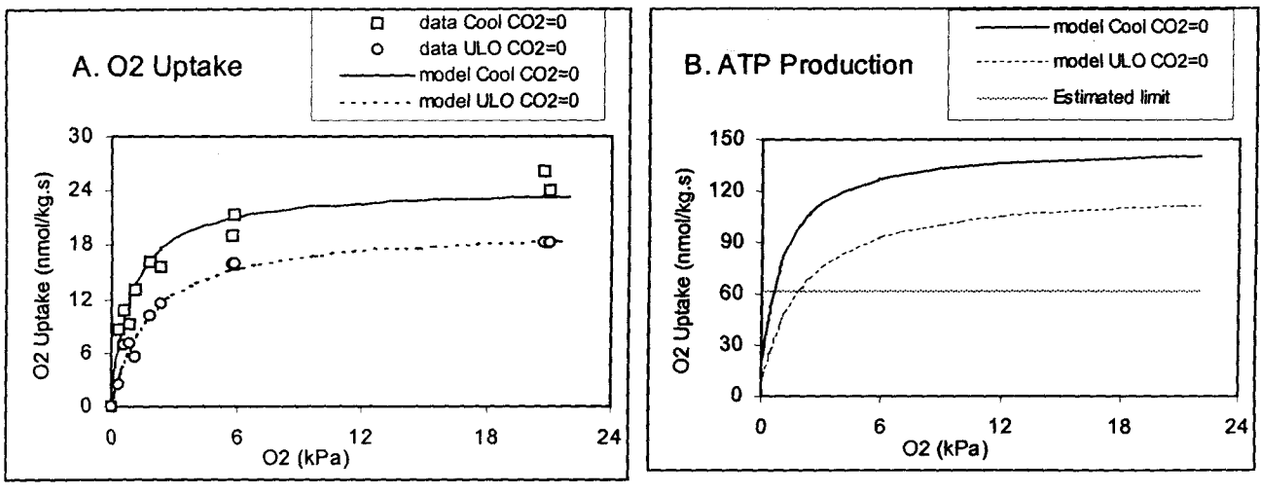


Figure 5. A comparison between pears stored for 5 weeks in ambient air or ULO. A: O_2 uptake. B: Calculated ATP production (nmoles/kg.s).

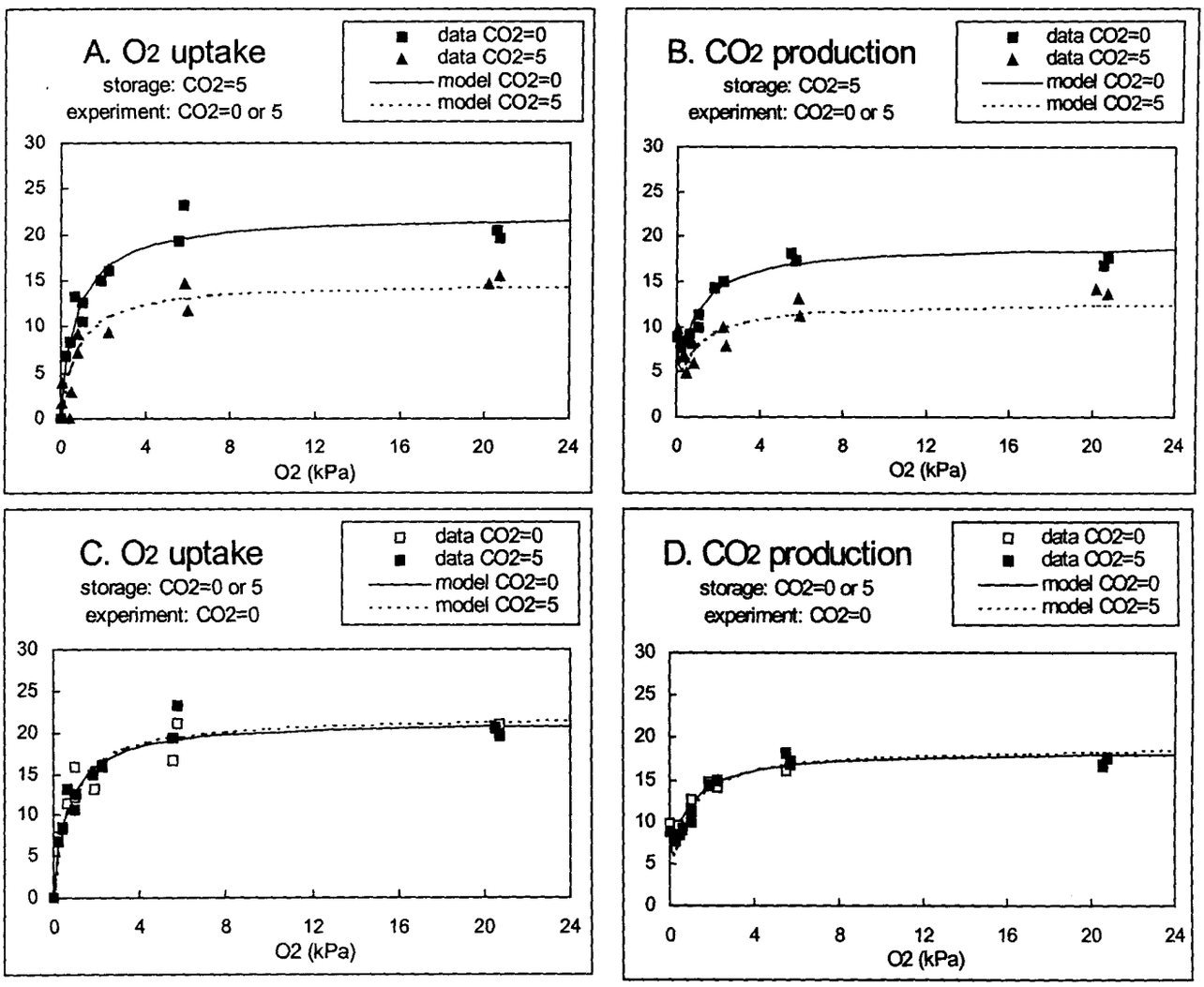


Figure 6. The comparison of the O_2- uptake (figures A and C) and CO_2- production (figures B and D) of pears after 5 months of storage at two conditions: CA (2 kPa O_2 with 0 kPa CO_2) or so called high CO_2 (2 kPa O_2 with 5 kPa CO_2). Figures A and B reveal that even after 5 months of storage 5 kPa still had a strong effect on gas exchange. Figures C and D reveal that no difference in gas exchange could be found between pears stored at o or 5 kPa for 5 months.

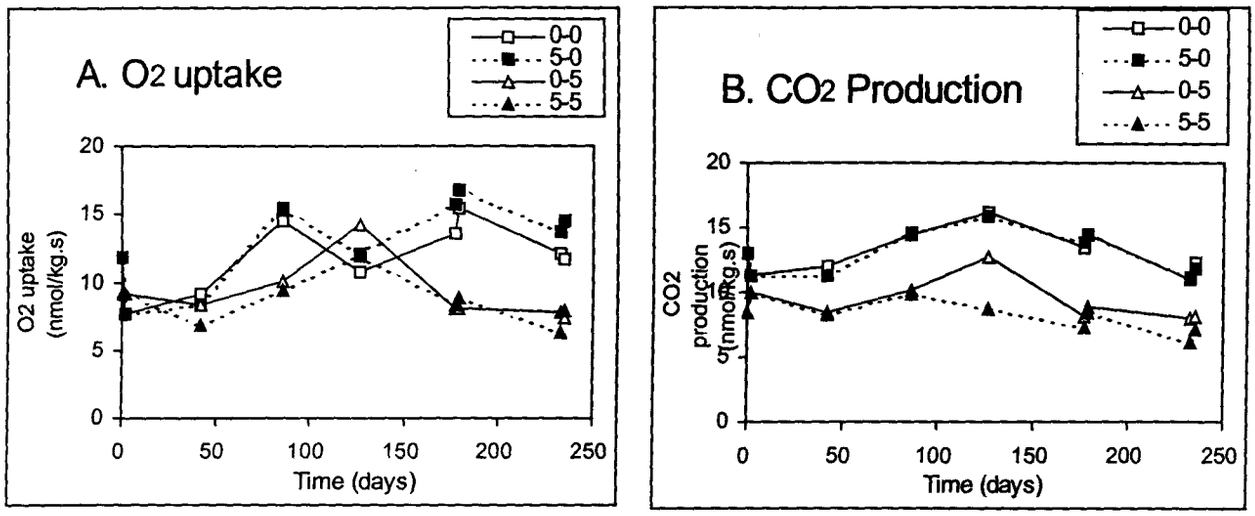


Figure 7. O_2 uptake (7A) and CO_2 production rates during the storage period. Measurements at 2 kPa O2 are shown in combination with 0 or 5 kPa of CO_2. Explanation: 0-0 = Storage at 0 kPa of O_2 and measured at 0 kPa of CO_2, 5-0 = Storage at 0 kPa of O_2 and measured at 5 kPa of CO_2, etc.

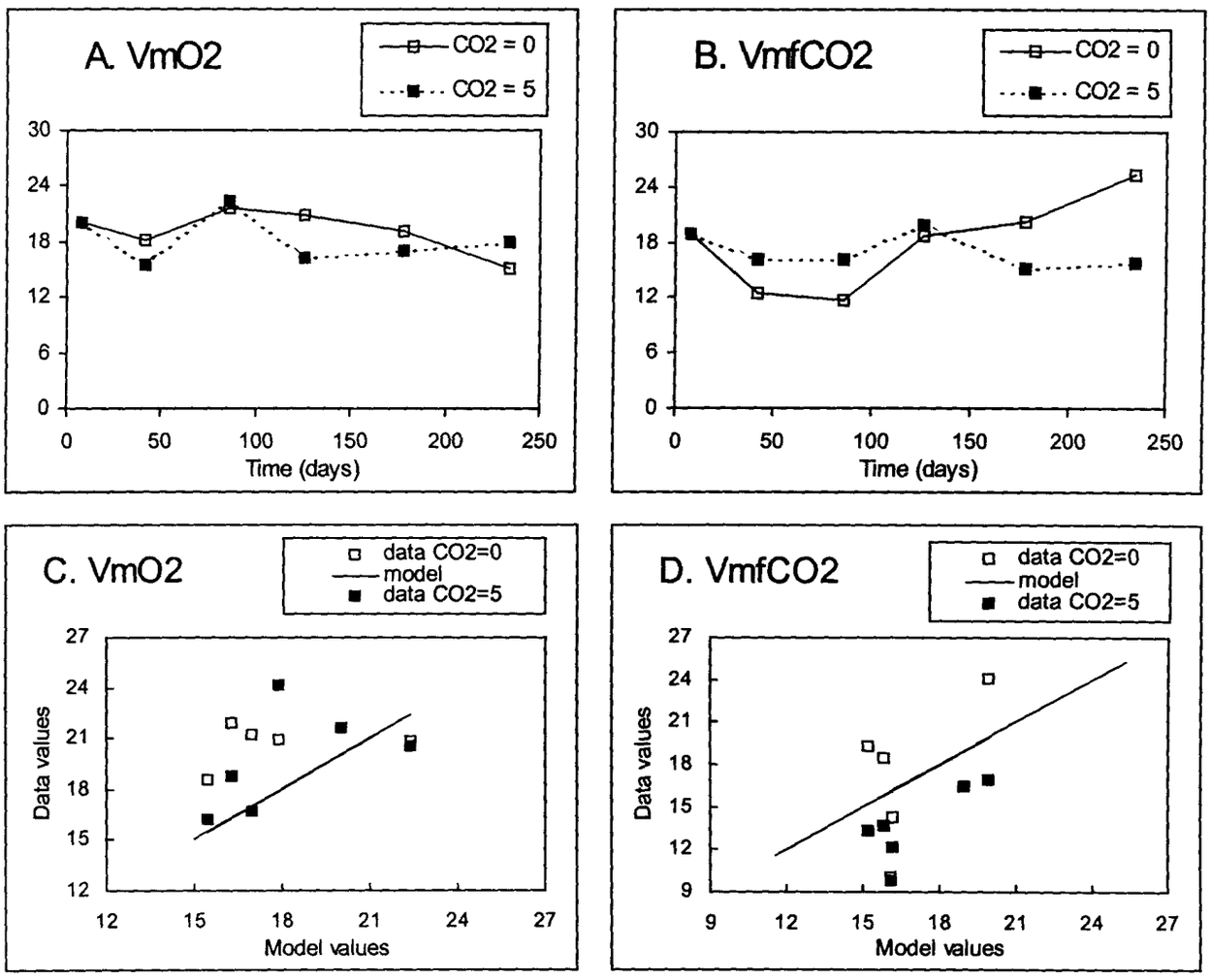


Figure 8. Changes in model parameters during the storage period. A: Vm_{O2}. B: Vmf_{CO2}. In figures C and D a comparison is made between fitted values and values actually measured throughout the storage season.

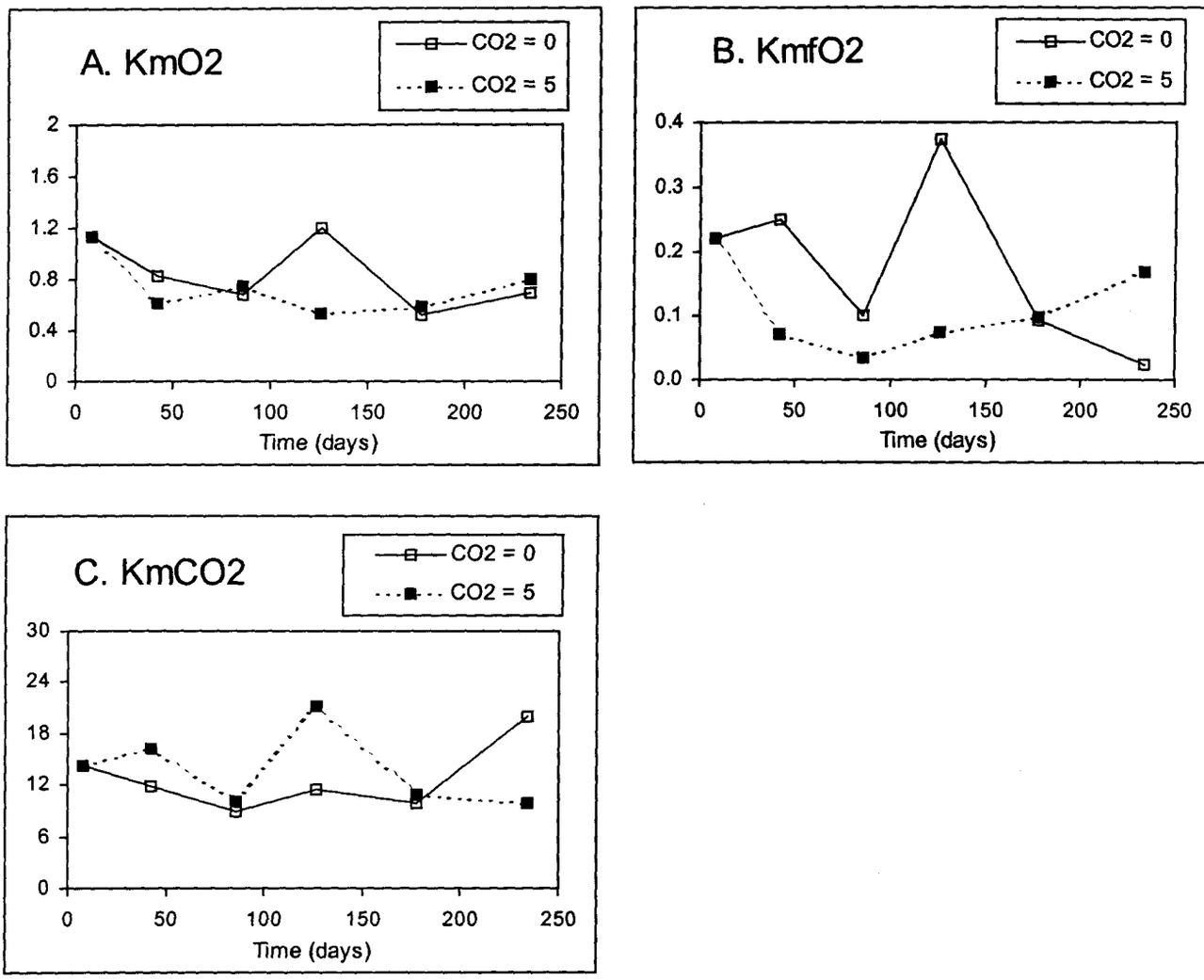


Figure 9. Changes in model parameters during the storage period. A: Km_{O2}. B: kmf_{O2}. C. Km_{CO2}.

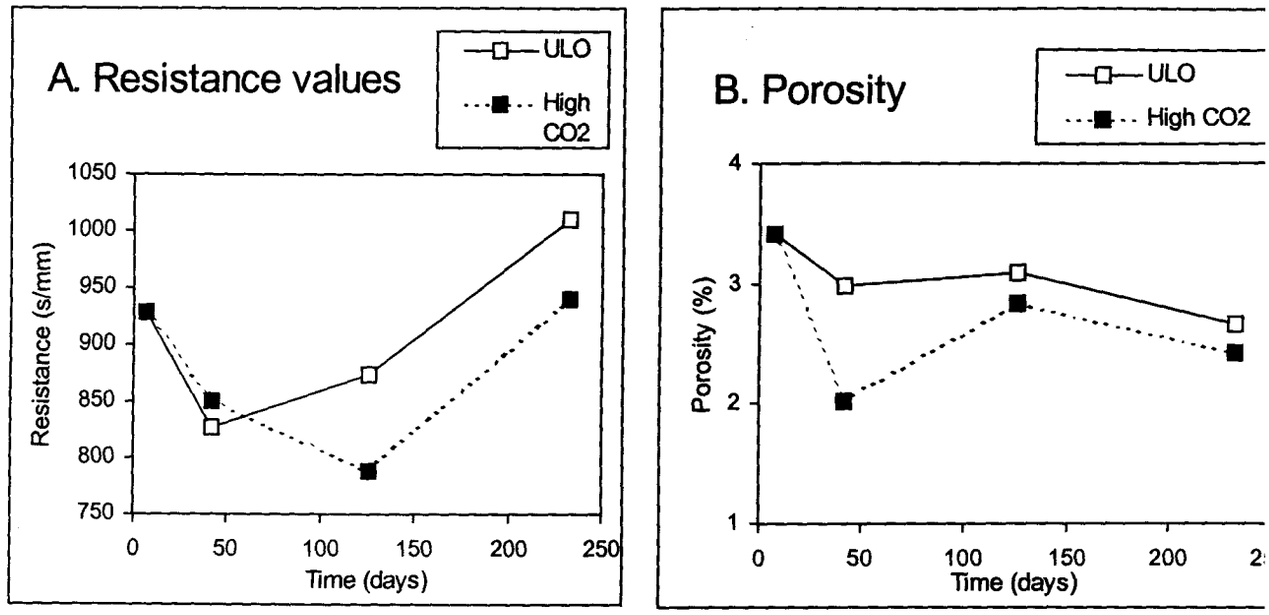


Figure 10. Changes in resistance to gas diffusion (A) and porosity (B) of pears during the storage period. Differences between pears stored at 0 kPa CO_2 ('ULO') or 5 kPa CO_2 ('high CO_2') are shown.

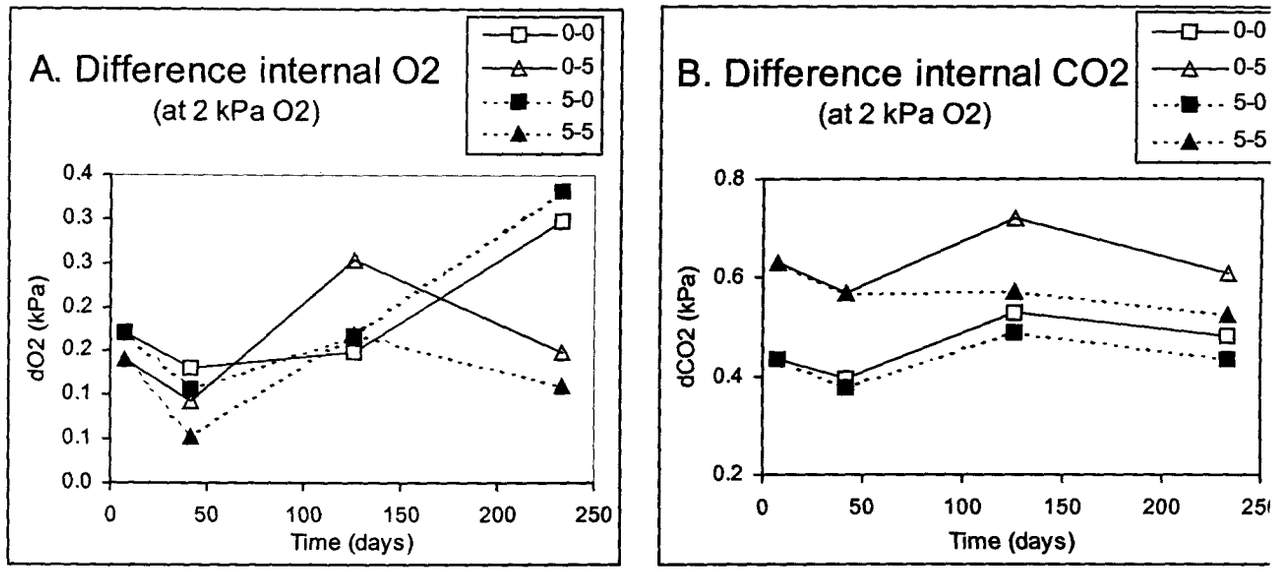


Figure 11. Changes in internal gas composition of pears during the storage period. Measurements at 2 kPa O2 are shown in combination with 0 or 5 kPa of CO_2. Explanation: 0-0 = Storage at 0 kPa of O_2 and measured at 0 kPa of CO_2, 5-0 = Storage at 0 kPa of O_2 and measured at 5 kPa of CO_2, etc. A: O_2. B: CO_2.

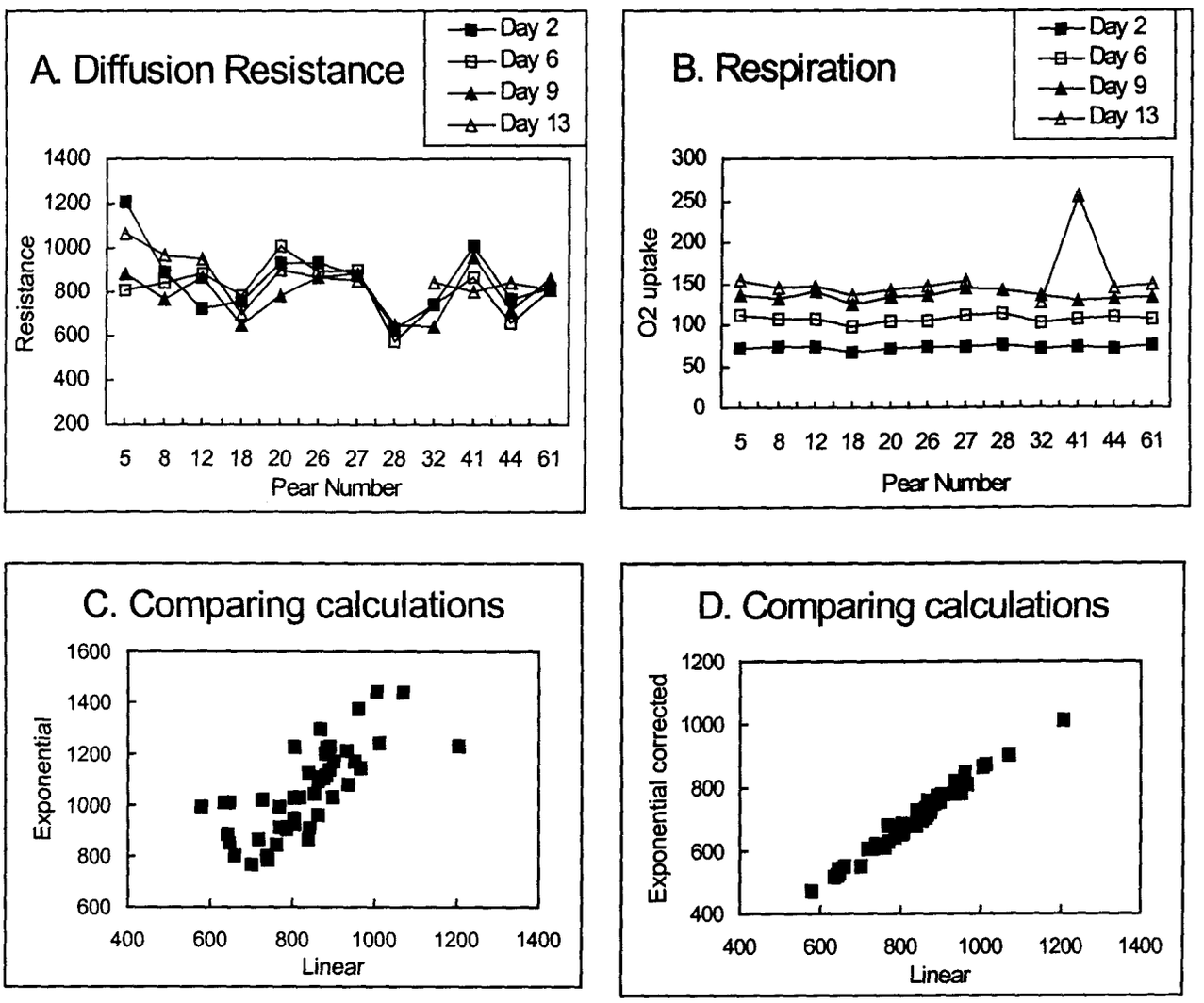


Figure 12. A. Variation in diffusion resistance (s/mm) caused by differences between individual pears and by ripening stage. A: Diffusion resistance (s/mm). B: Variation in O2 uptake (nmol/kg.s) caused by differences between individual pears and by ripening stage. 12 C and D: Comparing methods for calculating resistance. C. Results of the exponential analysis without a correction for the intern volume, D. Results of the exponential analysis with a correction for the intern volume. Both C and D are plotted against the results of the linear analysis.

Task 7: Destructive measurements

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	29
No. of man-month devoted already to the task:	9

Objectives

The objective of this task is to clarify the biochemical processes involved in the development of brown heart in pears. The research of ATO in the first year mainly focused on PPO-activity and the vitamin C content in pears stored at different gas conditions.

The hypothesis is that browning in pear tissue is induced by decompartmentation of intracellular membrane structures. Due to leakage of internal membranes, PPO's are combined with their substrate, resulting in browning of the tissue. Membrane damage can probably 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 á-tocopherol (vitamin E), ascorbate (vitamin C) and glutathion (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 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). Secondly, ascorbate is directly involved in the removal of alkoxyl (LO.) and peroxyl (LOO.) radicals (Chan, 1993). Thirdly, ascorbate is able to convert enzymatically formed *o*-quinones back to their precursor phenols (Nicolas *et al.*, 1994).

Material and methods

After 4 months storage samples were taken for PPO and polyphenol measurements. For determinations on total tyrosinase, pears (The Netherlands) were stored in a flow-through system (Peppelenbos and Jeksrud, 1998). Pears were stored under 2 kPa O_2 in combination with 0 kPa or 10 kPa CO_2. After 25 days of storage, samples were taken. Pears in Spain were placed in experimental chambers during 4 months at -1 C, 2 kPa ± 0.1 O_2 and 3 kPa ± 0.1 CO_2. Relative humidity was kept at 95%.

Image analysis system.

Thin slices (about 0.4 cm) were cut from the pear longitudinally with a double-knife cutter. From this profile the total area and the percentage brown area was measured immediately after cutting by computer image analysis (CIA).Measurements took place in a specially drafted CIA-box. Reflected light of 4 TL-bars (20 watt, colour 84) illuminate the object via a diffusor. A feed-back system stabilizes the light stream (with a photocell). The object was recorded with a colour KY-F30 3-chip CCD JVC camera, which was connected to an image operating chart. In this chart the video signal is digitalized and further analysed with the software. The software was developed by ATO-DLO (Schouten *et. al*, 1997). During visual judgement brown pears were classified in four classes: no browning (0), slight browning (1), moderate browning (2) and severe browning (3). In this paper classes 1 to 3 were taken together.

PPO assay using L-DOPA as a substrate.

Freeze-dried samples were ground in a mortar under liquid nitrogen and stored at -20 C. Pulverized samples were mixed with 100 mM NaPi buffer, pH 6.5. The samples were centrifuged and mixed with oxygen saturated buffer with L-DOPA (final concentration 16.7 mM). The increase in absorption was determined with a Perkin Elmer spectrophotometer at 478 nm using an extinction coefficient of 3313 $M^{-1} \cdot cm^{-1}$ (Wichers *et al.*, 1984) for the oxidation product. For measurement of total PPO 0.11% v/v SDS was added. For measurement of laccase 0.5 mM tropolone, a specific inhibitor for tyrosinase, was added (Kahn and Andrawis, 1985; Wichers *et al.*, 1984; Gerritsen *et al.*, 1994).

Analysis of variance (Genstat) was used to determine correlations between parameters, like harvest date, growing location, enzyme activities, polyphenol content, browning, carbon dioxide concentration during storage and size of the pears.

PPO assay using 4-methyl catechol as a substrate.

PPO was extracted from pear tissue by the acetone extraction method of Sciancalepore and Longone (1984). The acetone powder was stored in a desiccator until extraction. The extract was prepared by suspending 1 gram of acetone powder in 30 ml phosphate buffer (0.1 M, pH 7.0) with 0.5 mM cysteine. The extract was homogenised, filtered through two layers of miracloth and centrifugated at 40,000 G for 20 minutes at 4 C. The supernatant was loaded onto a Sephadex G-25 column (PD 10, Pharmacia), which was previously equilibrated with 20 ml phosphate buffer (pH 6.5). PPO was eluted with 3.5 ml of the same buffer. Activity was determined spectrophotometrically according to Sciancalepore and Longone (1984). The reaction mixture consisted of 1.9 ml citrate buffer (0.1 M, pH 5.3), 1.0 ml 4-methylcatechol (final concentration 0.02 M) in citrate buffer, and 0.1 ml of enzyme extract. The reaction was monitored for 3 min at 420 nm (30°C). The initial velocity was calculated from the slope of the linear part of the curve obtained.

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 homogenised in water. Cell remainders were removed by centrifugation (15-30 min., 11,000g). The homogenate (0.5 ml) was mixed with 0.5 ml 10% TCA to remove proteins. After vortexing and centrifugation (15-30 min., 11,000g) 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 min. the samples were centrifugated again and the extinction was measured in triplicate at 650 nm. Calibration was done with 0.0-1.0 mM tyrosine.

Ascorbic acid measurements

A Waters liquid chromatograph model 510 with a pharmacia LKB VWM 142 uv-vis detector (251 nm). A Waters Symmetry C-18, 3.9 x 150 mm column, particle size 5 ìm, with a Sentry Guard Column C-18 (Waters), was employed. Measurements were performed at 25 C. Mobile phase: 2.5 gram tetrabutylammoniumhydrogensulfate (z.s. Merck 818858) and 55 ml methanol (p.a. Merck 6009) dissolved in 942.5 grams Milli-Q water (Millipore) (Keijbets and Ebbenhorst-Seller, 1990). Before use the eluent was filtrated and degassed with a 0.45 ìm Millipore filter (HVLP 04700) Flow rate during measurements was kept 1 ml/min. Analyses were completed within 5.5 minutes including a post column elution time of about 1 minutes. As a standard 62.5 mg ascorbic acid (Sigma) was dissolved in 100 ml Milli-Q water (stock I). 1 ml of stock I was diluted in a 50 ml measuring glass with 5 ml 9.5% oxalic acid and 5 ml methanol (comparable with the extraction procedure)(stock II). A dilution series from stock II was stored on ice and kept in the dark before injection. Stocks were prepared freshly every day, although stock II is stable for more than one day when stored in the fridge. Fruits were peeled and the flesh was divided in a core and cortex sample. Both samples were divided into little cubes and immediately frozen in liquid nitrogen. The samples were crushed in a kitchen mixer. All the following steps were done in the refrigerator or on ice with the working place as dark as possible. Ten grams of sample were diluted with 5 ml 9.5% oxalic acid (Merck 100495), 5 ml methanol (p.a.) and 30 ml Milli-Q water. The mixture was homogenised with an ultra turrax mixer and filtered through fluted paper (Schleicher & Schull 595½). The filtrate was passed through a unit consisting of a 0.45 um sterile filter and a Sep-pak C18 column (Waters), and was directly injected in a manual-injector system with a 20 ul loop. HPLC measurements were done directly after the extraction procedure. Measurements took place at 251 nm. Obtained results were analysed by means of the Millenium HPLC manager (Waters).

Results and Discussion

Occurrence of brown heart

Pears from orchard 1, both stored at 0.5 and 3.0 kPa carbon dioxide, are more susceptible to brown heart than pears from orchard 2 (data not shown). This trend was observed for several years, and is confirmed in figure 13. Pears from later harvest dates are more susceptible than pears from early harvest dates, and 3 kPa carbon dioxide clearly induces brown heart during storage (figure 13). The initiation of brown heart was monitored while pears were stored at enhanced CO_2 atmospheres (3 kPa) and an increased temperatures (5 °C). Brown heart was already detected after five weeks in pears stored at these conditions (data not shown).

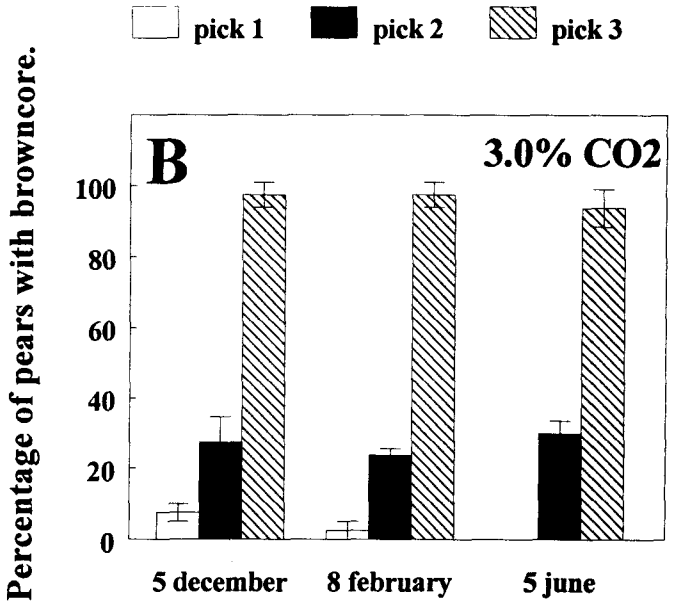


Fig.13. Influence of harvest date on the development of brown heart in Conference pears grown in orchard 1. Fruits were monitored at 5 December 1995, 8 February and 5 June 1996. Pears were grown in The Netherlands and stored at 3.0 kPa CO_2. Bars are the mean of four measurements (n=20) ± SE.

PPO, total polyphenol content and development of brown heart

Brown heart develops through the action of PPO (Scott and Wills, 1974). PPO catalyzes the formation of quinones, which polymerise into melanin products. We determined PPO activities in pears from two growing locations (orchard 1 and 2) and three harvest dates (pick 1, 2 and 3) stored under 0.5 and 3.0 kPa CO_2. After 4 months storage, tyrosinase (latent and active form) and laccase activities were determined. The same samples were used to determine the total polyphenol content. PPO activity did not, as expected, depend of gas conditions during storage, but we did find a correlation with browning of the cortex tissue of pears. Generally, all the measuring results taken together (different conditions, harvest dates and growing locations), the percentage of active tyrosinase (the quotient of active tyrosinase and total tyrosinase) is 4.2% on average, with a high SD of 4.2 (n=85). Tyrosinase activity appears to be inversely related to brown heart appearance, which is shown in figure 14A and 14B.

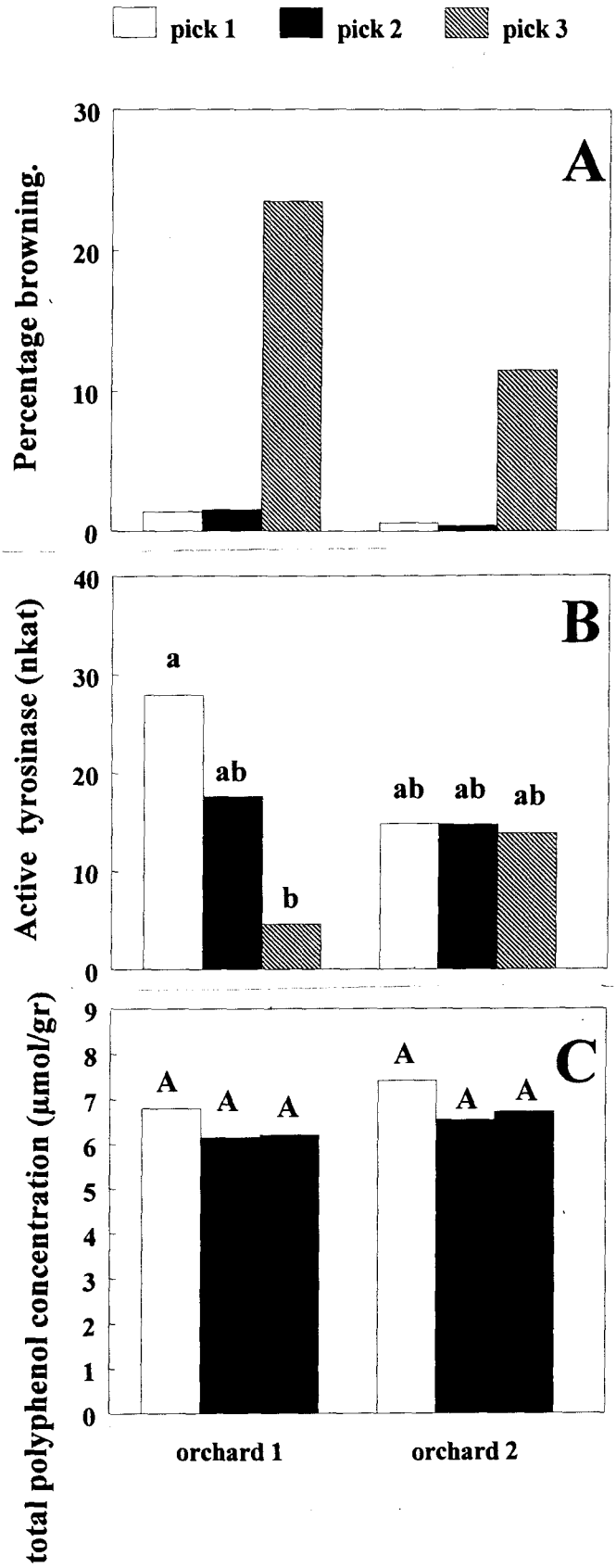


Fig. 14. Pears were stored at 2% oxygen and 0.5 or 3.0% carbon dioxide. Pears stored under both conditions were taken together in these figures. Samples of cortex tissue were taken after 4 months. Graph A shows the judgement of pears for brown heart. Browning was determined with a imaging system that calculates the area percentage of brown tissue in the lengthwise cut pear. Graph B: relation between tyrosinase activity, growing location (orchard 1 and 2) and harvest date (pick 1, 2 and 3). All activities are expressed in nkat. Difference between the first and the third bar of the grower-1-cluster is significant ($p<0.05$). Graph C: relation between total polyphenol content, growing location (orchard 1 and 2) and harvest date (pick 1, 2 and 3). Concentrations are expressed in ìmol/gr dry weight. Values do not differ significantly ($p<0.05$).

Pears from the third harvest date of orchard 1 show the severest incidences of browning (figure 14A). In figure 14B it is shown that these pears exhibit the lowest tyrosinase activity. A correlation between laccase activity and browning was not clear. Laccase activities are very low in pear tissue. No relation was found between polyphenol content and extent of browning, although shifts in concentrations of individual polyphenols can not be excluded on basis of these results. According to the results presented in figure 14C and other data, total polyphenol content is not related to browning of the cortex tissue, susceptibility for browning or the degree of browning. PPO activity or total polyphenol content are not related to the size of pears. Tyrosinase activity seems to be related to browning, not to gas conditions. In parallel, PPO activity was determined in Spanish pears (orchard 3), with 4-methyl-catechol as a substrate in stead of L-DOPA. Experiments were done to compare pears grown in different climates. Determination of catechol oxidase activity of PPO in Spanish, brown pears show the same results. PPO activity is decreased in brown pears.

Vitamin C in pears

In our 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 storage in the static system (2 kPa oxygen, carbon dioxide <0.5 kPa) the vitamin C content of pears decreases minimally in time (table 5). Atmospheric conditions during storage play a key role. Decreased oxygen concentrations (0.3 kPa) result in a 30% decrease of ascorbate in the cortex tissue and a 24% decrease in the core. Adding 10 kPa carbon dioxide decreases the ascorbate content with 60% compared to standard conditions (figure 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

Table 5. Vitamin C content of pears stored under normal conditions: 2 kPa oxygen, 0.5 kPa carbon dioxide (-1 C). Pears were from orchard 1 (second pick). Average values are given with standard deviations. January: n=8; November n=15.

Figure 15 shows the ascorbate levels in mixed samples: samples of five individual pears were put together. This method masks individual variations in vitamin C levels of the pears. Another point of criticism is that figure 14 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 16).

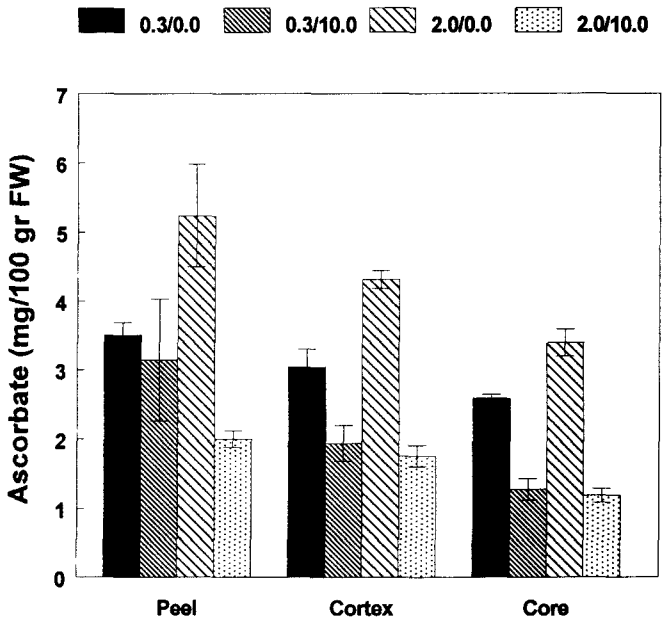


Figure 15. Ascorbic acid content of pears after 60 days storage under different conditions in the flow through system (5 C). Pears were from orchard 1 and harvested at the optimal picking date (2). Values are the mean of a duplicate experiment of mixed samples of 5 pears (n=2) with standard errors. Gas conditions within a cluster: 0.3 kPa oxygen, no carbon dioxide; 0.3 kPa oxygen, 10 kPa carbon dioxide, 2 kPa oxygen, no carbon dioxide (standard condition); 2 kPa oxygen, 10 kPa carbon dioxide. Clusters represent the peel, cortex and core of the fruit respectively.

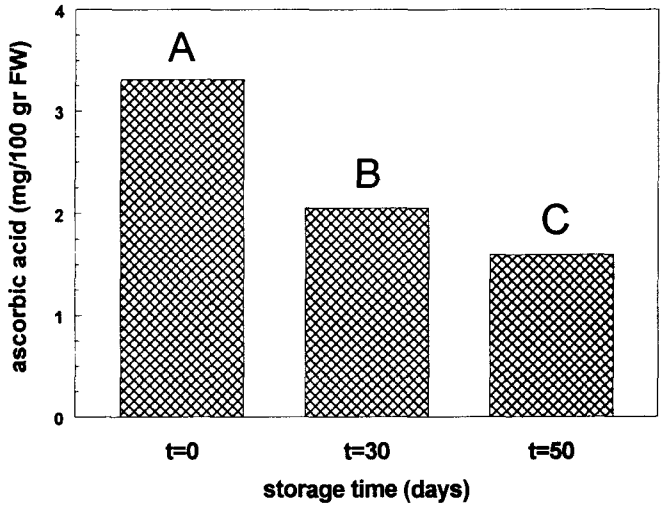


Figure 16. Ascorbic acid in pears during storage at 2 kPa O_2 and 10 kPa CO_2 in the flow-through system (5 C). Pears were from orchard 1 (optimal harvest date). Ascorbic acid content of individual pears was determined at t=0 (n=16), 30 days (n=20) and 50 days (n=20). Bars are the mean values of a duplicate experiment. Presented values differ significantly (p 0.05).

Ripening

One explanation for the differences in ascorbic acid could be the stage of ripening. Enhanced carbon dioxide concentrations inhibit ethylene production and as a consequence ripening of the fruit. According to Trautner and Somogyi (1978) ascorbic acid concentrations in pears depend on stage of ripening. Figure 17 shows the results of an experiment in which ripening has been monitored. The ascorbic acid content of pears of progressive ripening stage was correlated to different ripening parameters like firmness, respiration and ethylene production.

Pears from grower 2 were stored at 10 C. Oxygen concentration in the container was established at 21 kPa to induce ripening. Ascorbic acid levels of pears in combination with different ripening parameters were monitored in individual fruits for 15 days. During ripening at 10 C the firmness of the pears drops dramatically (figure 17A). Ethylene production and respiration values increase, as expected, during progression of ripening (figure 17B). In contradiction to the results of Trautner and Somogyi (1978) no clear decrease in ascorbic acid levels could be monitored. Ascorbic acid values of day 11 and 15 in figure 17A differ significantly from the values at day 0 (p 0.01). Therefore we conclude that differences in ascorbic acid concentrations in figure 15 cannot not be attributed to the stage of ripening.

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 sterilised 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 kPa oxygen and 10 kPa 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 destruction of fruit flesh. This tissue was distinguished from enzymatically 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 18 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.

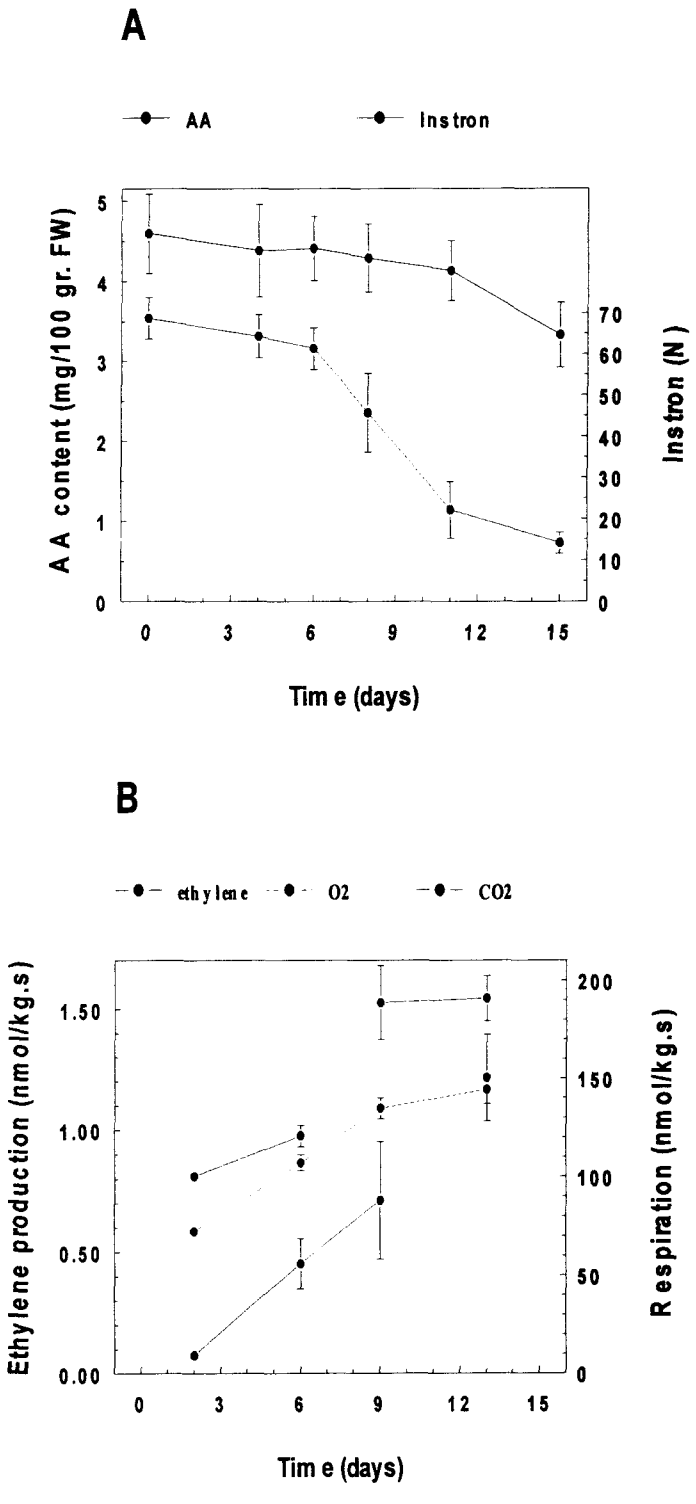


Figure 17. Ripening of Conference pears. Fruits were stored at 10 C and 21 kPa oxygen in the flow-through system. Relative humidity was kept 97-99%. During ripening different ripening parameters, respiration rate and ascorbic acid content were monitored. Graph A: (____) ascorbic acid content in mg/ 100 gr fresh weight (n=20) and (_ _ _) firmness (Instron) values in Newtons (n=20). Graph B: (_____) ethylene production in nmol/kg.s (n=12), (_ _ _) carbon dioxide production in nmol/kg.s (n=12), (. . . .) oxygen uptake in nmol/kg.s (n=12). All measurements were done on individual pears.

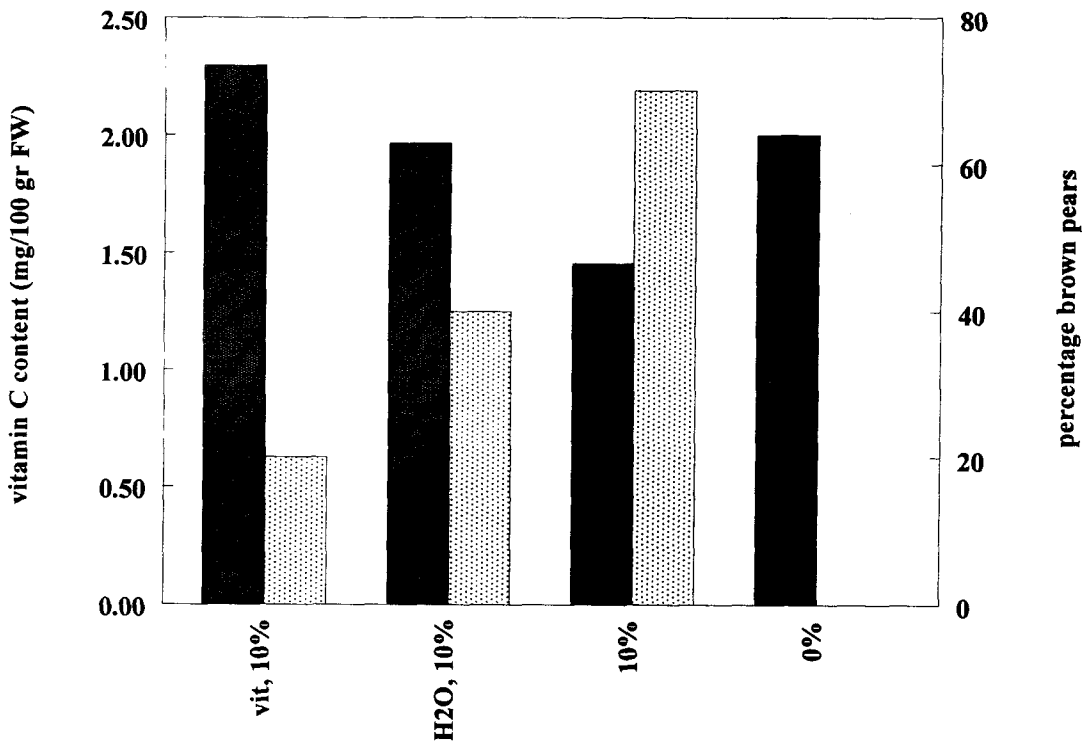


Figure 18. Pilot experiment. Pears were vacuum infiltrated with a 1% w/w vitamin C solution ('VitC') and water ('H2O'). After treatment pears were stored at 2 kPa oxygen and 10 kPa carbon dioxide in the flow through system. Pears stored without carbon dioxide served as a blank (0 kPa). After 4 weeks 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.

Conclusions

Browning in pears is induced by polyphenol oxidase. In stead of what was expected we did not find a relation between susceptibility for browning and polyphenol oxidase activity in pears. No significant differences in polyphenol oxidase activity could be recorded in pears from different harvest date, growing location or storage condition. Therefore another factor is responsible for the susceptibility for browning. We hypothesise that browning is induced by decompartmentation of internal membrane structures. After decompartmentation, polyphenols located in the vacuole can react with polyphenol oxidase, resulting in the browning reaction. Decompartmentation is likely a result of senescence. Damage to internal membrane structured can be a result of radical formation. Antioxidants play an important role in avoiding damage due to radicals. An abundant antioxidant in pears is ascorbic acid. Ascorbic acid levels in pears tissue are influenced by external and internal gas conditions. Possibly ascorbic acid can act as an indicator for damage. Preliminary data support this idea.

Task 9: Modelling

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	14
No. of man-month devoted already to the task:	1.0

Objectives

In this task the data and information of the other tasks are combined and integrated. First statistical analysis on the original data will be performed by all partners (guidelines were given). Modelling will take place on the analysed data. Two models will be constructed. The first model is a correlative model based on the relationship between climate, nutrition and Brown Heart data. The second model uses physiological processes like metabolic rates and energy fluxes. This model will be based on existing models that use enzyme kinetics (both oxidative and fermentative pathways) and diffusion characteristics. The model will calculate and predict the flow of O_2, CO_2 and ATP.

Material and Methods

The first model will be build using neural network techniques, in order to identify and clarify relationships. Only one output is needed; the probability of the Brown Heart disorder. To build the model, many independent data-sets have to be generated. The number of 404 data-sets was mentioned in the technical annex, and it was investigated whether this number could be increased. Partner 2 increased the number of harvest dates from 2 to 3, and partner 4 changed the number of orchards from 7 to 11, resulting in a total number of 488 (instead of 404 as was mentioned in the technical annex).

Table 6. Total number of independent data-sets.

Partner	Orchard	Subset	Harvest	Year	Total
2. IRTA	1	3	3	4	36
3. IVTPA	2	1	3	4	24
4. PFW	11	1	5	4	220
5. VBT	3	4	3	4	144
6. UHOH	2	3	3	4	64

PROJECT TOTAL					488

The second model is based on descriptions of actual physical and biochemical processes (respiration rates, diffusion resistances). Recently the most important elements of the model, describing respiration and fermentation rates in relation to various gas conditions, were developed (Peppelenbos and van 't Leven, 1996, Peppelenbos et al., 1996). Both models are used to predict optimal storage conditions for pears. Very likely every year other optimal gas conditions to be used in Controlled Atmosphere facilities will be predicted.

Results and Discussion

Physiological model

The results from the gas exchange and biochemical measurements (tasks 6 and 7) are currently being analysed to test the central hypothesis of reduced energy metabolism being the main factor for causing tissue disorders. A prediction on maintenance needs will be generated and compared between harvest dates, storage periods but also between partners. Another important factor that will be compared using the gas exchange models is how internal CO_2 concentrations are affected by harvest dates, storage periods or growing location.

Climate model

Assessment of climate data in relation to brown-heart data is just beyond the starting phase. Datasets have been generated out of the climate and brown heart data from the different partners.

Inconsistencies and errors in these data sets have yet to be clarified. After this stage a modelling path will be initiated. The modelling path will consist of two parts. The first part (*fast first look*) will be an explorative application of the neural network technique to obtain quick results upon the predictability of brown-heart occurrence. Because this is the first year of the project, only a limited set of examples is available and therefore the leave-one-out validation technique will be used to get a statistical valid optimal architecture. First a neural network will be set up for all available data giving a first impression on the quality of the different variables towards the prediction of brown-heart occurrence. The smallest combination of variables that gives the highest prediction will then be used. Not all the data of those variables will be of equal use and the amount of data should be narrowed down. This part of the process of data reduction should be based upon expert knowledge from the partners. As an example, it could be that there is an opinion among partners that the number of days with a temperature higher than 18 °C in the last two months before picking is important in brown-heart occurrence. That means that only the temperature data concerning during the last two months before picking should be considered and that it is possible to use two temperatures classes: higher and lower than 18 °C. In this way, using and testing a number of hunches, the setup of the neural network should be adapted and a performance boost could be achieved in predicting the brown-heart occurrence. This means that a inquiry has to be setup to archive a number of opinions that live among partners which could have a significance effect on brown-heart occurrence.
Second part (*correct look*) of the modelling part will use a neural network only in a final stage of the modelling to lower noise in data and get and optimal prediction performance. The basis will be a number of statistical tools, such as P(rincipal) C(omponent) R(egression), P(artial) L(east squares) R(egression), B(ayesian)N(etworks) and Decision Trees, that will be tested to get information on the structure of the climate and brown-heart data. By means of these statistical techniques an allowable data reduction and problem separation can be achieved. An allowable problem separation is possible if the problems are more or less independent. Expert knowledge should help this process. The advantage of problem separation is that not *one* model will be formulated to deal with the prediction of brown-heart occurrence with *one* statistical technique with possibly suboptimal performance but several smaller problems that can be tackled with (possibly) a different statistical technique for each small problem in order to get an overall optimal prediction.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997TO 31-05-1998

During the first year there were some difficulties with the Gas Chromatograph used, resulting in missing values for the measurements on diffusion resistance (after 86 and 177 days of storage). We also suspect that part of the bad fits found between data-sets and CO_2-production models can be largely attributed to the problem mentioned. To date the GC has been repaired and is functioning properly.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

The plans as described in the technical annex will largely be followed. Changes are mainly expected for task 7, biochemical measurements, where some very interesting findings on vitamin C are worth to be further analysed. First monitoring vitamin C during storage at different gas conditions, and the consequences of changing conditions during the storage period. Important questions are: can adaptation of gas conditions avoid browning, and what happens to ascorbic acid levels during adaptation? More fundamentally, we are interested in the effect of ascorbic acid on PPO, and, reciprocally, the effect of PPO on ascorbic acid. For example, is PPO able to break down ascorbic acid? Secondly, we will focus our attention on energy metabolism. Especially fermentation and the effect of carbon dioxide at ULO conditions. Enhanced carbon dioxide concentrations during storage suppresses energy production. For measurements on fermentation products we will cooperate with the Department of Molecular and Laser Physics of the Catholic University Nijmegen. This group is able to detect fermentative metabolites in the gas atmosphere at very low concentrations (ppt-ppb range).
Work on the first version of the physiological model, focussed on relating internal gas concentrations to energy metabolism and the onset of disorders, will start in the next period. Results of this analysis will be used for advice on measurements in the coming growing season.

E. DISSEMINATION

Paper

Veltman R.H., van Schaik A.C.R., 1997. Membrane damage in fruits possibly explains brown heart. Fruitteelt, 12: 12-13 (in Dutch).

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Lecture

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"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

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

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Participant no.: 2

A. PARTNER INFORMATION

Name and address
Name of the institute: IRTA
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Scientific team
Names of the scientists participating in the project:
- C. Larrigaudiere - Group leader
- A. Herrero - Orchard factors
- M. Vendrell - Research scientist
- I. Recasens - Profesor Physiology
- T. Casero - Mineral analisis
- I. Lentheric - PhD student

Objectives
As in the technical annex

Actions in the project
IRTA (partner 2) is particularly involved in the following tasks:
- task 1: cultivation of pears
- task 2: harvest of pears
- task 3: storage of pears
- task 4: variation in climate and orchard characteristics
- task 5: post-harvest treatments
- task 7: a- quality evaluation
  b- biochemical studies
  c- mineral analysis
- task 9: model development
- task 10: validation of the model

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

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives
In Spain, Conference pears seems less sensitive to Brown Heart (BH) disorder. This difference is likely the reflect of differences in cultural practices and/or growing conditions (climate, soil ....). Involvement of partner 2 in these conditions was justified by the following objectives:
- feed the model
- complete the model using its specific conditions.

Material and methods
Partner 2, IRTA, used 2 orchards of varied susceptibility for B.H:
- orchard 1: "Gimenels", low susceptibility
- orchard 2: "Albatarrech", high susceptibility

Pears were grown according to the local recommendations.
Differences between the two orchards were mainly focussed on difference in N_2 fertilisation, orchard 1 representing a low fertilised orchard (controlled procedure: 70 U / ha . year) and orchard 2 a high fertilised orchard (uncontrolled procedure, excessive organic fertilisation, > to 200 U/ ha. year).

Results and Discussion
- Significant differences in susceptibility for B.H were observed for the two orchards (see fig.1).
- As shown in fig.2 to 4, the incidence in B.H was not directly related to changes in quality parameters. At the reverse, the fruits with presented more damage (orchard 2), retained a better quality during storage.

Conclusions
- At the view of these results, the N_2 fertilisation seems to be an important factor involved in B.H occurrence. The higher the dosis, the higher the damage. In consequence, emphasis will be given on this aspect during year 98-99 (see part D).
- There is not a direct relationship between the evolution of the quality parameters (firmness, SSC and acidity) during storage and B.H occurrence. As a consequence, any quality parameter could be used to predict B.H incidence.

Task 2: Harvest of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives
Picking date is strongly related to the risk of Brown Heart, the higher the damage, the later the picking date. In consequence, experiments were carried out for 3 different picking date and for the two orchards. Effects of harvest date on quality parameters and biochemical status were investigated.

Material and methods
Fruits were picked at 1 week-intervals, the 04 Aug., 12 Aug and 18 Aug. for H1 (harvest 1), H2 and H3 respectively. Maturity was determined according to standard procedures analysing the ground colour, titratable acidity, firmness and sugars content (SSC value).

Results and Discussion
According to our results, harvest date seems to be an important factor which control the following parameters:
- maturity indexes:
As expected and as shown in table 1, maturity indexes varied for the 3 different picking date. There is no significant differences in ground colour and firmness. In contrast, both SSC content and acidity changed with increasing maturity and the variation in SSC was the inverse to that of acidity (increase in SSC content and decrease in acidity).
- physiological disorders:
At the view of our results (fig.1) and as expected, the occurrence in physiological disorder was directly related to harvest date, the higher the damage, the later the harvest date.
- antioxidant defense mechanism:
In accordance with the hypothesis that storage-related disorders like B-H may involved an oxidative stress, we studied the differences in antioxidative potential for the 3 different harvest date. This potential was determined analysing the activity of the enzymes mainly involved in this process,

Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Ascorbate Peroxidase (APX). Significant differences were found between harvest date and were as follows:
* SOD activity sharply decrease in the more mature fruits (fig.5)
* There is a slighter but significant decrease in CATactivity in the more mature fruits (fig.5)
* In contrast, both aspecific peroxidase activity (POD) and ascorbate peroxidase activity increase with increasing maturity (fig.6).
These results showed significant differences in antioxidant defense potential between the different harvest date. These differences are mainly:
* less capability to scavenge active oxygen species when the fruits are picked more mature
* a differential capability to metabolise the peroxides and particularly H_2O_2 (decrease in CAT activity and increase in POD activity) between the different maturity stages. These differences will induce at least a transitory accumulation of H_2O_2 likely related to membrane damage and B.H occurrence in the more mature fruits.
* a sharp increase in APX activity which probably caused in the more mature fruits a decrease in ascorbate content and as a consequence less antioxidant potential.

Conclusions
Results shown significant differences in antioxidant defense capabilities for the three different picking date. These results are of interest and may be related to the higher susceptibility which present the more mature fruits to B.H.

Task 3: Storage of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives
Fruits were stored in CA chambers at various CO_2 levels in order to study the underlying mechanisms involved in B.H occurrence.

Material and methods
Two different storage procedures were established:
- a standard storage, - 0.5 °C, 95 % RH, 2 % O_2 and 0.7 % CO_2, representative of conventional commercial conditions
- a stress storage, - 0.5 °C, 95 % RH, 2 % O_2 and 5 % CO_2, in order to induce B.H
Fruits were removed from storage at time 60 d, 120 d and 180 d.

Results and Discussion
Significant differences in B.H occurrence were observed for the two different storage conditions (fig.7 and 8), the higher the CO_2 level, the higher the damage.

Conclusions
Results are in accordance with the recent state of the art which classified the B.H disorder as a CO_2-induced problem.

Task 4: Variation in climate and orchard characteristics

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives

Two main objectives were established for partner 2:
- collect meteorological data in order to feed the predictive model
- analyse the effect of N_2 fertilisation (prior to Ca^{++} application) on B.H occurrence.

Material and methods

- Meteorological data for orchard 1 were directly checked on the site, for orchard 2, data were collected from a meteorological station near the orchard. Parameters checked were the following: T°C / day (min., max. and average), humidity in %, radiation in J s^{-1} m^{-1} and rainfall in mm / day.
- Experiment on fertilisation was realized in orchard 1 applying two different doses in N_2: 70 U / ha. year and 140 U / ha. year.

Results and Discussion

- meteorological data: influence of meteorological parameters on B.H incidence will be analysed later by the predictive model
- effect of fertilisation strategy:

* *effect on quality*: in general no significant differences in quality parameters (colour, firmness, SSC and acidity) were found for the two different fertilisation procedure.
* *effect on B.H incidence*: In conventional atmosphere (0.7 % CO_2 - fig.9A), both the 70U and 140U-treated fruits developed slight damages. In contrast, fruits stored at 5% CO_2 showed more damage (Fig.9B). The intensity of the damage depends on the maturity stage at harvest and on the storage duration. Slight differences in B.H occurrence were found between the 70 U 140 U-treated fruits.
* *effect on mineral composition*: Conference pears fertilised with low doses 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 2). This changes in mineral composition showed that Ca^{++} may be an important element in the control of B.H disorder.

Conclusions

N_2 fertilisation seem to be an important factor to take in consideration to understand and prevent B.H disorder. In this way, complementary studies will be planned next year. We will repeat the experiment using more doses (90 U, 180 U and 270 U) and examined the effect of Ca^{++} treatments on quality and B.H occurrence.

Task 5: Post-harvest treatments

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives

Partner 2 was involved in this task as follows:
- determine the effect of delaying time before cooling on quality and B.H incidence
- determine the effect of stress scenario (high CO_2 treatment prior to conservation) on B.H incidence.

Material and methods
After harvest (fruits were picked at commercial harvest maturity, H2), fruits were kept for 0, 1, 2, 4 and 6 days at 25°C and then stored in conventional CA chamber. After 2, 4 and 6 months of storage the effect of this prematuration procedure on B.H incidence and quality was established.
Due to technical problems, the study of the effect of high CO_2 pretreatment was not carried out this year. It is planned for the next year.

Results and Discussion
Prematuration of fruits after harvest caused significant changes in storage capabilities and the fruits kept at 25°C before storage showed generally:
- a greater decrease in firmness during storage (fig.10)
- some differences in acidity and SSC content were also found (fig.11A and 11B)
These changes are probably related to an induction of the maturation process during the stay at 25°C (increase in respiration and C_2H_4 production).
On an another hand, no significant changes in B.H incidence were observed

Conclusions
Results confirm that B.H incidence was more directly related to CO_2 concentration during storage than to the prematuration process. Results are in accordance to those of task 1 and showed that quality parameters cannot be used directly to predict the disorder. In this context, a difference should be made between the increase in maturity on the tree (see task 2), which involve a decrease in the endogeneous antioxidant defense mechanisms and an higher susceptibility to B.H disorder, and this prematuring process which has no effect. Further studies are needed to clarify this interesting aspect.

Task 7: Destructive measurements

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	12
No. of man-month devoted already to the task:	8

Objectives
7-a: Quality evaluation
Quality changes during storage were studied for the different orchards, storage conditions and harvest date. Correlations between changes in quality parameters and B.H incidence were established.
7-b: Biochemical studies
The aim of this work was to determine the underlying mechanism involved in B.H incidence. Two different studies were carried out:
- study of the biochemical changes due to the different storage conditions (0.7 % or 5 % CO_2)
- study of the biochemical changes which occurred in damaged fruits
7-c : Mineral content
Study on the mineral content and on it influence on B.H occurrence was already described in task 4 : Fertilisation/effect of mineral content.

Material and methods
Methods for the determination of ethane production, MDA and enzyme activities are similar to those described in references.

Results and Discussion
- quality evaluation:
In general there is no significant difference in the quality parameters between fruits stored at 5% and 0,7% CO_2. Quality parameters are not available to predict the sensibility to B.H.

- relationship browning/PPO/B.H:
As shown in our previous paper, there is not a direct relationship between enzymatic browning and internal disorder. PPO enzyme was not directly involved in B.H incidence.

- influence on membrane peroxidation:
Membrane peroxidation was analysed determining both the ethane and MDA content. In both case these products significantly increase during storage, showing that membrane peroxidation occurred during storage (fig.12 and 13). However, no significant difference was shown between the different atmospheres. As a consequence, the higher incidence in B.H at 5% CO_2 seem not to be directly related to an increase in peroxidative damage of membrane.

- Relation with fermentative metabolism:
As expected ethanol content increased steadily during storage (Fig.14). On the opposite, acetaldehyde content decreased especially during the first period of storage (Fig.15) and remained constant later. In both case, no significant differences were shown between fruits stored at 0.7% or 5% CO_2.
Changes in Alcohol Deshydrogenase (ADH) and Pyruvate decarboxylase (PDC) activities were also estimated during storage. ADH activity and, in a lesser extend PDC activity generally increased during storage (Fig.16). Again there is not a significant difference between fruits at 0.7% or 5% CO_2.
As shown in Fig 17, ADH activity was rather induced by the C.A system (decrease in O_2 and increase in CO_2 levels) than directly by the cold temperature (there is no activation of ADH in air). In contrast, a significant increase in PDC activity was noted in air.
In conclusion, the fermentative metabolism appear not to be directly involved in B.H occurrence.

- Relation with peroxide-scavenging enzymes:
- *Changes in SOD activity during CA storage*:
There is no significant change in SOD activity in less mature fruits (H1) during storage (fig 18). More mature fruits, however, exhibited a peak in activity at 120 days for H2 and at 60 days for the more mature fruits H3. In general, fruits stored at 5% CO_2 showed more activity than fruits stored at 0.7 %.
- *Changes in CAT activity*:
Immature fruits exhibited a significant decrease in CAT activity (Fig. 19). Decrease in activity is rapid and minima are obtained soon (60 days of storage). In more mature fruits, H2 and particularly H3, changes in activity were less important, the initial activity being already low at harvest. No significant differences were shown between the 0.7% and 5% CO_2 stored fruits.
- *Changes in APX activity*:
Results which concern APX enzyme seemed to be the opposite to those of CAT (fig. 20). Initial value were low in immature fruits and higher in the more mature fruits. APX activity increase during storage in immature fruits, but remained quite unchanged or slightly decreased in the more mature fruits, H2 and H3 respectively. In general, APX activity is slightly higher in fruits stored at 5% CO_2.
- *Changes in POD activity*:
Except for the more immature fruits (H1) stored at 5% CO_2 (Fig.21), for which POD activity decreased, there is no significant changes in activity during storage and between the two procedures of storage (0.7% and 5%).

- Biochemical changes in B.H damaged fruits:
The aim of this part of the work was to determine the biochemical changes and particularly the changes in ethane and enzyme activities in altered fruits. Fruits were classified using an index, according to a scale from 1 to 5, where:
index 1 : only the core is slightly altered (browning of carpelar vesicles).
index 2 : all the core is brown
index 3 : core and slight browning or cavities in the pulp
index 4 : core and important browning (20%< <50%) of the pulp or numerous cavities.
index 5 : core and severe browning of the pulp (>50%) or severe cavities.

Briefly and as shown in fig. 22 to 24, our results show :
- An important increase in ethane content in the more damaged fruits in accordance with a process of peroxidation of membrane.
- An increase of SOD activity in the more damaged fruits which can reflect a greater level of oxidative stress in these fruits.
- No changes in CAT activity which appeared to be unable to channel the peroxides (H_2O_2) generated by SOD enzyme.
- Slight decrease in peroxidase enzyme activities (POD and APX).

Conclusions
According to these results we can establish the following conclusion.
- there is no direct relationship between the evolution of quality parameters during storage and the incidence in B.H. In this way, quality parameters analysed were not suitable to predict this disorder.
- Peroxidative damage of membrane occurred during storage, but there is no evidence that it was an underlying mechanism to explain the higher incidence of disorders at high CO_2 levels.
- Fermentative metabolism is not clearly involved in B.H occurrence.
- Except for SOD activity which seemed to increase in damaging conditions (5% CO_2), there is not a clear evidence that peroxide-scavenging enzymes played a key role in the development of B.H disorder during storage.
- In contrast, studies realized on damaged fruits showed that peroxidative damage (↑ in ethane, ↑ in SOD activity) can likely be involved when the damage is visible. The contradiction shown between the stored but non damaged fruits for which peroxidative damage seem not to be relevant, and the damaged fruits for which peroxidative damage is involved, may reflect a transitory phenomenom only visible when the symptoms develop. Further studies are needed to clarify this result.

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C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997TO 31-05-1998

Herewith some changes from the original plan describe in technical annex.
. Task 4: Rather than a specific mineral application, we studied the more general effect of N_2 fertilisation on B.H incidence. At the view of your preliminary results, Ca^{++} seem to be an important factor involved in B.H occurrence. To confirm this result, other experiments are planned this new year in which we will repeat the experiment on N_2 fertilisation (3 dosis: 90, 180 and 270 U/ha.year) and examined the effect of Ca^{++} preharvest treatment.
- Task 3 - storage: Due to technical problems (lack of microchambers), IRTA has not been able to assure the same cooling period for the different picking dates (fruits picked more immature were kept longer in cold before establishing CA conditions than more mature fruits). In our opinion this difference will not modify the physiological response of the fruits during storage.

- Task 5 - pretreatment: Due to others technical problems, the study which concern the effect of high CO_2 pretreatments has not been studied this year. Experiment will be planned this new year.
- Task 7-b - polyamines: We slightly changed research plan of this task. In accordance with others partners the focus has been on radical scavengers and peroxidative damage. Influence of polyamines will be studied this new year.
- Task 7-b - LOX activity: Developed protocols were not reproductible. We will try to develop new protocols this year to carried out this task.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

Task 1: The same orchard will be used
Task 2: No changes - 3 harvest dates
Task 3: No changes . same storage conditions
Some microchambers will be likely used to estimate the short-term physiological response of fruits to high CO_2 levels (5%).
Task 4: Two different experiments will be carried out:
- effect of N_2 fertilisation on quality and B.H incidence
- effect of Ca^{++} treatment (3 different treatment) on quality and B.H incidence
Task 5: The effect of CO_2 pretreatment (CO_2 shocks) on quality and B.H incidence will be studied
Task 7:
7-a: quality parameters will be checked during storage
7-b: biochemical approach will confirm or establish:
- the role of membrane peroxidation (membrane status and peroxide content) on a long-term basis and in case of availability of the microchambers also on a short-term basis
- the relationship between quality, BH occurrence and polyamines content
- the effect of high CO_2 pretreatment on the physiology of Conference pear and particularly its relationships with fermentative metabolism, quality and B.H incidence.
7-c: mineral analysis: mineral analysis will be carried out especially for the experiments which concern the effect of N_2 fertilisation and Ca^{++} pretreatment.

E. DISSEMINATION

Articles in international reviews:
C. Larrigaudiere, I. Lentheric and M. Vendrell. 1998. Relationship between enzymatic browning and internal disorders in controlled-atmosphere stored pears. Accepted for publication in: Journal of the Science of Food and Agriculture.

Congress:
Partner 2 will participate to the 11th FESPP (Federation of European Societies of Plant Physiology) congress which will take place in Varna (Bulgaria) from 7 to 11 September 1998 presenting a poster titulated: "Late harvest induced changes in antioxidant defence mechanisms in Conference pears".

F. ANNEX

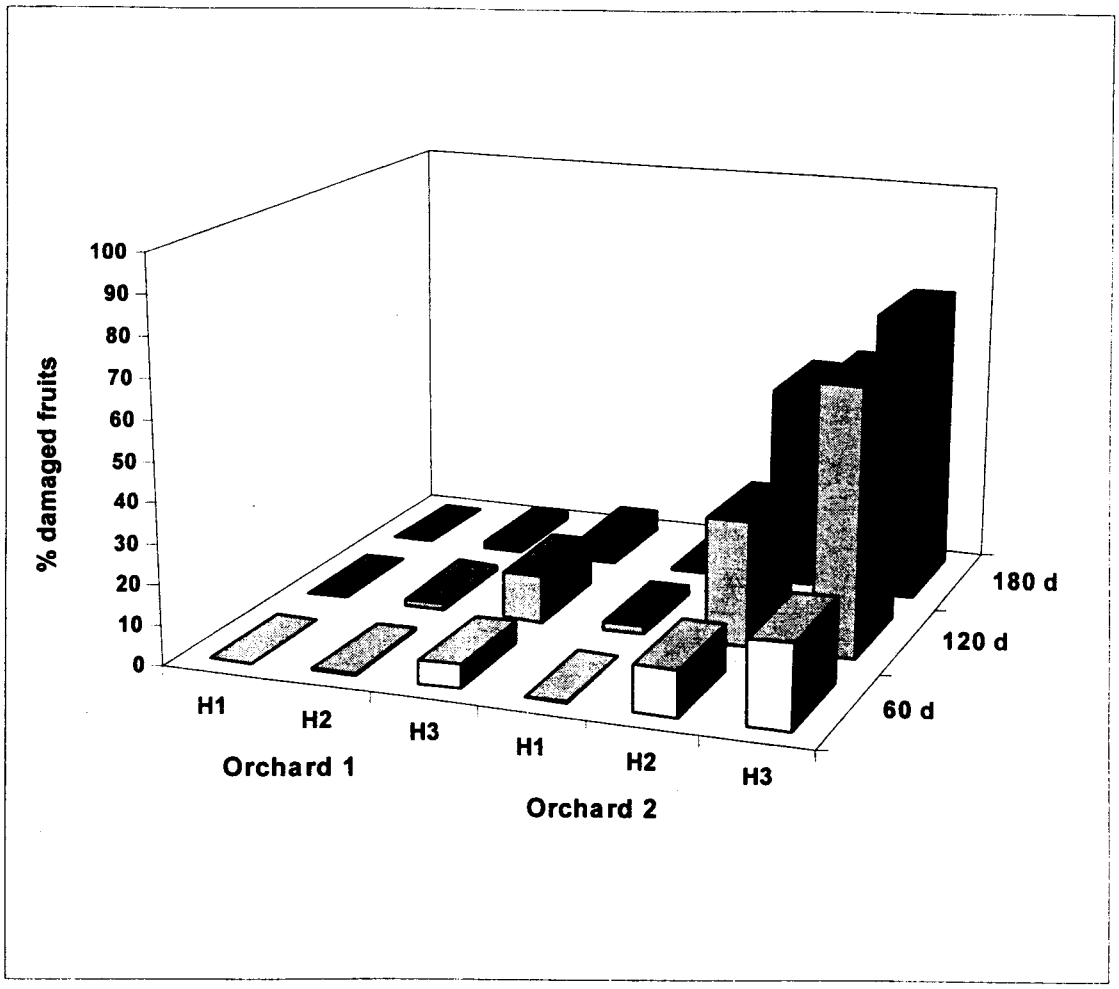

Fig.1 : Influence of orchard location on B.H incidence in Conference pears stored at 5% CO2

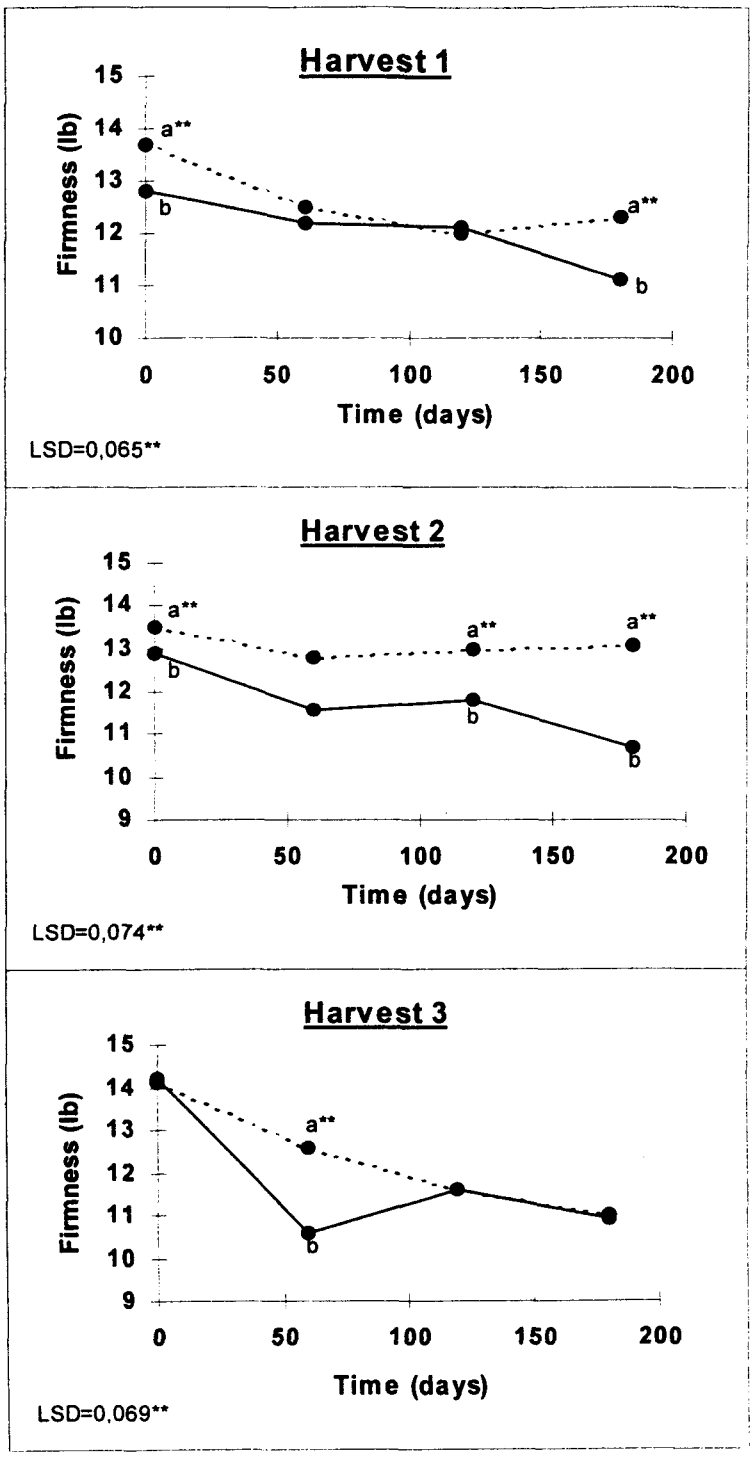

Fig. 2 : Changes in firmness during storage as a function of orchard origin
Full line : orchard 1, Gimenels; dotted line : orchard 2, Albatarrech

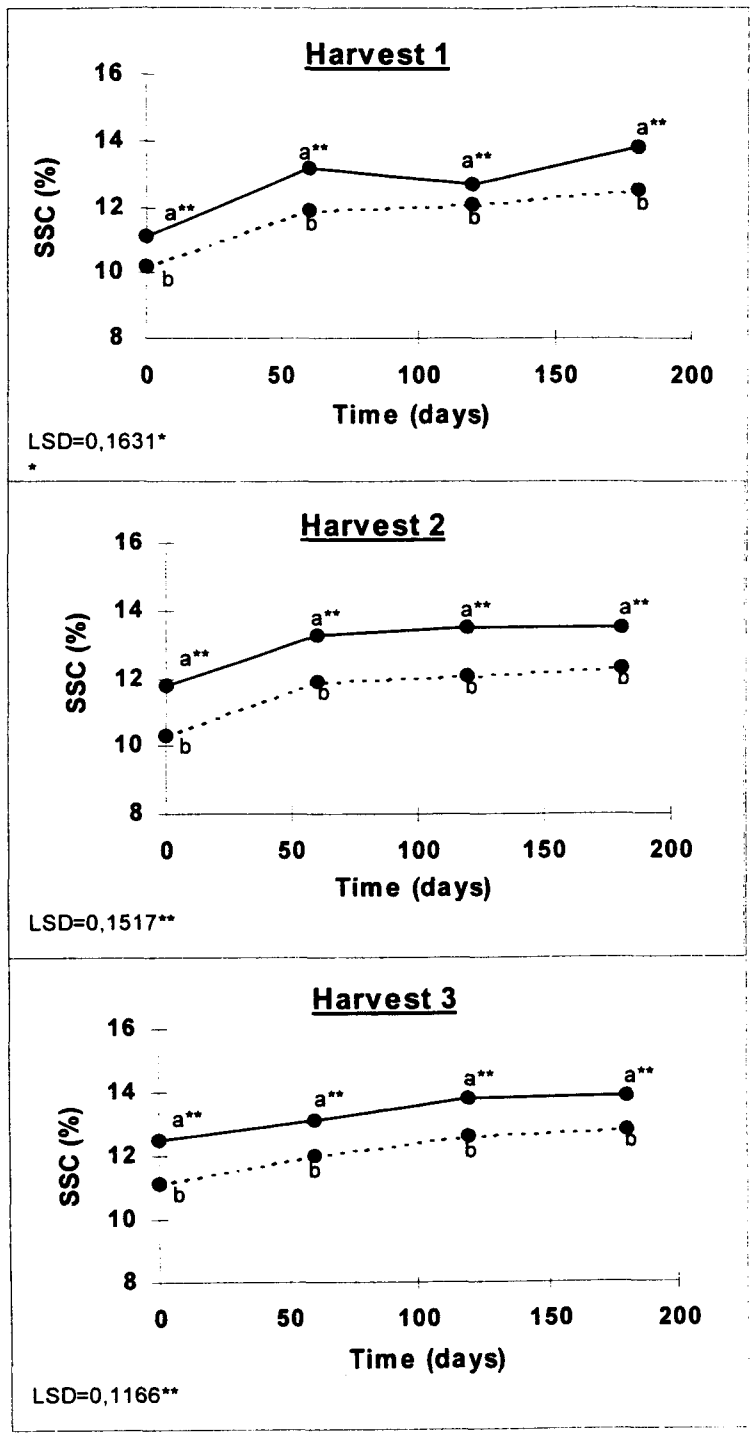

Fig. 3 : Changes in sugar content (%SSC) during storage as a function of orchard origin.
Full line : orchard 1, Gimenels; dotted line : orchard 2, Albatarrech

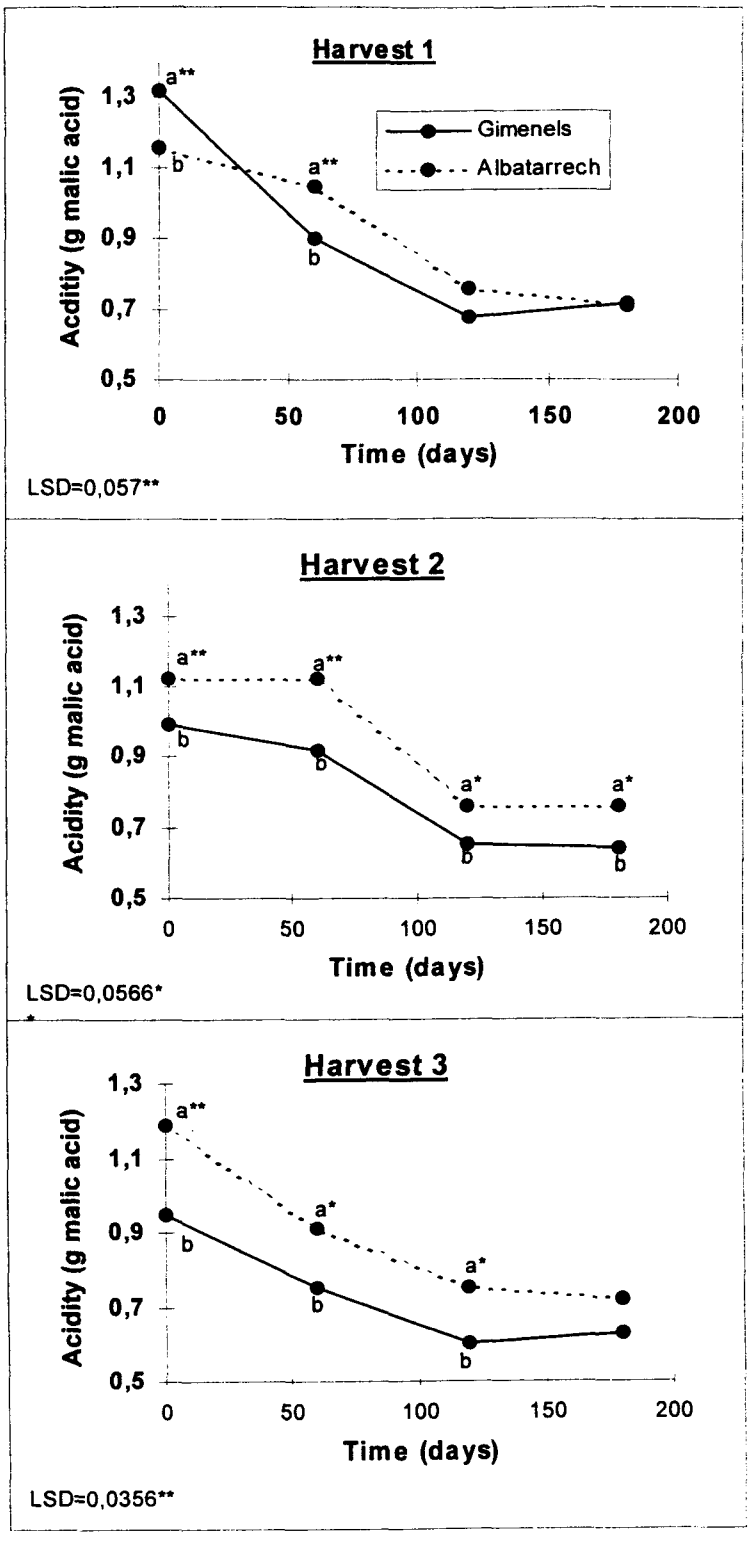

Fig. 4 : Changes in acidity during storage as a fonction of orchard origin
Full line : orchard 1, Gimenels; dotted line : orchard 2, Albatarrech

	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 1: Changes in maturity parameters in Conference pears harvested at three different picking date.

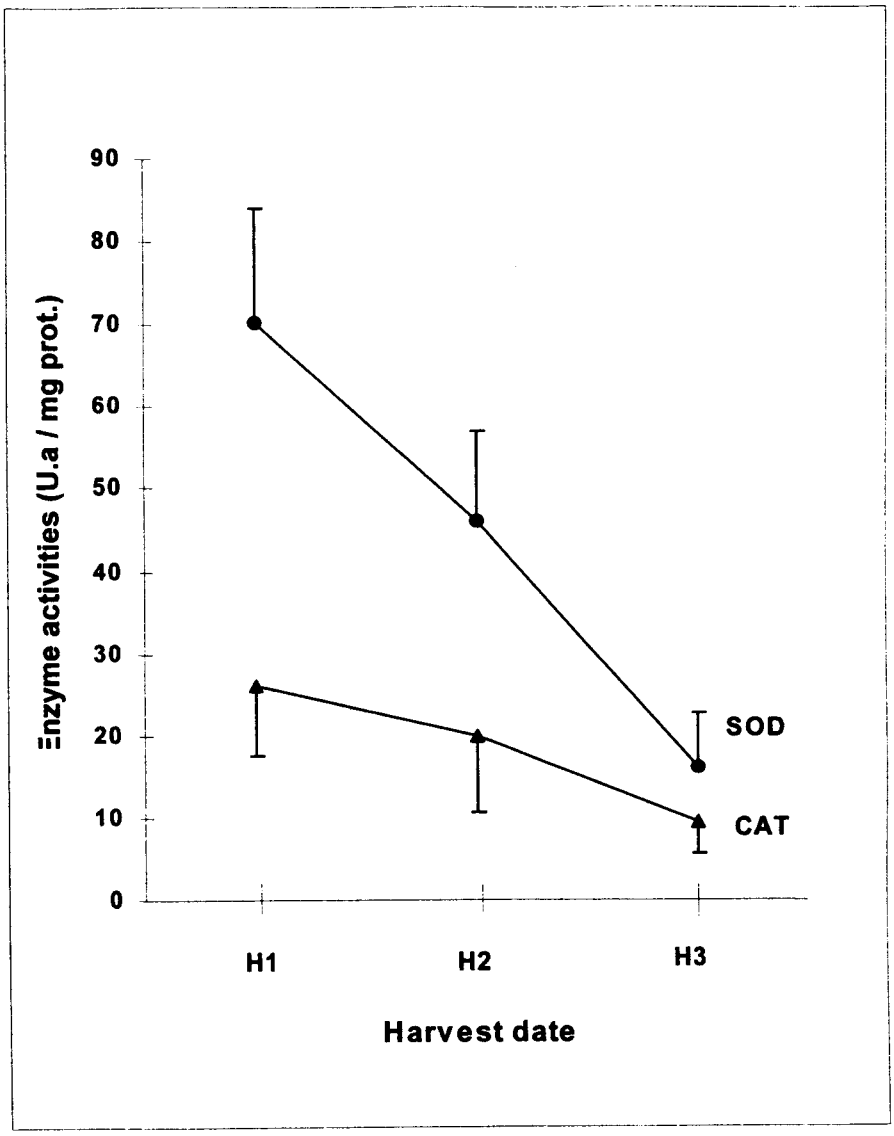

Fig.5: Changes in Superoxide Dismutase (SOD) and Catalase (CAT) activities as a function of harvest date.

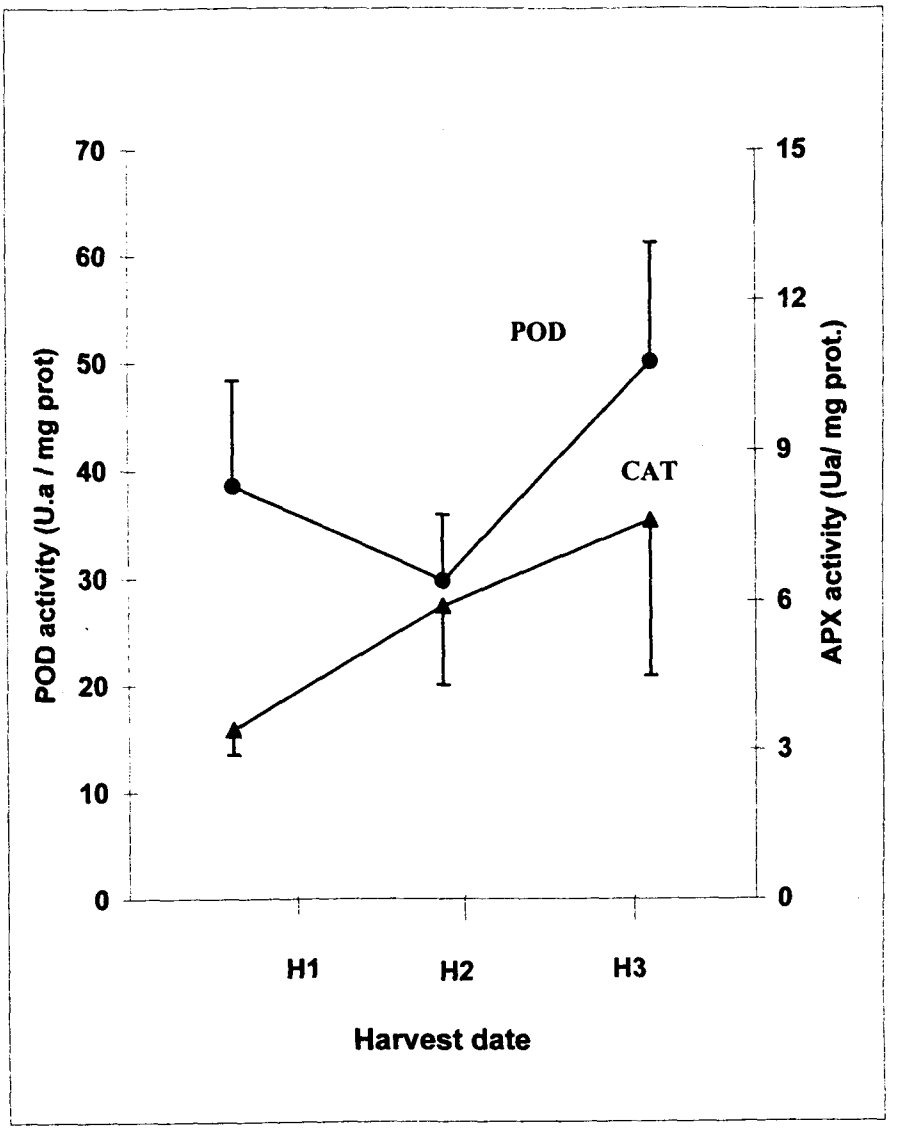

Fig.6: Changes in Ascorbate Peroxidase (APX) and Peroxidase (POD) activities as a function of harvest date.

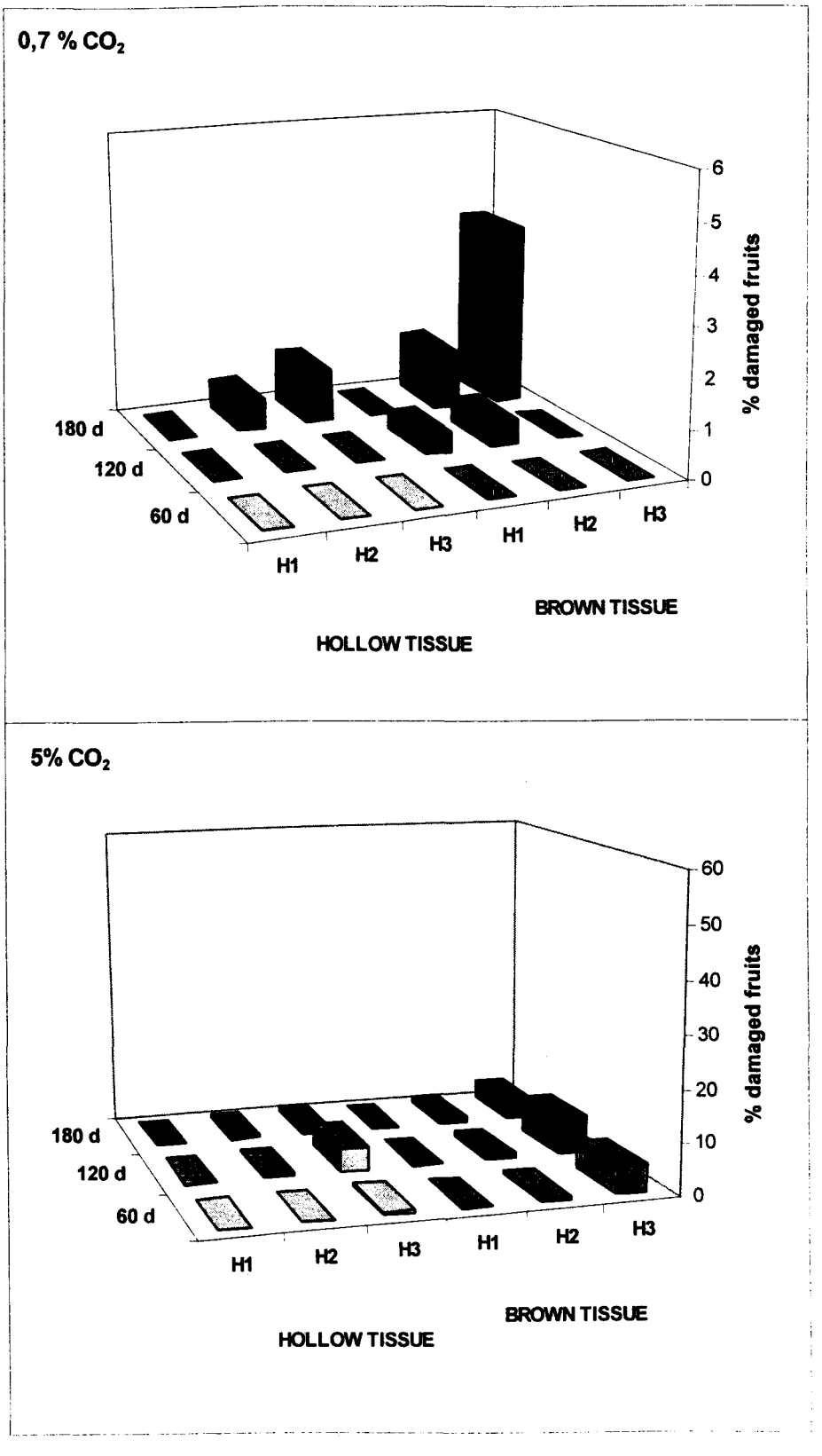

Fig. 7 : Effect of CO2 level in CA storage on B.H incidence - orchard 1

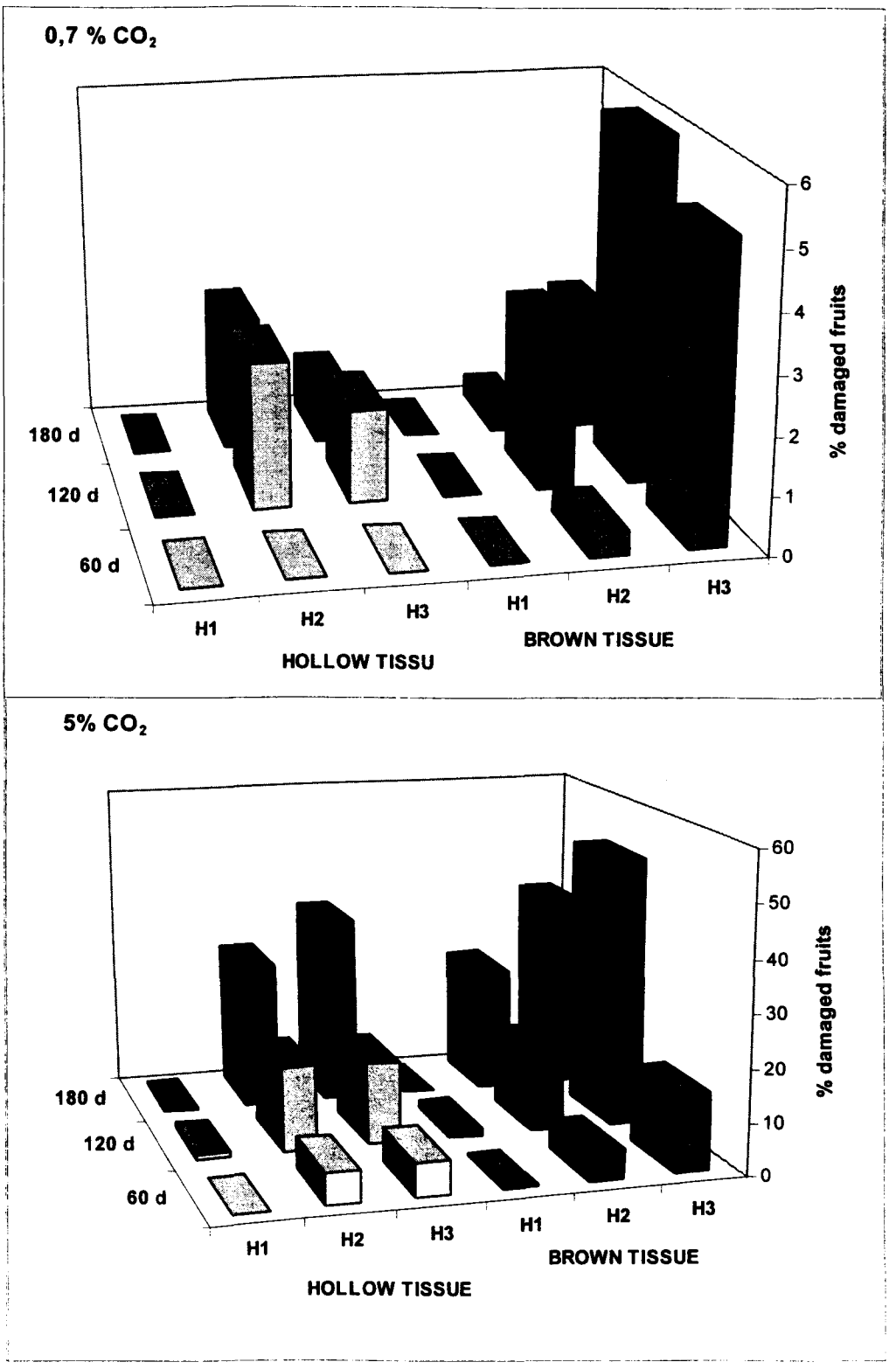

Fig. 8 : Effect of CO_2 level in CA storage on B.H incidence - orchard 2

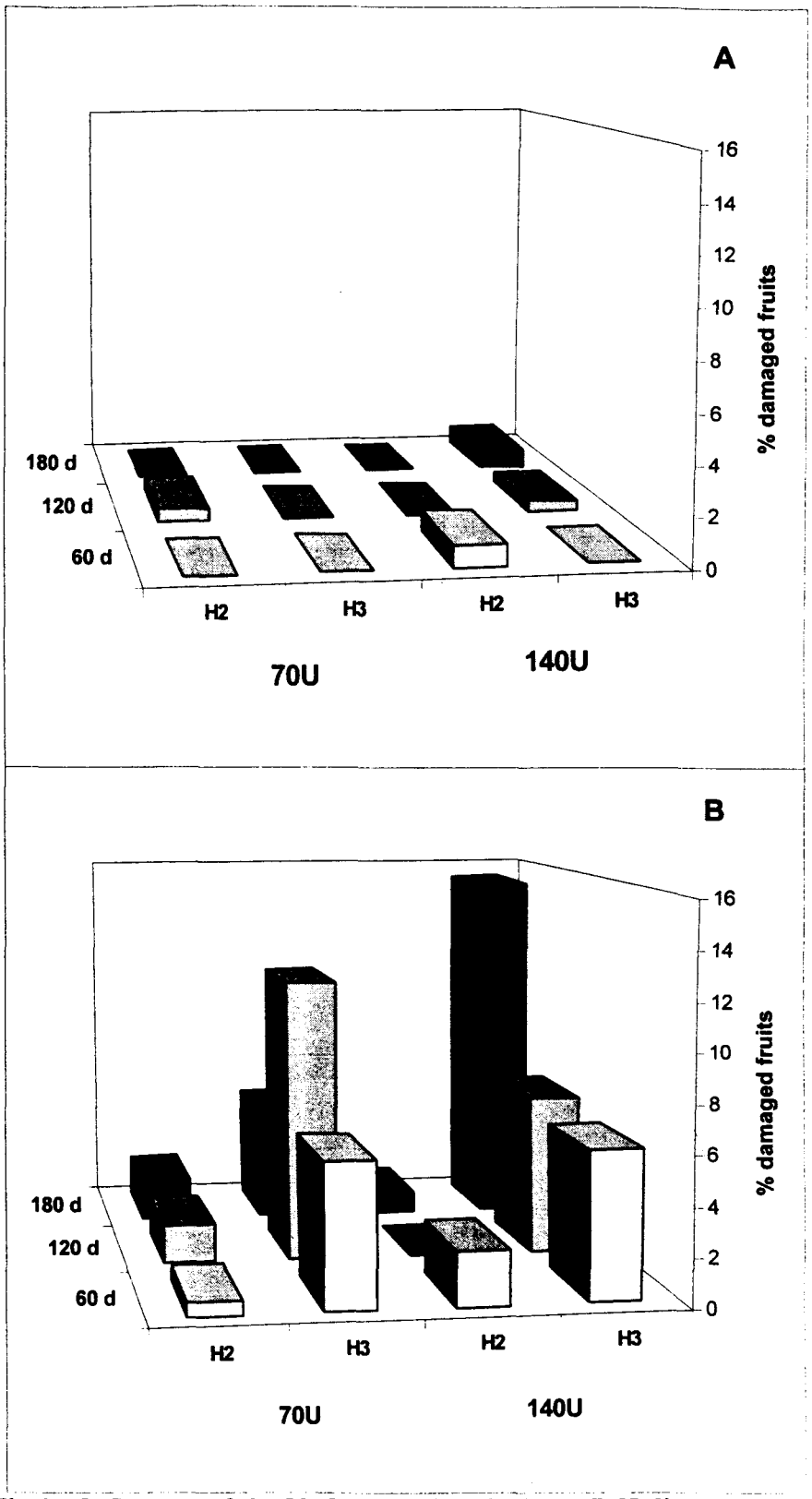

Fig.9 : Influence of the N_2 fertilization dosis on B.H disorder.
70 U : 70U/ha.year 140U : 140U/ha.year.
A- Fruits stored at 0,7% CO_2
B - Fruits stored at 5% CO_2

	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 2: Change in mineral composition as a function of N_2 fertilization, 70U: 70U/ha.year , 140U: 140U/ha.year

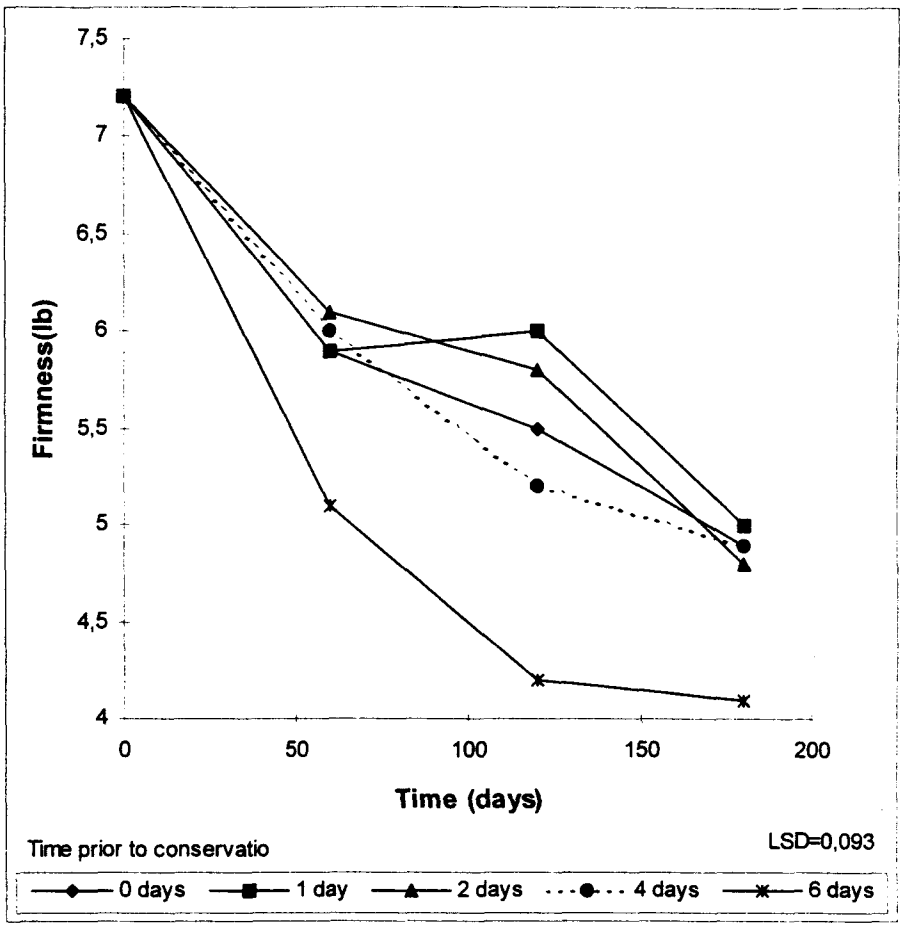

Fig. 10 : Effect of the prematuration procedure (0,1, 2, 4 and 6 days at 25°C) on the evolution of firmness during storage

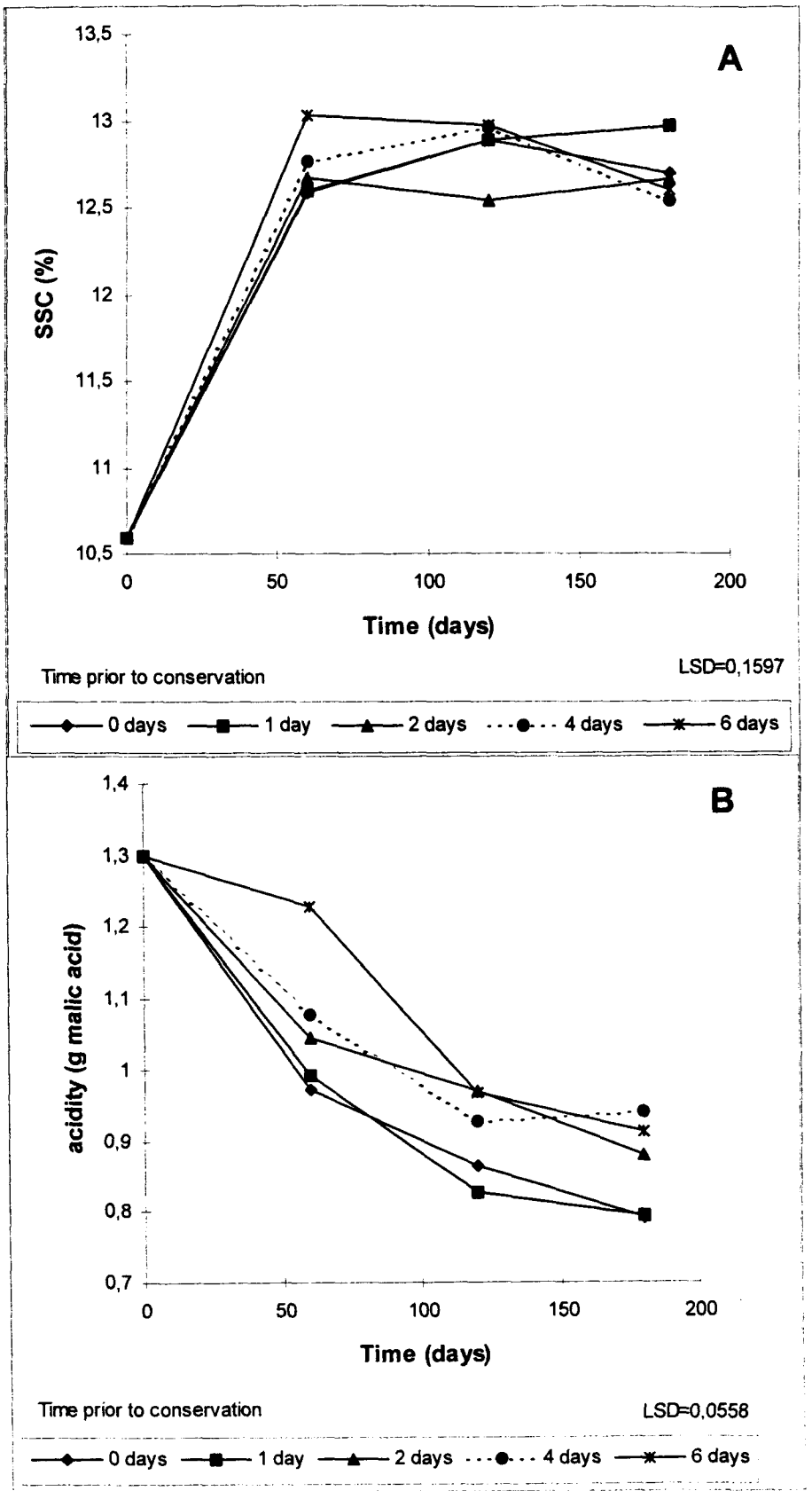

Fig. 11 : Effect of the prematuration procedure (0,1, 2, 4 and 6 days at 25°C) on the evolution during storage of : A : sugars content, B: acidity

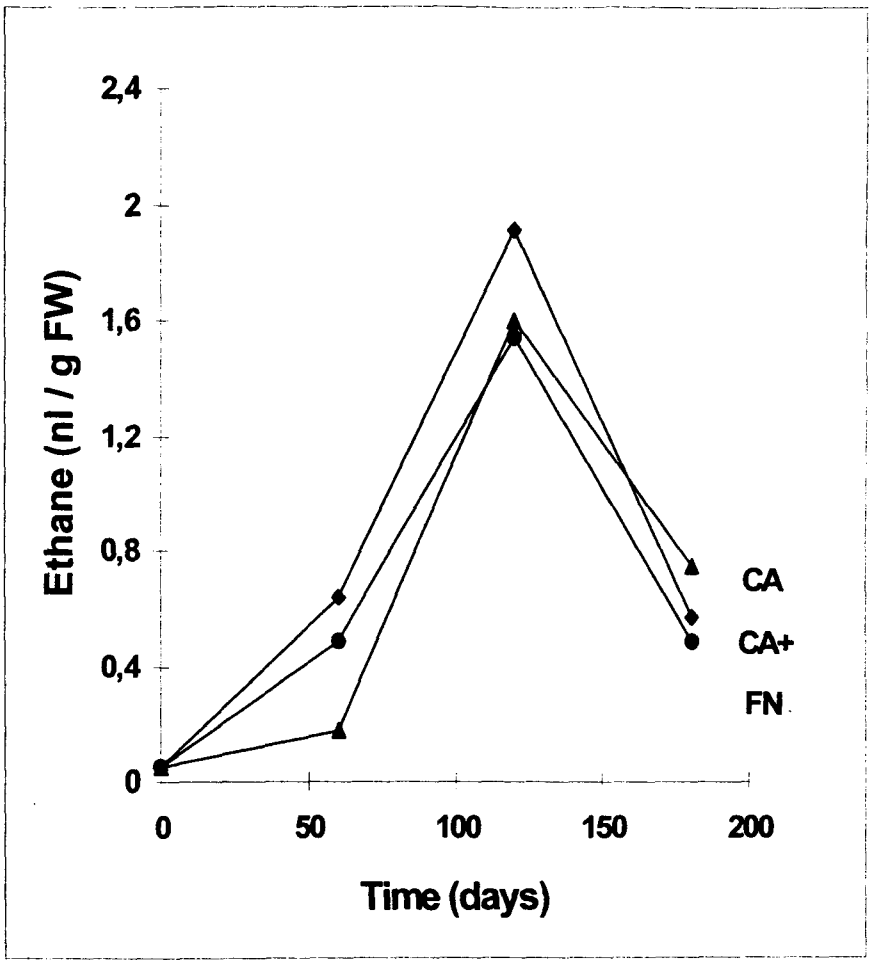

Fig. 12: Change in ethane production in fruits stored at various CO_2 regimes, FN: air, CA: 0.7 % CO_2 and CA+: 5 % CO2.

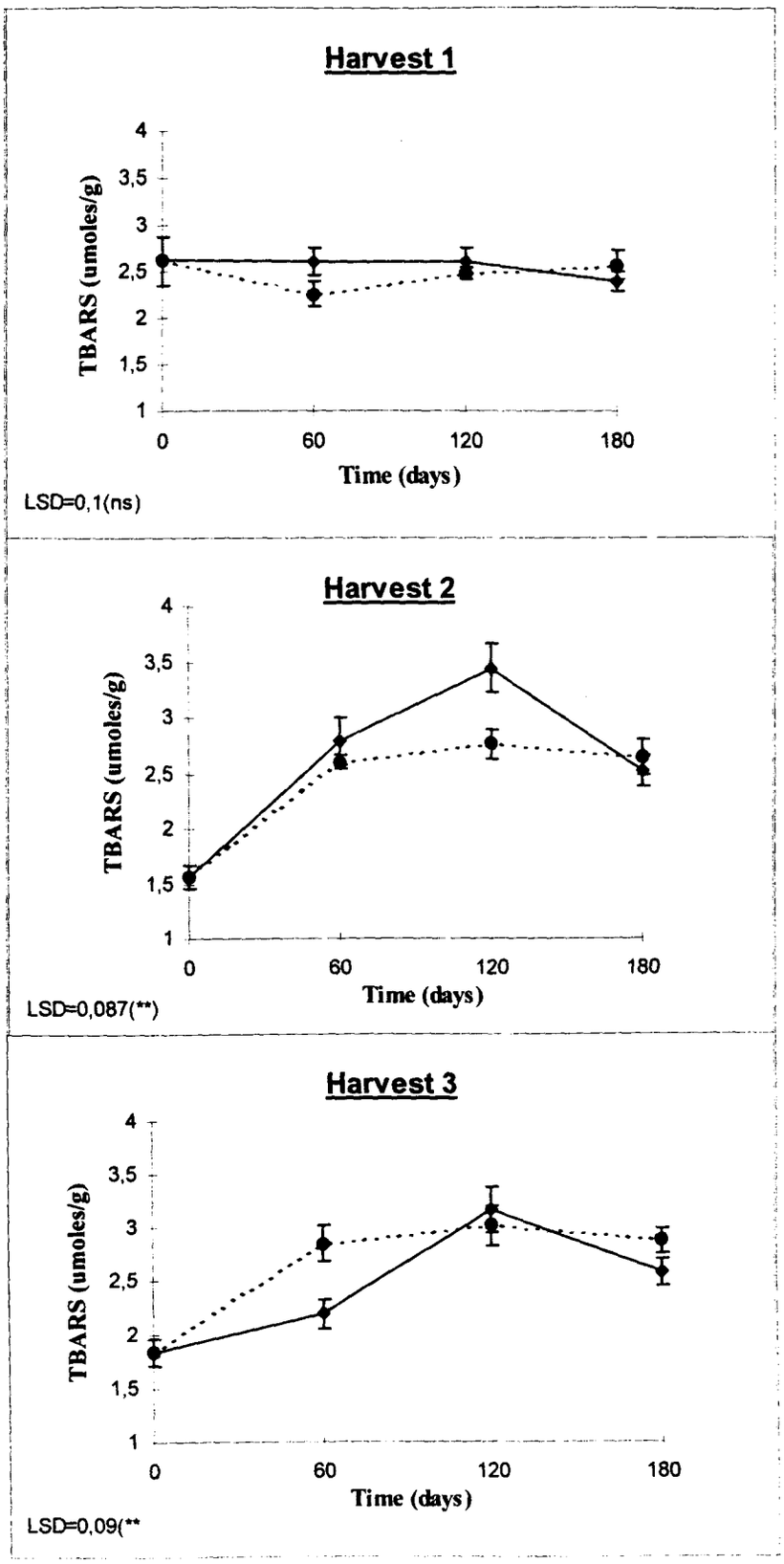

Fig. 13 : Changes in thiobarbituric reactive substances (TBARS) content during storage at various CO2 levels : 0,7% (full line), 5% (dotted line)

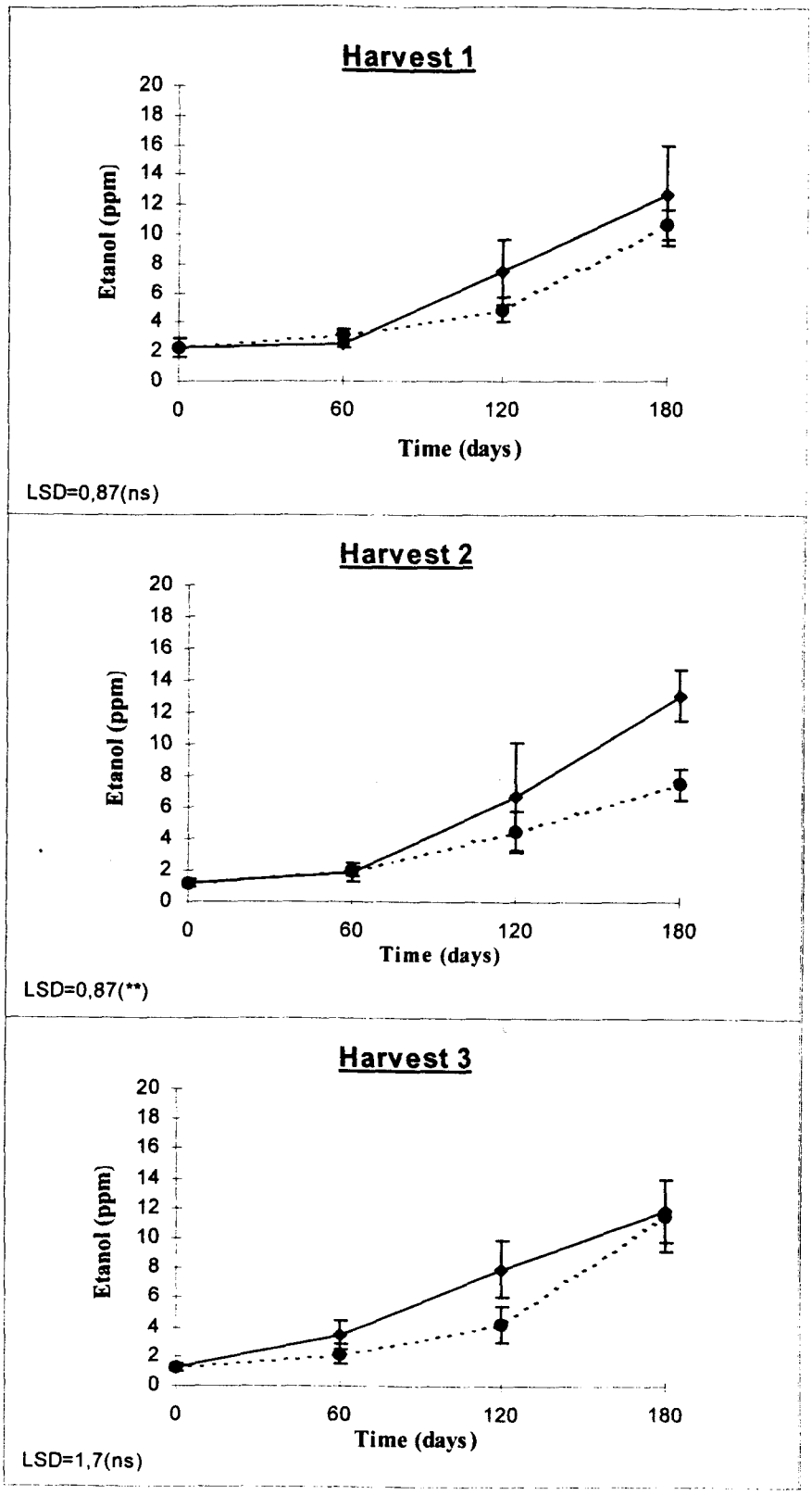

Fig. 14 : Changes ethanol content during storage at various CO2 levels : 0,7% (full line), 5% (dotted line)

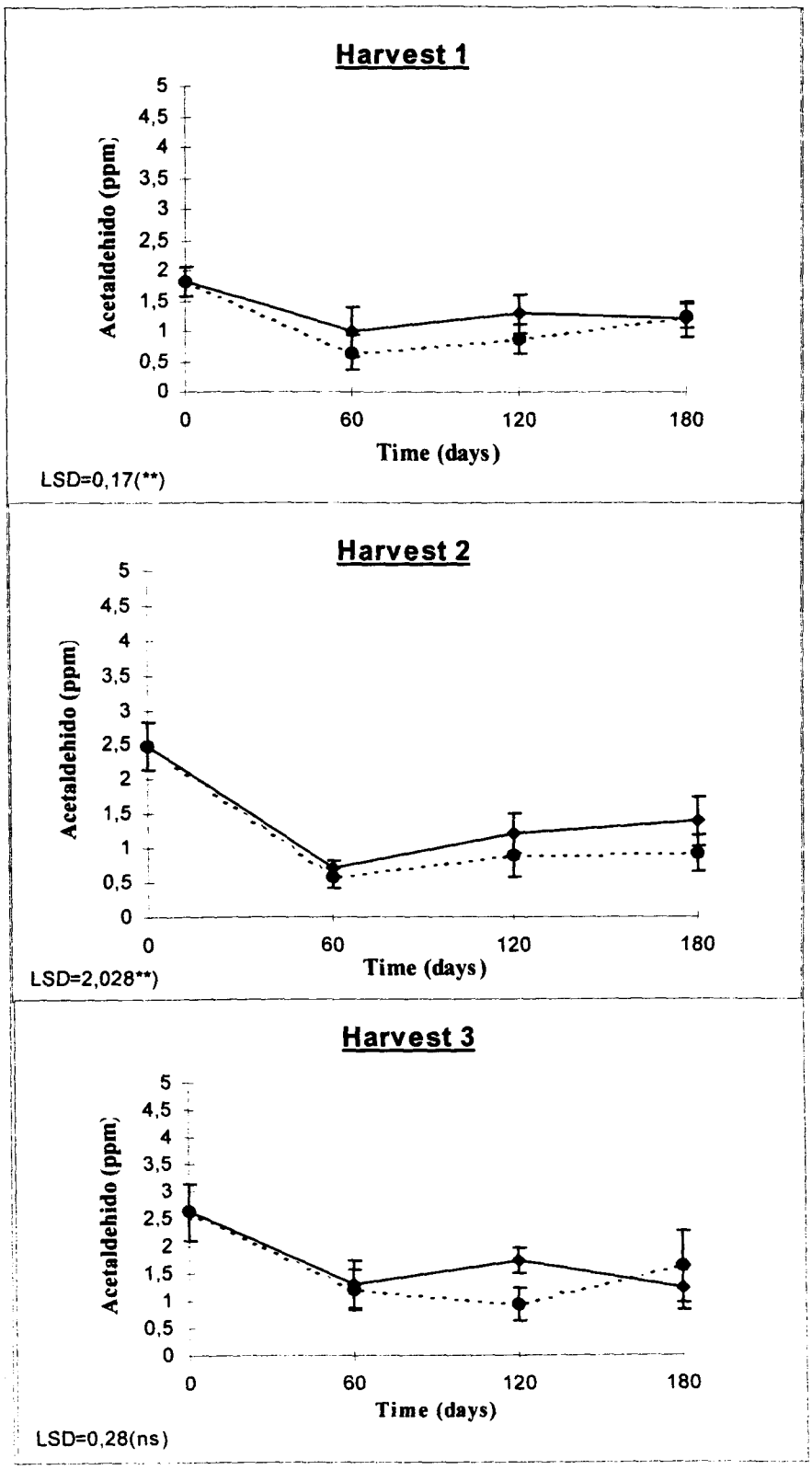

Fig. 15 : Changes acetaldehyde content during storage at various CO2 levels : 0,7% (full line), 5% (dotted line)

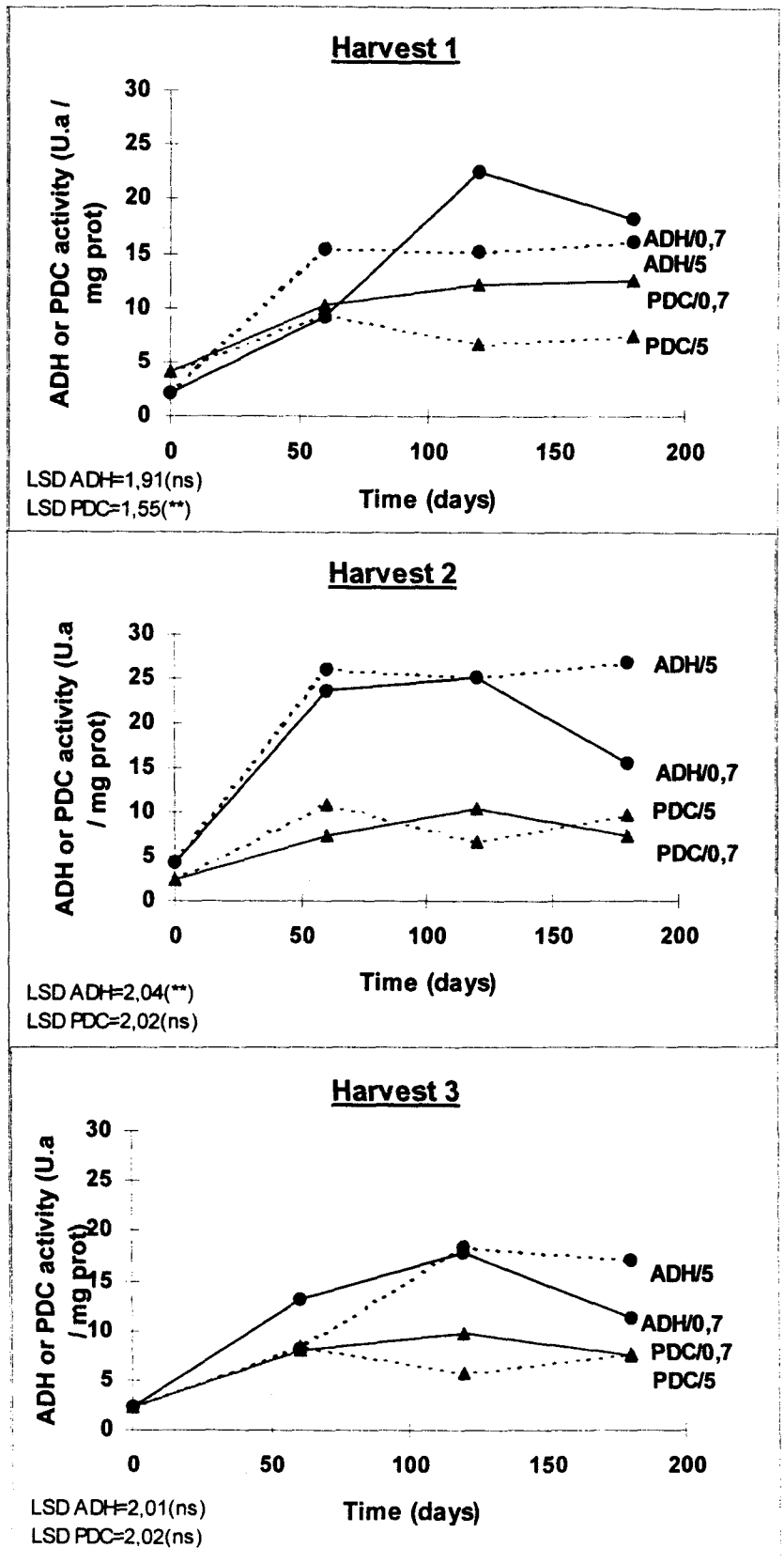

Fig. 16 : Changes ADH and PDC activities during storage at various CO2 levels : 0,7% (full line), 5% (dotted line)

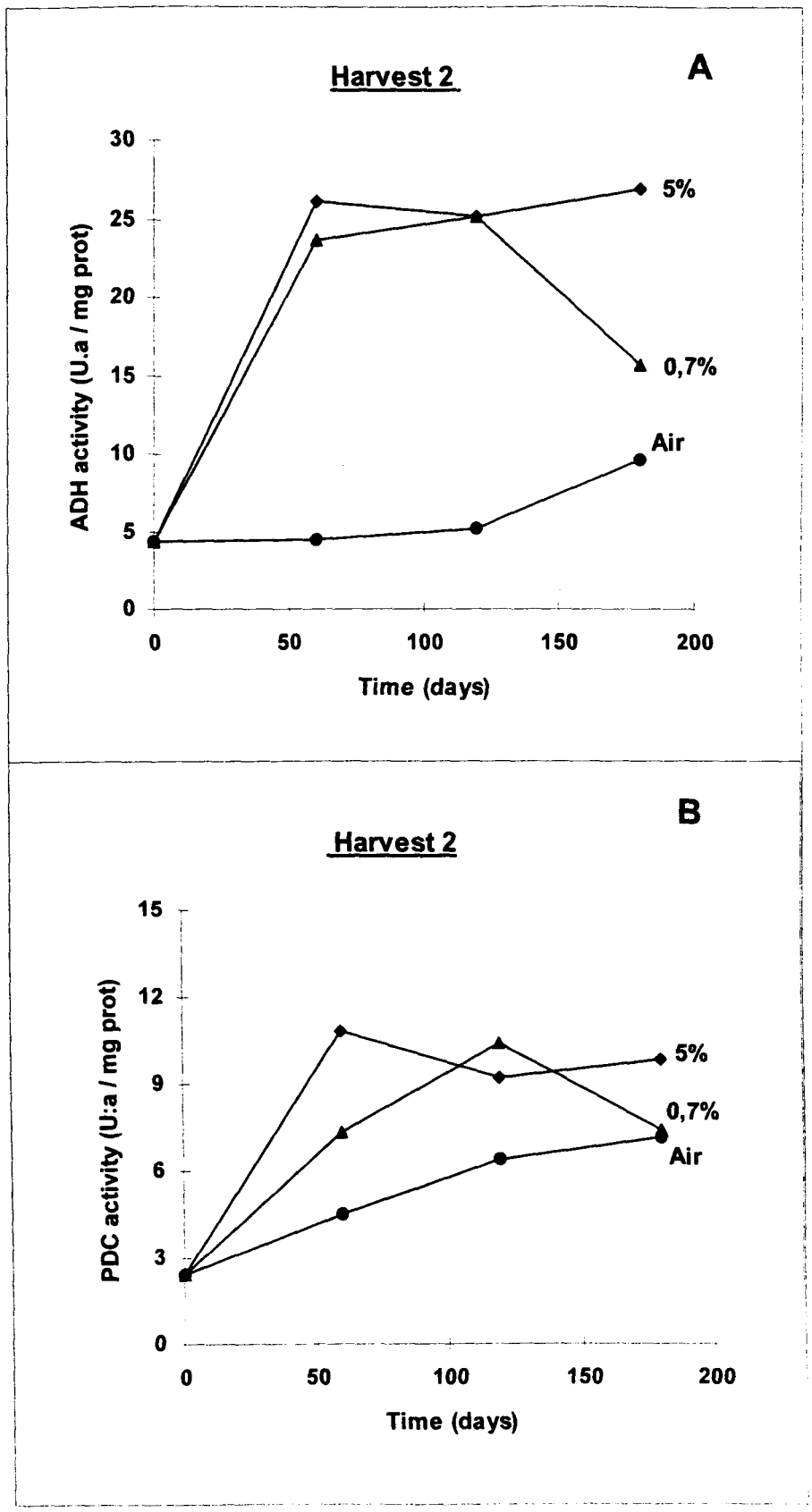

Fig. 17 : Effect of storage atmosphere (air, 0,7% CO2 and 5% CO2) on: A : ADH activity, B : PDC activity

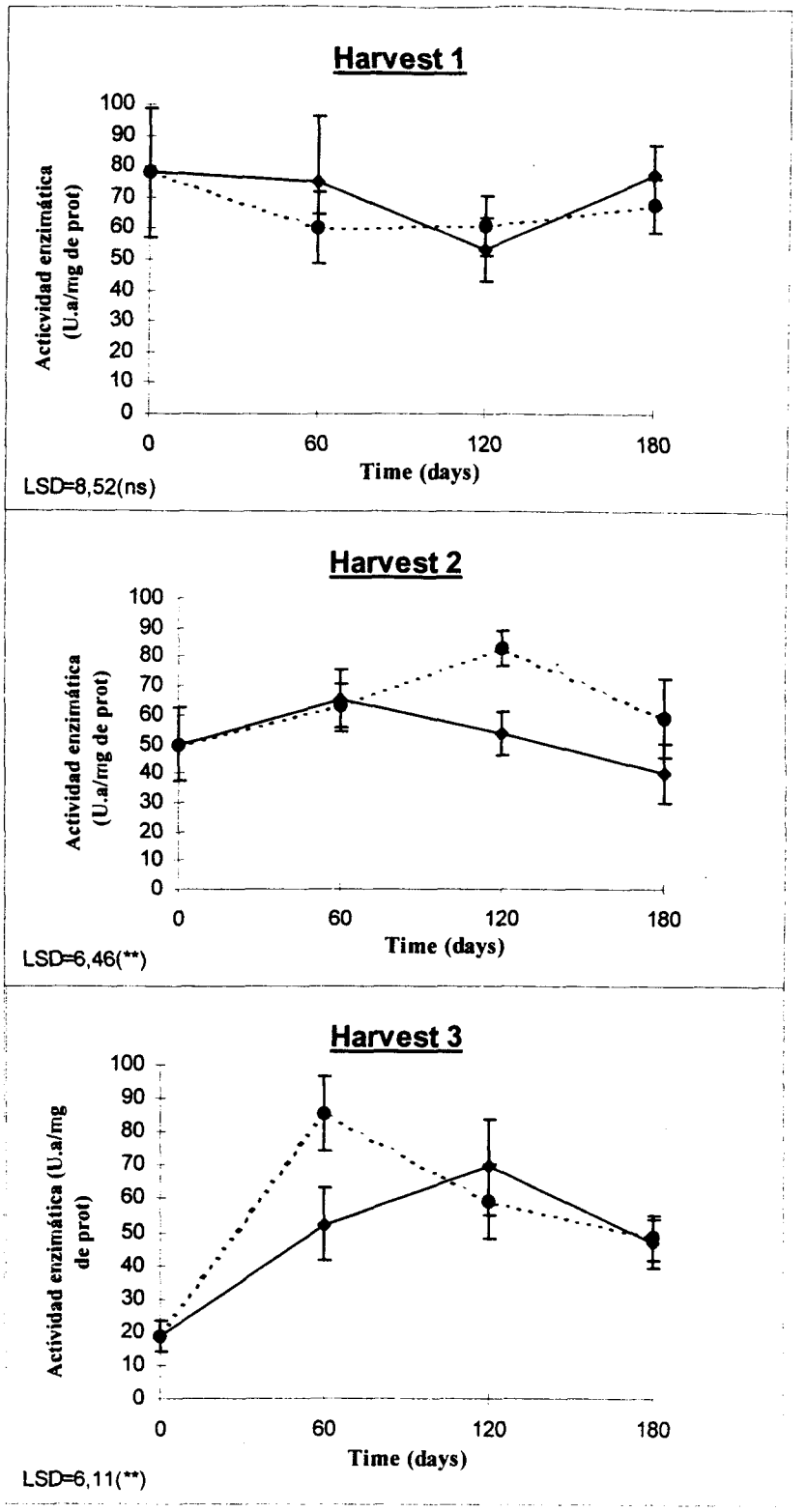

Fig. 18 : Evolution of Superoxide dismutase (SOD) activity during storage at various CO_2 level : 0,7% (full line) and 5% (dotted line)

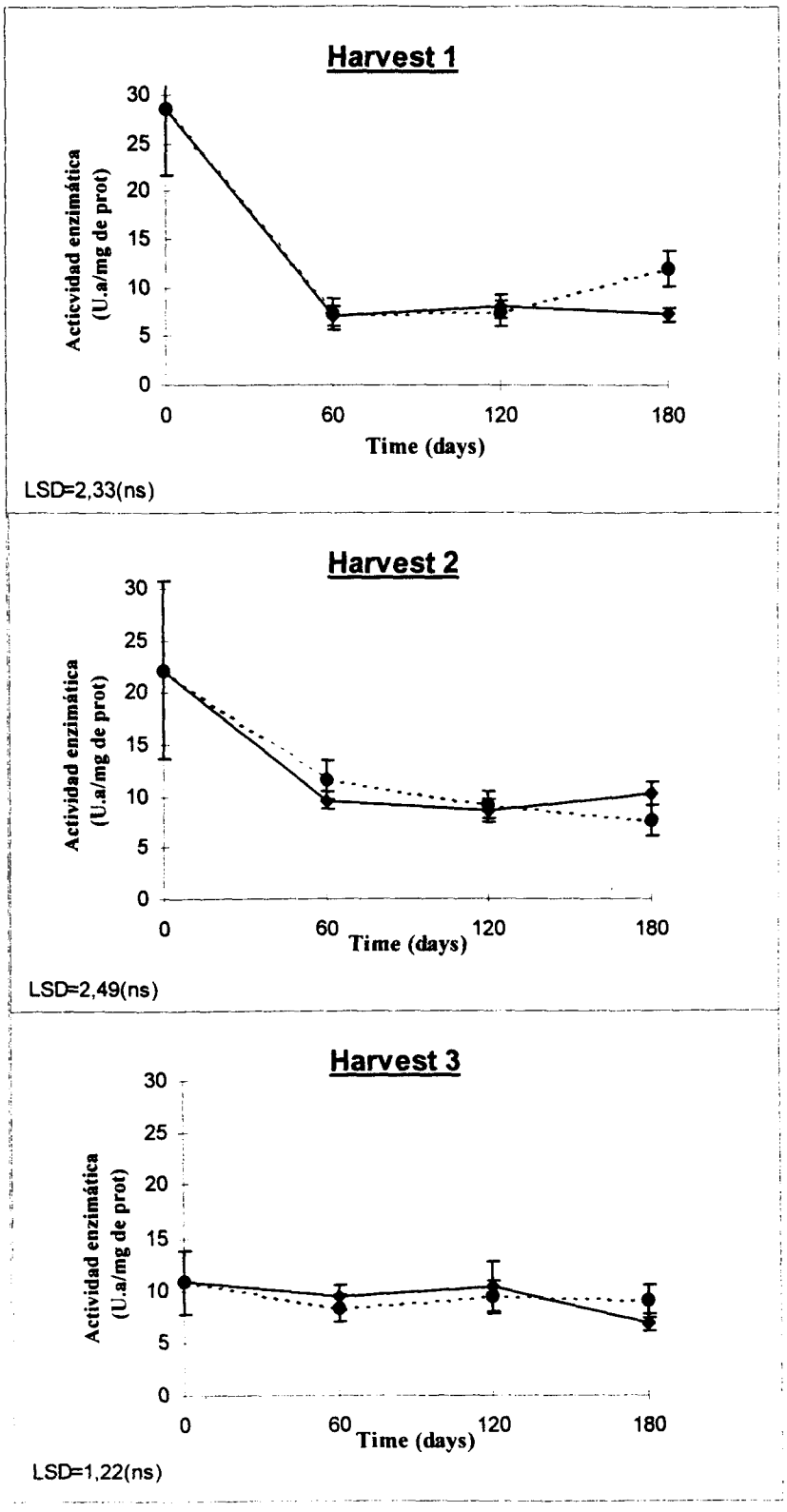

Fig. 19 : Evolution of Catalase (CAT) activity during storage at various CO_2 level : 0,7% (full line) and 5% (dotted line)

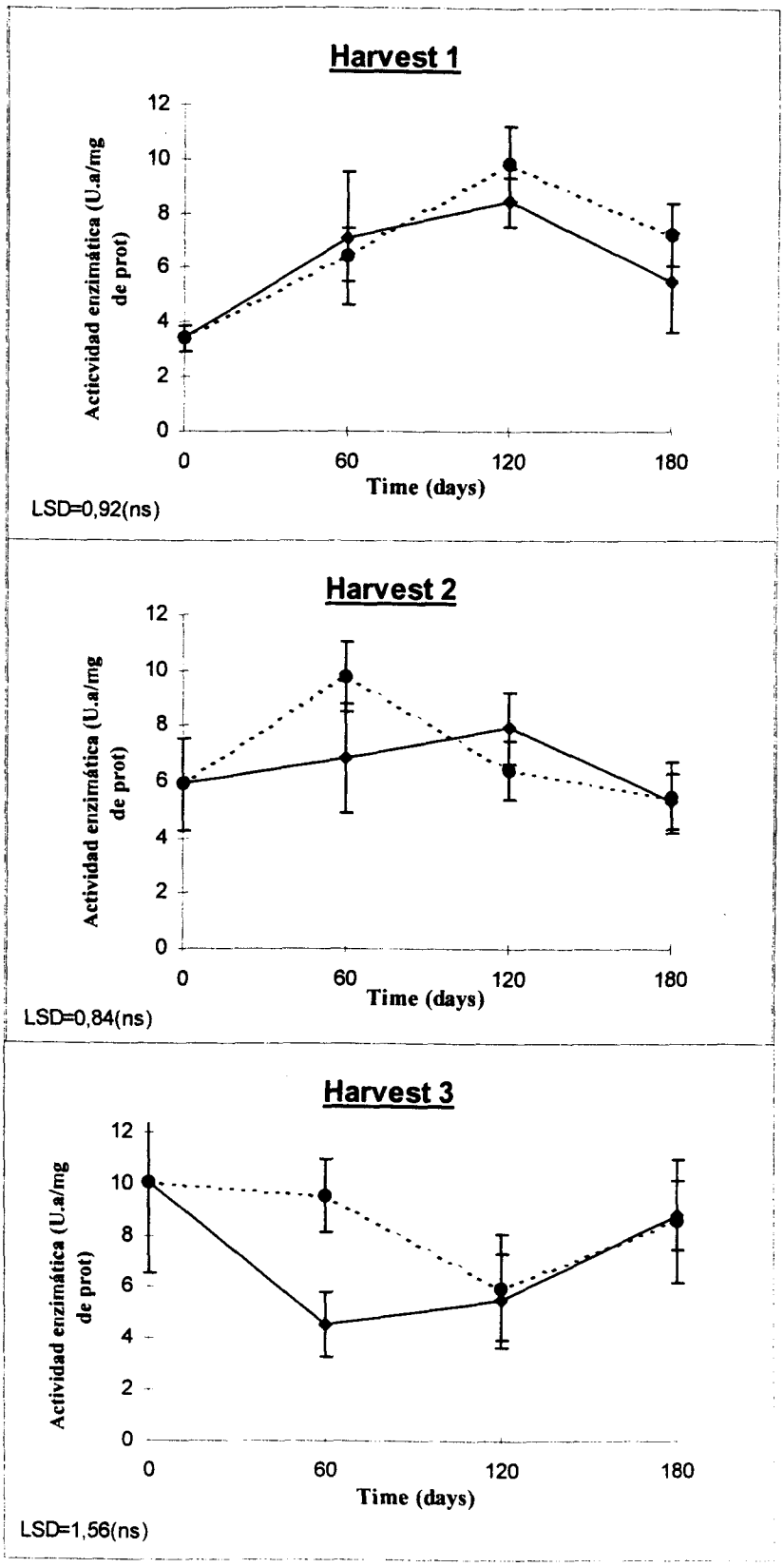

Fig. 20 : Evolution of Ascorbate Peroxidase (APX) activity during storage at various CO_2 level : 0,7% (full line) and 5% (dotted line)

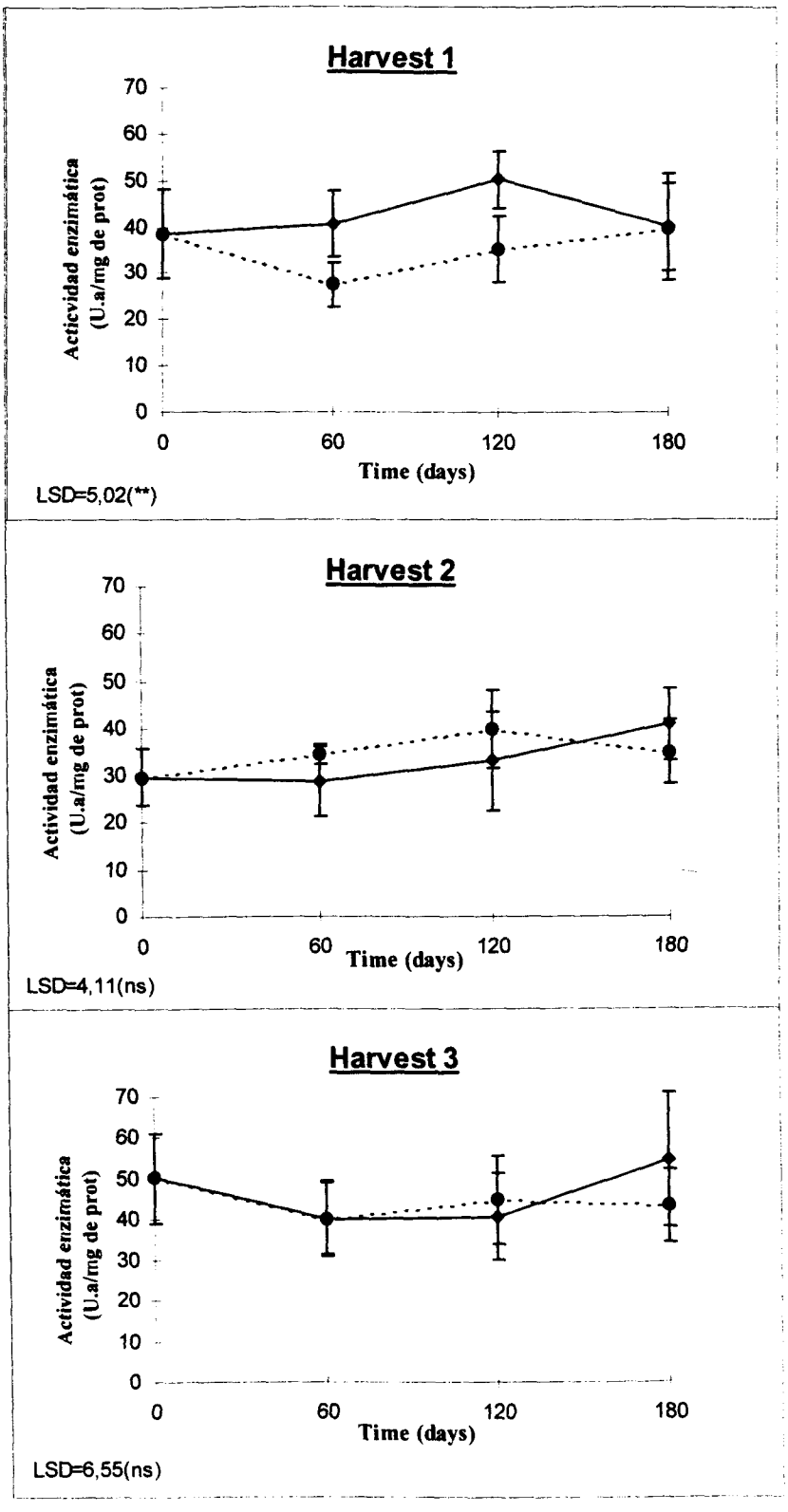

Fig. 21 : Evolution of Peroxidase (POD) activity during storage at various CO_2 level : 0,7% (full line) and 5% (dotted line)

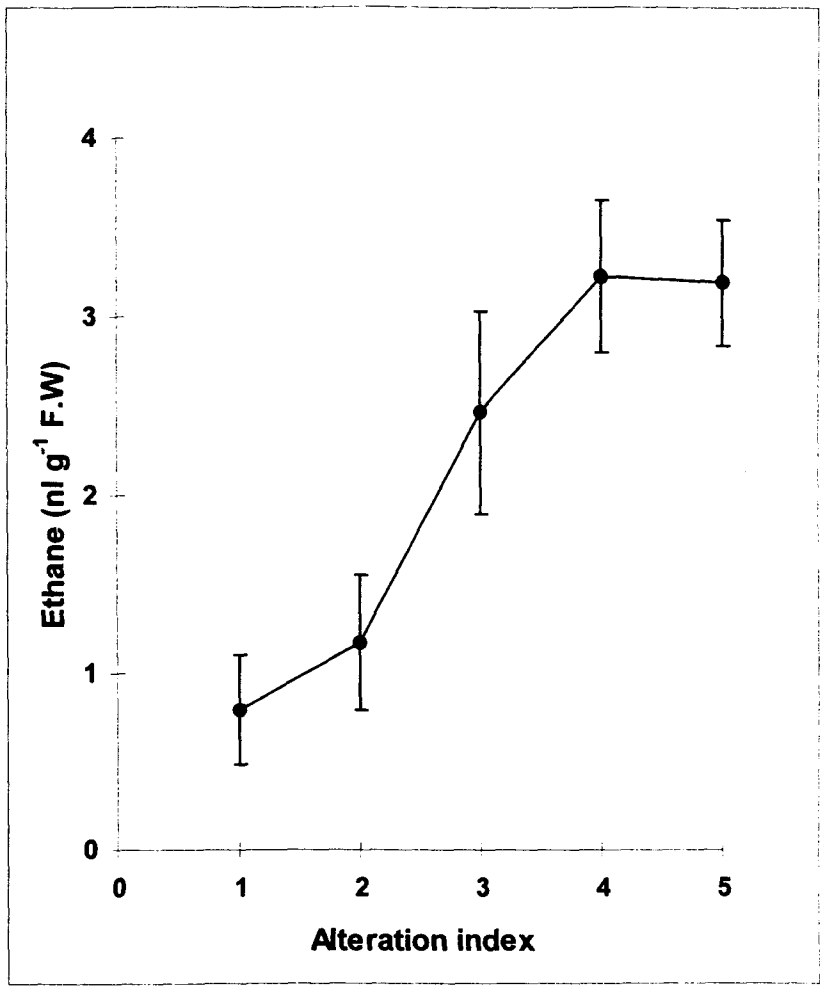

Fig.22: Change in ethane production in relation to the intensity of the damage.

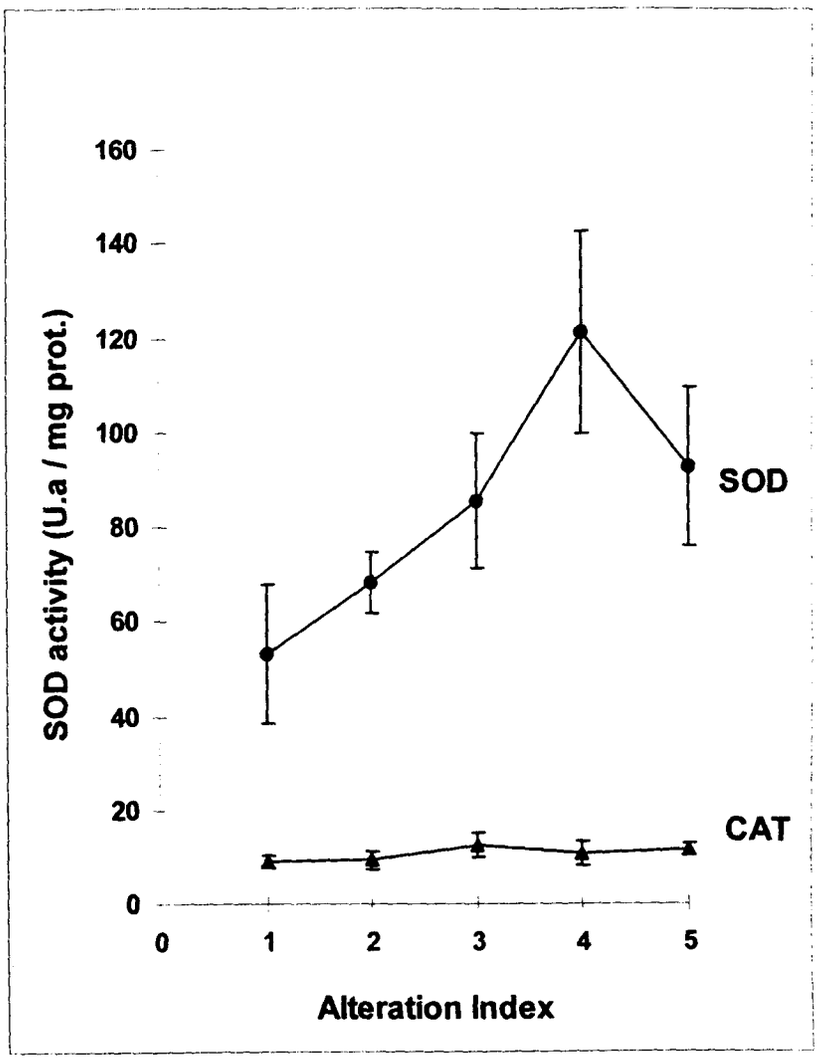

Fig.23: Changes in Superoxide Dismutase (SOD) and Catalase (CAT) activities in damaged fruits. Damage is classified according to an index.

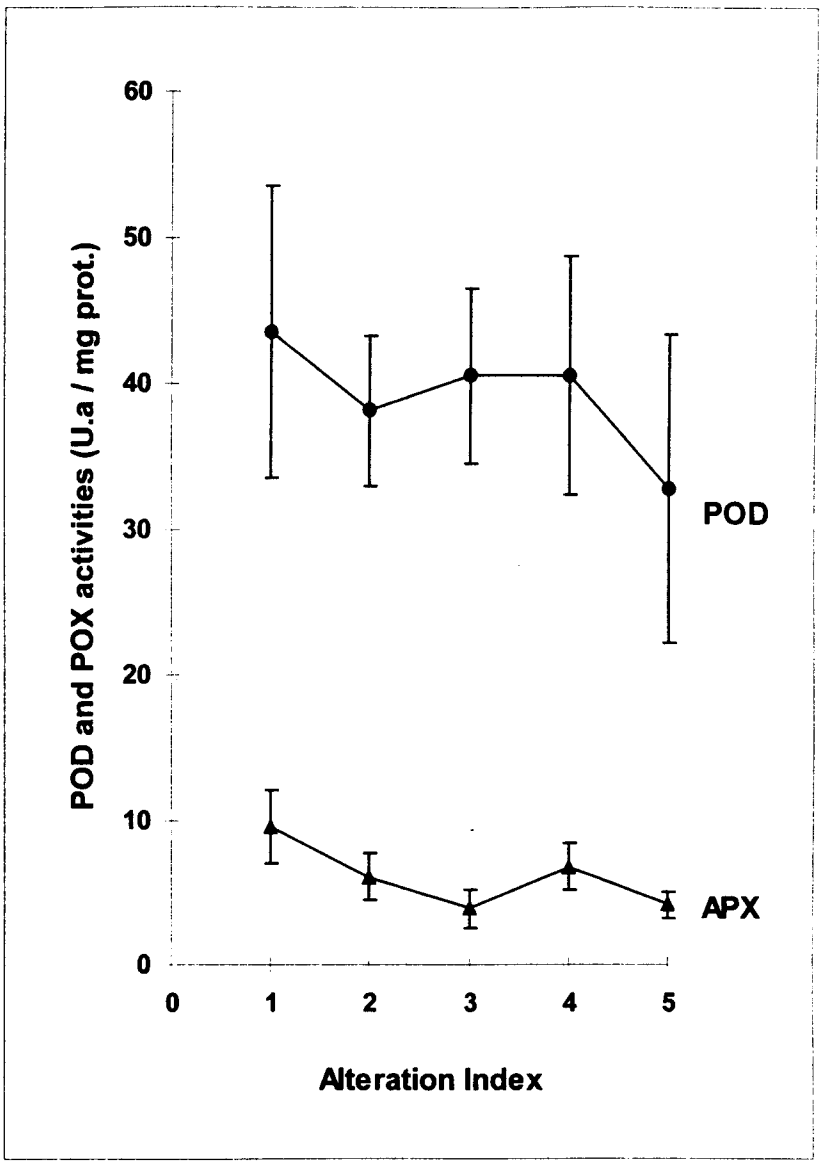

Fig.24: Changes in Ascorbate Peroxidase (APX) and Peroxidase (POD) activities in damaged fruits. Damage is classified according to an index.

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

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

Coordinator: Dr. H.W. Peppelenbos
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Participant no.3:
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Istituto sperimentale per la Valorizzazione Tecnologica dei Prodotti Agricoli
(Research Institute for Agricultural Products Technologies)
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Contractor

A. PARTNER INFORMATION

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ITALY

Scientific team

- Dr. Paola Eccher Zerbini
- Dr. Alberto Pianezzola
- Dr. Anna Rizzolo
- Maurizio Grassi
- Gianni De Colellis.

Objectives

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

Actions in the project

Research work at IVTPA is focused on the relationships between orchard factors (fruit maturity, mineral content) and storage disorders. The work programme includes:
Task 1 - selecting orchards;
Task 2 - picking fruit at different dates;
Task 3 - storing in experimental CA containers;
Task 4 - recording meteorological variables and agronomical features in selected orchards;
Task 6 - setting up the system and methods for gas exchange measurement;
- respiration measurements at harvest and during storage.

Task 7 - analysis of fruit at harvest to evaluate fruit maturity;
- analysis of fruit after storage to evaluate quality by common physical and chemical analysis and sensory analysis to evaluate eating quality during post-storage ripening;
- control of the occurrence of storage disorders;
- setting up the methods for biochemical analysis of fermentative metabolites in the pear tissue.

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

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

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	0.4
No. of man-month devoted already to the task:	0.1

Objectives

Obtaining fruits of known history and growing conditions.

Material and methods

Orchards. Two orchards were selected in a pear growing region (Campogalliano, in 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 was treated for scab and 'brusone'; no treatment was made with growth regulators. Full bloom occurred on 1st April. Severe frost (several degrees below zero) occurred some days after bloom (18 April), with fruits already set. Fruits 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 was treated for scab, 'brusone' and Psylla. Full bloom and severe frost occurred as in orchard 1. 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: $K= [(dl + dq) /2] \times h$.

On each tree the number of fruits visible on one side of the canopy was photographed and counted.

Results and Discussion

Yield capacity. The mean number of fruits produced by trees and yield capacity are reported in Table 1. The yield capacity K seems to be influenced by different planting distances. The number of fruits was affected by the early frost.

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.

Conclusions

Planting density seems to describe well the yield capacity of the trees as estimated by the Winter method, and it is easier to measure. So the yield (number of fruits per tree) can be referred to the number of trees per Ha.

Task 2: Harvest of the pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	3.2
No. of man-month devoted already to the task:	0,4

Objectives
The development of a predictive model requires to obtain fruits of known maturity, presumably with different susceptibility to brown heart.

Material and methods
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 the 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 recognise orchard, harvest, block and tree. The fruits were transported to IVTPA for analysis and storage.

Results and Discussion
Quality of fruits. Analysis at harvest are reported in Table 2. Fruit size and starch hydrolysis increased with harvest time, while firmness decreased. Relative Intercellular Space Volume (RISV) seems to be inversely related to the number of seeds, in fact the fruits of Malaguti orchard had no seeds and had a higher RISV.

Conclusions
Harvesting pears with one week interval resulted in fruit with different maturity, as indicated by weight, firmness, starch hydrolysis and Streif index and different susceptibility to brown heart, as requested.

Task 3: Storage of the pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	5.0
No. of man-month devoted already to the task:	0,8

Objectives
The development of a predictive model requires to store fruits in different known controlled armosphere conditions, which are likely to favour or to prevent the Brown Heart disorder.

Material and methods
Storage. At IVTPA after harvest the pears were left in a cold room at 0/-1 C for one week, then they were stored in controlled atmosphere containers with 2 % oxygen and 0.7 or 5 % CO_2, in the same cold room for 8.5 months.
Two more boxes per orchard and harvest were also stored in each of two commercial stores. In one of them (Campofrigo) after cooling pears for 3 (first harvest fruits) to 1 weeks, the fruits were stored in CA with 4.5 % O_2 + 1.5 % CO_2 for 5 months, then for 1 month with 1.5 % CO_2 and O_2 decreasing from 21 % to 9 %.
In the other (Eurofrutta) after cooling for 17-2 days, the fruits were stored in CA with 1.4% O_2 + 2.4% CO_2 for 40 days, then in 3 % O_2 + 2 % CO_2 for 4.5 months.

Results and Discussion
In the IVTPA storage, 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.
Brown Heart was present in 10% of pears stored in 2% O_2+0.7% CO_2, and in 18% of fruits stored in 2% O_2+5% CO_2.

Conclusions
The gas concentrations used for storage fulfil the requirement of enhancing or reducing the development of Brown Heart.

Task 4: Variation in climate and orchard characteristics

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	0.4
No. of man-month devoted already to the task:	0.1

Objectives
The development of a predictive model requires to know the variations in climate during the growing season between the different orchards and countries.

Material and methods
Meteorological data. Data from March to August 1997 were obtained from two stations. The precipitation data were recorded by one station located in orchard 2, belonging to Consorzio Fitosanitario Obbligatorio Modena. Minimum, maximum and average temperature at a height of 2 m and global radiation (daily integral of energy flux in the 0.3-3000 nm range with a solarimeter Kipp & Zonen) as MJ/m^2 were recorded by the other station, belonging to the Osservatorio Geofisico del Dipartimento di Scienze dell'Ingegneria, Università di Modena (Geophysical Observatory of the Engineering Sciences Dept. of the University of Modena)) located at Marzaglia, at 3 km distance from the orchards. The data from both stations were obtained free of charge for scientific use, provided that the institutions are mentioned when publishing the results.

Results and Discussion
Monthly means are reported in table 3. Frost occurred several times in April during and after bloom. Its effect was worsened by the wind.

Conclusions
It is necessary to compare results from all the partners in order to reach conclusions.

Task 6: Measuring gas exchange and diffusion rates

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	20.0
No. of man-month devoted already to the task:	11,7

Objectives
The development of a predictive model requires the assessment of pear physiology related to gas exchange and diffusion rates.

Material and methods
Setup of the 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 sensible (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 rates 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, and 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. Automatic gas-tight fittings (4 mm diameter, Metalwork Italia) were inserted in the two holes used for inlet and outlet of gas mixtures. 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 analysed 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 analysed 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 types of syringes were tested: The so called "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.

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 analysed. The oxygen decreased more slowly than theoretically 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 analysed 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 analysed at closing and after 1, 2, 3, 4, 5 h. 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 . 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. Based on these results, it was decided to wait for 16 - 24 hours before doing respiration measurements.
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. 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.
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 or 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 due to different gas pressures and temperature between calibration and sample gases. So 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 same gas mixtures, 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. 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, indicating that diffusion was occurring. At the end of stratification experiments, some balloons 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: 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 balloons 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. 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 in the surrounding air the 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. 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: 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 analysed after 5, 24, 30, 48 hours; then the same atmosphere was renewed, and gas was analysed after 24, 30 and 48 hours. 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). The second trial reproduced the situation of respiration measurements of stored fruits: pears, which had been equilibrated for 48 hours either in 2%O_2+5%CO_2 or 2%O_2 + 0.7% CO_2 were put in jars with air or N_2 and gas composition was analysed after 6, 24 and 48 hours. 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.

First method for respiration measurement (set up at harvest). Each pear was put in a jar, the jar cap was tightly closed, and flushed at 110 l/h for 10 min through the fittings with one of four gas mixtures: air, N_2, 2 O_2 + 0.7 CO_2 (2+0.7), 2O_2 + 5 CO_2 (2+5). 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 the respiration time of 16-24 h. The jars were kept at -1 C. For gas sampling, 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.

Second procedure for gas sampling and injection in the GC loop (used in December). After the evidence of insufficient gas tightness of rubber balloons, they were no longer 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 the entrance of oxygen due to the building of 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. After transport to the GC laboratory, the syringe was slightly put in pressure by pushing the plunger before opening and injecting into the GC.

Third procedure for gas sampling and injection in the GC loop (used in March). A new method was devised to sample gas from the respiration jars without using syringes, because the latter could not avoid undue entrance of oxygen. The jars, which were kept at -1 C for 24 h, were taken in the GC laboratory at 20 C for analysis. The temperature difference caused an immediate increase of pressure inside the jar, which was sufficient to transfer a gas sample directly from the jar to the GC inlet valve.

Calculation of gas exchange rates. The total amount (%) of O_2, CO_2 and N_2 were corrected to 100% in one step. In the respiration measurements made at harvest and in December, no correction was made for actual pressure, assuming standard pressure conditions. In the measurements of December, a correction was made where nitrogen apparently increased during the respiration time. In fact the per cent N_2 should remain equal (if only aerobic respiration occurs) or decrease (in case of fermentation). A correction was also made for the entrance of air in the sampled nitrogen atmosphere, due to the use of syringes. In March a pressure measuring instrument was bought before the last respiration measurements. Actual pressure and temperature were recorded in steady state conditions before each sampling, and the data were corrected for actual pressure and temperature.

Diffusion rates. The measurement of diffusion rates was not originally planned in the first year of the project, but the evidence of diffusion occurring during respiration measurements induced to advance it to this year. Diffusion rates were measured following the paper of Banks (1985) by preloading fruits with a certain amount of Neon in the atmosphere (20 ml per jar in December, 7 ml per jar in March) during the equilibration before respiration measurement. The gas sampling for diffusion followed the exponential model in December and both the linear and exponential models in March. A dedicated detection system for Neon had to be bought. Unfortunately for Ne analysis the gas sampling has still to be done with syringes.

Fruits. At harvest one fruit for each block, orchard and harvest was used for each of the four respiration atmospheres. During storage in December and March respiration and diffusion measurements were made on one fruit per block, orchard, harvest and storage atmosphere in each of three respiration atmospheres: the same as that in storage, N_2 and air.

Results and Discussion

Respiration measurements. At harvest in many jars strange results were found, as O_2 increased and CO_2 decreased; in some cases O_2 was found in N_2 jars (Table 4). Some of these effects are due to diffusion (e.g. the reduction of CO2 in the 2+5 atmosphere), some may be due to leaks in the jar and to gas permeability of the rubber balloons, and many are due to the use of syringes for gas sampling and injection in the GC. The only measures which are less influenced by these disturbances are those made in air. In December, with the elimination of the rubber balloons and with the corrections adopted for the calculations, the results were slightly better, but still the use of syringes allowed undue entrance of air in the sampled gas. In March the new method for direct sampling from the jar allowed finally to record 0% O_2 in all the nitrogen atmosphere samples without further corrections. Moreover, the measurement of pressure inside the jars allowed to check their tightness during the experiment and to correct the problem, thus eliminating leakage in the respiration measurement phase. The results of March indicate that oxygen consumption is lower in 2+5 atmosphere than in 2+0.7, while CO_2 production in N_2 is higher than in the other atmospheres.

Diffusion resistance. Diffusion resistance was calculated by two methods, linear and exponential (R-exp and R-lin in Table 5), but the two methods were not correlated (r=0.006). If the value of diffusion resistance by the exponential method was calculated using the internal gas volume as indicated by Banks (1985) (R-exp-Ne in Table 5) instead of that indicated by Baumann and Henze (1985), then the correlation with the linear method improved slightly (r=0.38). The results however are very variable, and the method still needs to be refined.

Conclusions

The equipment and method for gas exchange analysis was successfully set up as regards gas tight containers, time of flushing, gas sampling, time of equilibration before respiration measurement, time of respiration. Jars and fittings proved to be gas tight, and the measurement of pressure allows to detect any leaks due to incorrect closing of the cap. Only for diffusion measurements, which must be carried out within seconds after the closure, leaks cannot be detected in time. The method set eventually for gas sampling for respiration measurement prevents undue entrance of air in the sample and allows to measure the low amounts of gases involved in respiration. There is still a certain variability in the responses of the GC, which can be partially reduced by improved calibration procedures. The measurement of diffusion rates needs still some improvement.

Task 7: Destructive measurements

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	20.0
No. of man-month devoted already to the task:	5.7

Objectives

The development of a predictive model requires the assessment of pear physiology related to fermentation metabolism and to mineral content, and the evaluation of the quality of fruit after storage.

Material and methods

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 same measurements were made in January and at the end of storage in May, after 5 and 8.5 months' storage at IVTPA. In January only 6 fruits were examined per orchard, harvest and storage atmosphere, while in May 20 fruits.
At the end of storage, after one week at 18 C, 20 pears per treatment were subjected to sensory analysis with a laboratory panel of 10 experienced people, who evaluated the intensity of eight attributes (firmness, juiciness, graininess, sweetness, acidity, astringency and aroma) on unstructured scales. Data from each panellist were standardised.
After storage of one (IVTPA) or two (commercial stores) boxes of pears per orchard, harvest and storage atmosphere were examined for storage disorders immediately after storage and after one week at room temperature.

Biochemical studies. 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 homogenised. All the portions were frozen and kept at -20 C. Lactic acid, ethanol and acetaldehyde were also analysed in fruits examined in January for quality.

Mineral analysis. Mineral analysis was planned one month before harvest and at harvest. On July 17 some fruits were sampled for mineral analysis. Two fruits / tree were sampled from 6 trees in each group (harvest time), obtaining a total of 144 fruits (2 fruits x 6 trees x 3 groups x 2 blocks x 2 orchards). The fruits were frozen for subsequent preparation and analysis. Pooled samples were obtained by pooling the fruits of the same group and block.
Mineral analysis at harvest was made on the same fruits used for quality evaluation.

Statistical analysis. The data were processed by analysis of variance using the GLM procedure of SAS System.. Per cent data were subjected to angular transformation.

Results and Discussion

Quality analysis. For the results of quality analysis at harvest see under Task 2. Main differences in the quality of fruit during and after storage were found in firmness and colour (Table 6). Pears stored in 2+5 were significantly firmer than those stored in 2+0.7 both in January and in May. In May firmness decreased slightly with harvest delay in 2+5 stored pears, and increased with harvest delay in 2+0.7 stored pears. Firmness was higher in pears from orchard Malaguti than from orchard Rinaldi. Skin colour was greener in 2+5 atmosphere throughout the storage. In January greenness decreased with harvest delay, but in May no difference in colour was significant as regards harvest.
As regards sensory analysis after shelf-life, significantly different means are reported in Table 7. Only a few sensory variables were affected by the treatments. Sensory firmness was significantly affected by storage atmosphere and harvest time: after shelf life on the average the pears stored in 2+5 were firmer than those stored in 2+0.7. The firmest fruits were 1st harvest fruits in the former atmosphere, and 3rd harvest fruits in the latter. Second harvest fruits were the less firm after shelf-life. Sensory juiciness and acidity decreased with harvest delay.
Storage disorders found in pears were scald, decay and brown heart. Decay was present with low incidence, without remarkable differences between treatments. Scald was different in the two orchards: it was higher in orchard Rinaldi than in Malaguti, and it increased with harvest delay (Table 8). At the end of storage scald incidence was slightly lower in fruits stored in Eurofrutta than in Campofrigo, but after shelf-life this difference disappeared. Brown heart incidence was not different in the two orchards but it was significantly affected by harvest time and storage conditions: it increased with harvest delay, and it was higher in Eurofrutta than in Campofrigo, and in 2+5 than in

2+0.7 (Table 9). Brown heart incidence was higher at IVTPA than in commercial stores: this could be due to the different length of storage, or to the different storage conditions, or to the different amount of fruits available.
As regards the development of brown heart during storage, which was checked on the same fruits used for respiration measurements and biochemical studies, no difference was found with time of storage in the frequency of affected fruits between December and March (Table 10). Only the appearance of the disorder changed with time, increasing from barely detectable as black points in the flesh, to clearly visible as large cavities or spongy tissue, and increasing the amount of browning. The region where the disorder begins to occur is clearly defined: at least 0.5-1 cm below the skin, and outside the core.

Biochemical studies. In fruits subjected to respiration measurement ethanol content significantly increased on the average with decreasing oxygen and with higher CO_2 in the respiration atmosphere (Table 11). However ethanol content in fruits subjected to respiration in nitrogen atmosphere is lower after 2+5 storage than after 2+0.7 storage, indicating perhaps adaptation to fermentation inducing atmospheres. During storage ethanol content increases with time, and it is higher on the average with the 2+5 storage atmosphere (Table 12). In the latter atmosphere, 2nd and 3rd harvest fruits had a much higher ethanol content than first harvest fruits, while at harvest and in the 2+0.7 atmosphere the differences were negligible (Table 13).
Lactic acid content was higher in fruits stored in 2+5, while that of fruits stored in 2+0.7 was not different from that found at harvest (Table 14). Lactic acid content was also significantly affected by harvest time, and was generally lower in first harvest fruits (Table 15). Acetaldehyde content was much less variable between treatments than ethanol, with the exception of fruits of block 2 stored in 2+5 and examined in December, which showed higher amounts of this compound.

Mineral analysis. K content was not affected by any of the treatments (Table 16). Ca content significantly decreased from July to harvest time, and during harvest time. Ca content was lower in Malaguti orchard than in Rinaldi orchard, and the opposite was true for Mg content. Mg increased from July to August, but during harvest time it varied without a definite trend. The ratio K/Ca followed closely and inversely the trends of Ca content: it was higher in Rinaldi orchard, and increased irregularly with time. No relationship was apparent between mineral content and brown heart.

Conclusions
According to these results, brown heart depends mainly on the maturity of pears and on storage conditions. No differences were found between orchards in this respect. Brown heart seems to be not related to mineral content. First occurrence of the disorder can be detected already in December. Brown heart is related to fermentative activity as indicated by the higher ethanol content in the storage treatment with higher incidence of brown heart. By acting on gas concentrations in storage it will be possible to reduce brown heart incidence.

Task 10: Validation of the models

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	0

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

Conclusions
In the first year no model validation is possible, because the first predictions are generated halfway the second year.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997 TO 31-05-1998

Task 6. The equipment and methodology had to be set *ex novo*. Main problems for respiration measurements were:
1) Our gas chromatograph (GC) is different from that of the other partners who all have the same type of GC, so exchange of information with expert partners regarding calibration methodology etc. was not possible. A great amount of time and work was spent in testing different possible causes of errors.
2) With our GC the analysis takes 6 min, while with the GC of partners it takes less than 2 min: this means the impossibility to analyse a large number of samples.
3) The GC laboratory is quite distant from the storage room, so it took some time to transfer gas samples from the storage room to the laboratory. It was not possible to accumulate many samples in order to reduce transport time, because leak problems could increase with the delay from sampling to analysis.
4) The type of analysis is such, that generally it is not possible to correct errors or mistakes, and so repeat the analysis. So we were compelled to reduce the number of gas exchange measurements during storage of pears. On the other hand, diffusion measurements which were not scheduled in the first year, were anticipated to this year. For diffusion measurements a different GC had to be found for Ne analysis, and the methodology had to be set, which still needs some improvement. The time and amount of work requested by diffusion measurement and Neon analysis is another limiting factor for the number of samples which can be analyzed.

Task 4. Some difficulties were encountered in the application of the Winter method for evaluating tree load.

Task 7. The methodology for the measurement of juice density and for lactic acid analysis was set up, and that for ethanol and acetaldehyde analysis was improved. The setting up of methods, especially for HPLC lactic acid analysis, was time consuming.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

In the second year it is planned to repeat the work done in the first year, with some changes:
Task 1 - only one orchard will be selected, as no differences in Brown heart were found between orchards.;
Task 2 - as in the first year
Task 3 - as in the first year, but a larger number of fruit will be stored, because it is planned to increase storage space at IVTPA;
Task 4 - as in the first year
Task 6 - as in the first year. The diffusion resistance measurement will be tested with different methods.
Task 7 - as in the first year except for biochemical analysis: vitamin C will be analysed instead of fermentative metabolites. If more fruits will be available, more attention will be dedicated to the first months of storage, when the disorder begins to occur.
Task 10- as in the first year

E. DISSEMINATION

Eccher Zerbini P., M. Grassi. Influenza dell' irrigazione sulla qualità delle pere Conference conservate in atmosfera a basso ossigeno. Atti IV Giornate Scientifiche S.O.I., Sanremo, 1-3 aprile 1998.

F. ANNEX

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

		ORCHARD					
		M : 18-years-old trees, 1905 trees/Ha			**R** : 6-years-old trees, 1250 trees/Ha		
		block			block		
Harvest	Data	1	2	Mean M	1	2	Mean R
1	n.fruits/tree	131	98	125	147	130	139
	K=(dl+dq)*h/2	39802	43388	41595	48735	50231	49444
	trunk section cm^2	164,04	174,05	169,04	63,47	67,08	65,18
2	n.fruits/tree	67	50	59	84	139	114
	K=(dl+dq)*h/2	35401	35479	35440	53230	51202	52269
	trunk section cm^2	165,43	208,90	187,17	70,94	76,16	73,41
3	n.fruits/tree	42	70	55	85	91	88
	K=(dl+dq)*h/2	34751	35185	34955	49315	47762	48538
	trunk section cm^2	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)	n° of seeds per fruit	% of fruit with seeds
Rinaldi	12-aug	219.08	218.62	1.002	1.068	6.131	70	98	-	95
Rinaldi	20-aug	231.44	230.16	1.006	1.057	4.824	73	102	3.1	65
Rinaldi	27-aug	273.50	272.75	1.003	1.051	4.546	76	163	0.75	55
Rinaldi	*mean*	*241.34*	*240.51*	*1.004*	*1.059*	*5.167*	*73*	*121*	*1.9*	*72*
Malaguti	12-aug	183.45	183.52	1.000	1.056	5.364	65	101	-	45
Malaguti	20-aug	214.37	214.33	1.000	1.072	6.665	68	105	0.15	10
Malaguti	27-aug	233.24	234.33	0.996	1.061	6.114	71	109	0.55	40
Malaguti	*mean*	*210.35*	*210.73*	*0.999*	*1.063*	*6.048*	*68*	*122*	*0.35*	*32*

Orchard	harvest date	L*	a*	b*	hue (rad)	chroma	firmness (N)	starch hydrol. (%)	soluble solids (° Bx)	Streif index
Rinaldi	12-aug	57.70	-16.14	36.74	1.983	40.13	63.2	4.75	12.9	1.051
Rinaldi	20-aug	60.62	-16.26	36.00	1.993	39.51	63.1	9.75	13.4	0.492
Rinaldi	27-aug	60.49	-15.95	36.16	1.985	39.53	60.6	16.50	12.4	0.302
Rinaldi	*mean*	*59.60*	*-16.12*	*36.30*	*1.987*	*39.72*	*62.3*	*10.33*	*12.9*	*0.476*
Malaguti	12-aug	56.00	-16.02	34.87	2.000	38.37	66.1	3.75	11.8	1.522
Malaguti	20-aug	55.86	-14.07	33.20	1.970	36.06	66.7	10.00	11.9	0.572
Malaguti	27-aug	59.30	-15.84	34.76	1.997	38.20	61.7	11.50	12.0	0.455
Malaguti	*mean*	*57.05*	*-15.31*	*34.28*	*1.989*	*37.54*	*64.8*	*8.42*	*11.9*	*0.660*

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

Table 3. Meteorological data (monthly means) recorded near the orchards during the 1997 season.

DAY	*temperature at 2 m*			*precip.*	*global radiation*	*humidity at 2 m*		
	Min (°C)	Max (°C)	Aver (°C)	mm	MJ/m^2	Min	Max	Aver
April	2,9	18,6	10,8	0,8	19,8	76	34	62
May	10,0	24,7	17,6	0,8	21,5	76	45	69
June	15,0	26,3	20,4	4,1	19,8	87	68	83
July	15,2	29,9	22,5	0,8	24,9	81	50	75
August	16,5	30,1	22,9	0,4	20,2	83	50	73

Table 4. Respiration measurements at harvest and during storage.

storage time	orchard	storage atmosphere	harvest	O_2 µmol/(kg.h)				CO_2 µmol/(kg.h)			
				respiration atmosphere				*respiration atmosphere*			
				2+0.7	*2+5*	*air*	*N2*	*2+0.7*	*2+5*	*air*	*N2*
at harvest	Malaguti	-	1	323,96	26,64	-65,68	55,31	32,45	-183,34	69,99	87,76
			2	-46,22	-10,95	-56,52	22,73	45,64	-97,48	62,24	64,13
			3	11,96	-16,73	13,33	18,42	32,24	-72,04	48,18	59,64
	Rinaldi	-	1	-1,96	-9,20	-80,58	55,88	18,86	-147,55	54,10	45,35
			2	-33,81	106,91	-51,33	2,66	34,54	-91,95	50,71	50,42
			3	11,19	-5,93	-28,65	17,07	34,80	-63,16	56,94	56,29
december	Rinaldi	2+0.7	1	-29,55		-22,91	0,00	29,74		22,91	34,80
			2	-25,07		-26,65	0,00	25,07		26,65	39,54
			3	-24,50		-20,59	0,00	24,50		20,59	31,48
		2+5	1		-8,08	-24,75	0,00		8,34	24,75	31,02
			2		-27,07	-31,25	0,00		27,07	31,25	39,88
			3		-44,23	-36,87	0,00		44,23	36,87	44,19
march	Rinaldi	2+0.7	1	-24,18		-43,15	0,00	38,71		33,05	44,16
			2	-33,64		-34,94	0,00	29,38		29,10	35,45
			3	-20,10		-38,67	0,00	31,68		31,96	54,26
		2+5	1		-5,74	-55,27	0,00		39,41	35,45	49,06
			2		-13,17	-35,22	0,00		34,29	48,49	58,31
			3		-8,59	-46,17	0,00		32,17	41,09	56,88

Table 5. Resistance to gas diffusion calculated by different methods: by exponential analysis without correction for internal volume (R-exp) and with correction for internal volume (R-exp-Ne), and by linear analysis (R-lin). Vi and Vi-Ne are internal volume calculated respectively according to Baumann and Henze (1985) , and to Banks (1985). Each value is the mean of 6 fruits.

storage time	harvest	storage conditions	Vi cm^3	Vi -Ne cm^3	R-exp s/cm	R-exp-Ne s/cm	R-lin s/cm
	1	2+0.7	12,13	33,60	6805	2768	
	2	2+0.7	16,64	39,68	5894	2602	
	3	2+0.7	25,40	43,28	5006	2887	
	mean	2+0.7	17,97	38,90	5901	2743	
Dec							
	1	2+5	14,40	24,37	12938	8331	
	2	2+5	13,22	18,86	11736	8268	
	3	2+5	14,37	20,92	11200	8164	
	mean	2+5	14,00	21,38	11958	8254	
	1	2+0,7	11,78	16,03	7528	6729	4749
	2	2+0,7	13,39	19,88	8976	5858	7366
	3	2+0,7	15,84	18,21	10556	8064	5567
	mean	2+0,7	13,78	18,16	9108	6893	5962
March							
	1	2+5	10,83	16,63	5398	3136	2492
	2	2+5	22,54	29,79	10231	6184	6246
	3	2+5	17,08	32,20	6663	5039	4973
	mean	2+5	17,24	26,89	7576	4904	4719

Table 6. Means for qualitative variables during and after storage for fruits harvested at different dates from two orchards and stored in different conditions.

storage time	storage conditions	orchard	harvest	firmness (N)	L*	hue (rad)	chroma	soluble solids (°Brix)	titratable acidity (meq/10g)
			1	60.7	60.30	1.871	37.09	12.7	
		Malaguti	2	55.1	64.63	1.856	40.88	14.1	
			3	54.4	64.41	1.816	40.02	13.6	
			m	*56.8*	*63.11*	*1.848*	*39.33*	*13.5*	
	2+0.7		1	34.7	63.62	1.889	39.43	13.1	
		Rinaldi	2	35.7	65.58	1.864	40.90	13.9	
			3	33.9	65.28	1.878	41.47	14.1	
			m	*34.8*	*64.82*	*1.877*	*40.60*	*13.7*	
		mean		45.8	63.97	1.862	39.97	13.6	
january									
			1	60.8	59.90	1.924	38.02	13.4	
		Malaguti	2	62.1	57.09	1.894	35.39	13.6	
			3	59.3	62.57	1.853	37.96	14.3	
			m	*60.7*	*59.85*	*1.890*	*37.12*	*13.8*	
	2+5		1	40.0	60.55	1.926	37.96	14.1	
		Rinaldi	2	38.3	62.90	1.872	38.81	14.0	
			3	40.7	64.98	1.885	40.23	14.6	
			m	*39.6*	*62.81*	*1.895*	*39.00*	*14.2*	
		mean		50.2	61.33	1.892	38.06	14.0	
			1	51.3	63.50	1.865	38.89	12.9	2.30
		Malaguti	2	50.5	62.72	1.884	38.68	13.3	1.84
			3	53.5	62.01	1.849	37.94	13.4	2.39
			m	*51.8*	*62.74*	*1.866*	*38.50*	*13.2*	*2.15*
	2+0.7		1	47.9	65.54	1.826	40.18	13.3	2.03
		Rinaldi	2	51.3	65.66	1.895	40.41	13.4	1.90
			3	50.4	65.60	1.857	40.48	14.1	1.82
			m	*49.9*	*65.60*	*1.859*	*40.36*	*13.6*	*1.92*
		mean		50.8	64.17	1.862	39.43	13.4	2.05
may									
			1	66.8	59.91	1.899	36.59	13.2	2.24
		Malaguti	2	63.0	60.56	1.944	37.48	13.6	1.96
			3	60.9	61.14	1.926	36.23	13.7	1.79
			m	*63.5*	*60.54*	*1.923*	*36.77*	*13.5*	*2.00*
	2+5		1	59.2	63.10	1.908	38.01	13.3	1.40
		Rinaldi	2	61.1	62.83	1.903	38.58	13.6	1.97
			3	60.8	62.47	1.901	37.89	14.0	1.38
			m	*60.4*	*62.80*	*1.904*	*38.16*	*13.3*	*1.58*
		mean		61.9	61.67	1.913	37.46	13.6	1.79

Table 7. Sensory analysis of pears: significantly different means of standardized data .

storage atmosphere	harvest	firm	juicy	acid
	1	50	51	60
2+0,7	2	40	45	45
	3	43	48	48
	mean	44	48	51
	1	53	59	54
2+5	2	50	53	49
	3	64	45	45
	mean	56	52	49

Table 8. Incidence of scald after 7 months of storage and 1 week of shelf-life in pears harvested at different times and stored at IVTPA in different conditions. Effect of orchard and harvest.

orchard	harvest	%
	1	7,4
Malaguti	2	6,9
	3	6,7
	m	7,0
	1	41,0
Rinaldi	2	51,2
	3	74,1
	m	55,5
	1	24,2
	2	29,0
	3	40,4

Table 9. Incidence of Brown Heart (%) in pears harvested at different times and stored in different conditions.

storage atmosphere	storage time	harvest			
		1	2	3	m
Campofrigo	march	0,0	0,0	0,5	0,2
Eurofrutta	march	0,0	4,0	4,6	2,9
2% O_2+0,7% CO_2	may	4,0	6,6	20,2	10,3
2% O_2+5% CO_2	may	6,0	29,5	41,8	25,8

Campofrigo = 4.5% O_2 + 1.5 CO_2 for 5 months, then air;
Eurofrutta = 1.4% O_2 + 2.4% CO_2 for 40 days, then 3% O_2 + 2% CO_2.

Table 10. Incidence of Brown Heart (%) during storage in pears used for respiration and biochemical analysis.

		% Total Brown Heart			
		1	2	3	m
dec	2+0,7	0,0	0,0	16,7	5,6
	2+5	0,0	33,3	66,7	33,3
jan	2+0,7	0,0	0,0	0,0	0,0
	2+5	0,0	0,0	50,0	16,7
march	2+0,7	0,0	0,0	33,3	11,1
	2+5	0,0	50,0	50,0	33,3
		% cavity			
		1	2	3	m
dec	2+0,7	0,0	0,0	16,7	5,6
	2+5	0,0	33,3	66,7	33,3
jan	2+0,7	0,0	0,0	0,0	0,0
	2+5	0,0	0,0	50,0	16,7
march	2+0,7	0,0	0,0	33,3	11,1
	2+5	0,0	16,7	50,0	22,2
		% browning			
		1	2	3	m
dec	2+0,7	0,0	0,0	0,0	0,0
	2+5	0,0	0,0	0,0	0,0
jan	2+0,7	0,0	0,0	0,0	0,0
	2+5	0,0	0,0	0,0	0,0
march	2+0,7	0,0	0,0	0,0	0,0
	2+5	0,0	33,3	16,7	16,7

Table 11. Significantly different means for ethanol headspace content in fruits from orchard Rinaldi harvested at different dates and examined for respiration at harvest, in December and in March.

storage conditions	respiration conditions	ethanol (mg/100g)
at harvest	2+0,7	0,1209
	2+5	0,1438
	air	0,1341
	N2	0,5182
2+0,7	2+0,7	2,0604
	air	1,6967
	N2	8,1642
2+5	2+5	3,8340
	air	2,5280
	N2	6,4523
	2+0,7	1,4727
	2+5	2,5688
	air	1,7034
	N2	5,9238

Table 12. Significantly different means for ethanol and acetaldehyde headspace content during storage in fruits from orchard Rinaldi harvested at different dates and kept only in 2+5 or 2+0.7 atmosphere. Effect of storage time.

storage conditions	storage time	acetaldehyde (mg/100g)	ethanol (mg/100g)
-	at harvest	0,2783	0,1303
2+0,7	nov	0,6946	1,2861
	jan	0,8401	1,7949
	mar	0,5809	2,7701
	m	0,7055	1,9694
2+5	nov	1,5810	3,3717
	jan	0,8646	2,0277
	mar	0,7794	4,2579
	m	1,0605	3,2147
	nov	1,1378	2,3289
	jan	0,8523	1,9113
	mar	0,6802	3,5140

Table 13. Significantly different means for ethanol headspace content in fruits from orchard Rinaldi harvested at different dates and kept only in 2+5 or 2+0.7 atmosphere. Effect of harvest.

harvest	storage conditions	ethanol (mg/100g)
1	at harvest	0,0943
	2+0,7	2,5132
	2+5	1,9867
2	at harvest	0,0759
	2+0,7	1,6087
	2+5	3,8602
3	at harvest	0,2510
	2+0,7	1,7561
	2+5	3,6949

Table 14. Significantly different means for lactic acid content in fruits from orchard Rinaldi harvested at different dates and examined for respiration at harvest, in December and in March.

storage time	harvest	lactic acid (mg/100g FW)
at harvest	1	10.076
	2	23.530
	3	15.347
dec	1	24.541
	2	23.116
	3	20.970
march	1	13.671
	2	22.774
	3	28.936
	1	16.849
	2	23.091
	3	22.128

storage conditions	lactic acid (mg/100g FW)
at harvest	16.243
2+0,7	18.554
2+5	26.116

Table 15. Significantly different means for lactic acid content during storage in fruits from orchard Rinaldi harvested at different dates and kept only in 2+5 or 2+0.7 atmosphere.

storage time	harvest	storage conditions	lactic acid (mg/100g FW)
	1	-	10.570
at harvest	2	-	37.973
	3	-	10.675
	1	2+0.7	8.350
	1	2+5	15.603
Dec	2	2+0.7	30.585
	2	2+5	42.785
	3	2+0.7	12.928
	3	2+5	37.907
	1	2+0.7	47.054
	1	2+5	6.896
Jan	2	2+0.7	23.824
	2	2+5	29.735
	3	2+0.7	19.369
	3	2+5	31.904
	1	2+0.7	7.836
	1	2+5	6.753
March	2	2+0.7	14.753
	2	2+5	20.409
	3	2+0.7	40.135
	3	2+5	16.905

Table 16. Mean values of mineral content of Conference pears one month before harvest and at harvest.

time	orchard	harvest	K (mg/100g f.w.)	Ca (mg/100g f.w.)	Mg (mg/100g f.w.)	K/CaF
		1	106,79	2,43	7,44	45,65
	Malaguti	2	142,11	2,67	14,29	55,56
		3	125,94	1,80	11,79	70,62
17-jul-97		*m*	*123,55*	*2,31*	*10,88*	*56,38*
		1	112,87	3,59	4,33	31,40
	Rinaldi	2	131,15	3,13	6,50	42,37
		3	120,14	3,30	7,34	36,37
		m	*121,39*	*3,34*	*6,06*	*36,71*
		1	155,59	1,88	12,83	86,01
	Malaguti	2	138,64	1,62	11,27	93,48
		3	104,98	1,66	10,63	63,19
at harvest		*m*	*133,07*	*1,72*	*11,58*	*80,89*
		1	87,13	2,06	10,55	43,04
	Rinaldi	2	127,13	2,06	10,65	63,39
		3	121,45	1,79	9,73	69,38
		m	*111,90*	*1,97*	*10,31*	*58,60*
	Malaguti		128,12	2,03	11,22	68,15
	Rinaldi		116,64	2,66	8,18	47,66
17-jul-97			122,51	2,80	8,57	46,94
at harvest			122,49	1,85	10,94	69,75
		1	115,08	2,49	8,71	51,18
		2	134,76	2,37	10,68	63,70
		3	118,13	2,14	9,87	59,89

Methods

Ethanol and acetaldehyde measurements

Sample preparation: 9-11 g of fruit pulp is sliced, homogenised, 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 µ m 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 homogenised in a 100 ml centrifuge tube in the presence of 25 ml of water (HPLC degree); then the suspension is centrifuged at 6000 g for 20 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. The conditions of HPLC analysis were: column: Inertsil C8 (4.6 x 250 mm, 5µm); mobile phase: 0.2 M H_3PO_4 ; flow rate: 0.8 ml/min, column temperature: 20 C (ambient); detection: UV 210 nm; sample amount: 20 µl.

Method for measuring Relative Internal Space Volume (RISV) used at IVTPA.

Fruit density. The volume of fruit is measured underwater as described by Baumann and Henze (1983). The axis of the fruit is positioned vertical instead of horizontal. Weight and volume are measured with an electronic toploader (0.01 g precision). Fruit density is obtained by dividing the weight of fruit by that underwater.

Juice extraction. The fruit or a part of it is put in a plastic PE bag and frozen at -20 C for later analysis of juice. The frozen fruit in bag is taken out of the freezer and put in a cold room at +2 C for slow thawing overnight. The plastic bag prevents condensation of water on the fruit. Then the fruit is taken at room temperature, taken out of the bag and pressed to extract juice.

Juice density. Exact volume of juice or distilled water is taken with a precision positive displacement pipette (Microman, Gilson Medical Electronics; maximum volume 250 µl) with a piston inside the capillary for ejecting the liquid. With the micropipette 250 µl of distilled water are sampled and weighted on an analytical balance (0.0001 g precision). Measures are repeated until two weights of the same sample differ less than 2 mg. It is necessary to wash the pipette with the liquid 2-3 times before obtaining stable measurements. With the micropipette 250 µl of juice are sampled after 'washing' the pipette 2-3 times with the same juice, and weighted. Every 5 measurements of juice samples, the capillary of the micropipette is changed. Whenever the capillary is changed the measurement of the weight of 250 µl of distilled water is repeated. Juice density is obtained by dividing the weight of 250 µl juice by that of 250 µl water measured with the same capillary.

RISV. Relative Internal Space Volume (%) = 100 x (1- fruit density/juice density).

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 4 total cost: (50%)	337.4 kECU	*EC contribution to partner no. 5:*	168.7	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

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A. PARTNER INFORMATION

Research Station for Fruit Growing
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4475 AN Wilhelminadorp
The Netherlands

Scientific team
Dr. A. de Jager
B.Sc. F.P.M.M. Roelofs

Objectives
See Technical Annex

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

7a. Quality evaluation
7c. Mineral analysis
8. Non-destructive measurements
10. Model validation
11. Dissemination of results

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

Task 1. Cultivation of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	0.5

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

Material and Methods
Experiments were conducted in existing orchards. At first, seven orchards were selected representing cases from insensitive to very sensitive to brown heart. Since, as a consequence of spring frost, most orchards contained only parthenocarpic pears, four orchards were added in a later phase, making a total of 11 orchards. These orchard were located in the following three areas: Zeeland (south western part), Hoekse waard (south of Rotterdam) and Betuwe (central part between rivers Rhine, Waal and Maas). In these orchards treatments were carried out according to the special purpose. In all experiments the basic unit consisted of a field of at least three adjacent trees. The growers were asked to follow their normal cultivation program unless this interfered with a special treatment.

Results and Discussion and Conclusions

See the other tasks

Task 2. Harvest of Pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	4.0
No. of man-month devoted already to the task:	1.0

Objectives
See other tasks

Material and Methods
Pears were picked weekly at five dates, from two weeks before optimum harvest date to two weeks after. Optimum harvest date was estimated according to firmness. A firmness of 6.3 kg with 7 mm plunger (penetrometer) is considered to represent the end of the 'picking window' for optimum harvest period. Fruits were harvested on September 5th, 12th, 19th, 25th and October 2nd for the experiments of task 3, 4(1) and 5 and on September 2nd, 9th, 16th, 23th, 30th for the experiments of task 4(2), 4(3) and 4(4). These dates are represented in this report by pick 1 to 5.

Immediately after harvest samples of 25 fruits were used for quality and maturity measurements for each (pre-harvest) object. Fruit weight, specific weight and density (Mettler PM 600 in gram), ground colour (Minolta CR 300 in L*a*b), firmness (radial and axial) (Instron, 7mm plunger in 2 sec to 8 mm depth), starch (visual, step 1-10), total soluble solids concentration (TSS, with Atago DBX 55 in □Brix) and acidity (% malic acid) were measured at each harvest date. Mineral analysis were done on the samples of August 19th (one month before estimated optimum harvest date) and September 19th (estimated end of safe picking period). Mineral analysis, total soluble solids and acidity were measured in a mixed sample, the other measurements were done on individual fruits.

Fruits were stored in containers of about $1m^3$. Each container represented one treatment and was filled with fruits of one picking date of several orchards. Spatial arrangement of the boxes in the container was at random with respect to orchard. Storage conditions were normally reached within 24 hours and were monitored by measurements each two hours.
From each harvest date all fruits from three trees were randomised and then (in the standard scenario) stored for 7 days at -0.5 °C if not stated otherwise. Then storage was continued at -0.5 °C, 2% O_2 and 0.5 % CO_2.

Results and Discussion and Conclusions
See the other tasks

Task 3: Storage of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	18.8
No. of man-month devoted already to the task:	4.7

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

Material and Methods

For fruits from all harvest dates, after 7 days at -0.5 □C, storage was continued at
(1) -0.5 □C, 2% O_2 and 0.5 or 5.0% CO_2 or
(2) at -0.5 □C, 4 or 7% O_2 and 0.5% CO_2
Incidence of brownheart and quality evaluation was done after storage till March 3 (1) or April 8 (2).

For the study of dynamics of brownheart development ripe fruits (pick 5) of 7 orchards were stored - 0.5 □C, 2% O_2 and 0.5 % CO_2 after 21 days cooling at -0.5 □C. The orchards were selected according to experience to represent the range from insensitive to very sensitive. Fruits, one box of 17 kg per orchard, were removed from CA storage each two weeks during a period of 20 weeks. At each removal pears were inspected internally for brownheart.
Degree of brownheart was scored in three classes of brown and three classes of cavities relating to the stages shown in figure 1 and figure 2. From this an index was calculated, both for cavities and browning, as follows: index = % class 1 + (3 * % class 2) + (6 * % class 3).

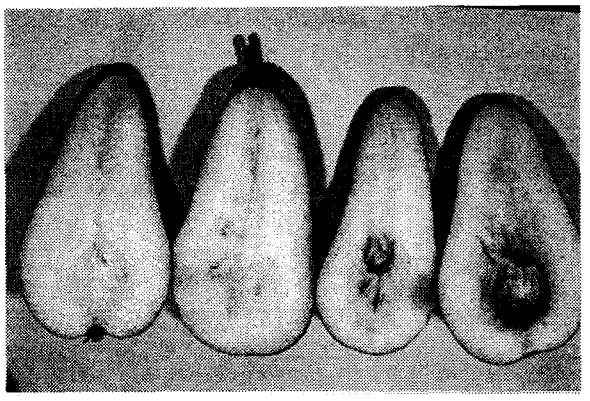


Figure 1, Minimum level of cavities of severity class 1, class 2, class 3 and an example of maximum percentage cavitie.

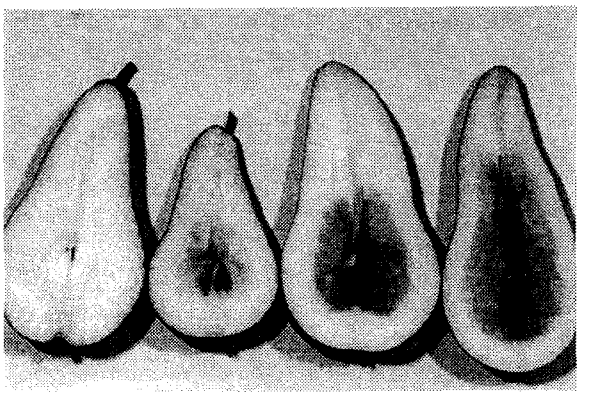


Figure 2, Minimum level of internal browning severity class 1, class 2, class 3 and an example of maximum percentage browning.

Results and Discussion

(1) Comparison between CA-storage at 0.5 and 5%CO_2.

Disorders

The incidence of disorders increased enormously by an increase in CO_2 level from 0.5 to 5.0% (table 1), especially at later picking dates. The effect of the increase in CO_2 level equals the effect of picking one to two weeks later.

Table 1. The effect of increasing CO_2 level from 0.5 to 5% on the total incidence of disorders (% of pears affected).

	pick 1	pick 2	pick 3	pick 4	pick 5
0.5% CO_2	0.4 a	0.2 a	6.1 a	17.0 b	62.6 d
5.0% CO_2	0.5 a	2.7 a	44.0 c	86.1 e	90.0 e

($P < 0.001$)

Cavities

In both conditions cavities occurred form the second pick on (figure 3). Differences between both storage conditions increased at later harvests. The decrease in cavity incidence at the latest pick is a little unexpected. Comparison of the different classes of cavity (severity)
suggests that there is more or less a constant ratio between them (Appendix A: Table 3.1.a). The cavity index enables a better discrimination between treatments when differences are still small.

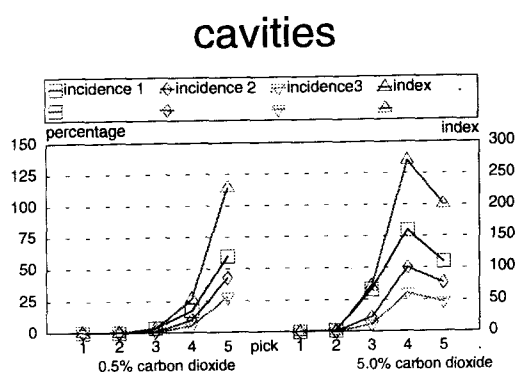


Figure 3. Percentage of fruits showing cavities after storage at two different storage conditions (-0.5 °C, 2% O_2 and 0.5 or 5.0 % CO_2); lines represent three different degrees of cavity and a cavity index composed of these three indices.

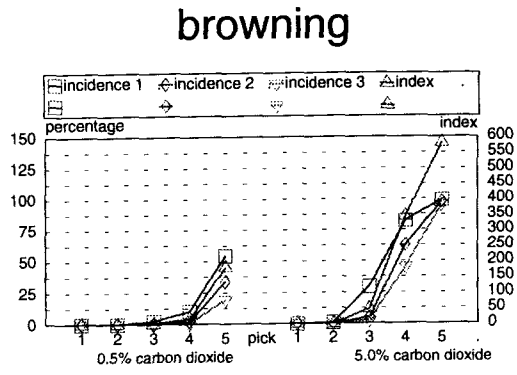


Figure 4. Percentage of fruits showing internal browning after storage at two different storage conditions (-0.5 °C, 2% O_2 and 0.5 or 5.0 % CO_2); lines represent three different degrees of internal browning and a browning index composed of these three indices.

Internal browning

Internal browning is influenced much stronger by CO_2 than cavity formation (figure 4). At 5% CO_2 browning was already detected in fruits of the first pick, whereas at 0.5% CO_2 it was found from the second pick on (Appendix A: Table 3.1.b) Furthermore, a much larger part of the fruits form pick 5 showed very severe symptoms of browning after storage in 5% CO_2 as compared to storage in 0.5% CO_2. At the former condition nearly all fruits from the last pick showed some internal browning.

Loss of weight

The mean loss of weight during the whole storage period (mainly loss of water) was 3.3% (table 2). Statistical analysis shows a significant effect of harvest date and storage condition (interaction) but in the basic dat a pattern is not easy detected. It seems that at low CO_2 loss is lower at later harvest while at high CO_2 loss is higher at later harvest.

Table 2. Loss of weight in fruits from 5 harvest dates at two levels of CO_2 concentration in CA-storage.

	pick 1	pick 2	pick 3	pick 4	pick 5
0.5% CO_2	4.3 c	3.2 abc	3.9 bc	2.7 abc	2.5 ab
5.0% CO_2	3.3 bc	1.7 a	4.0 bc	3.7 bc	3.8 bc

(F pr = 0.04)

Rot

Percentage of rot in storage was significantly ($p < 0.001$) influenced by level of CO_2 and harvest date (table 3). It was higher at the high CO_2 level (3.6% compared to 1.9%) and increased with later picking, especially pick 4 and 5.

Fruit weight
The average fruit weight after storage was not different at the two conditions. Picking later resulted in an average weekly increase in fruit weight of 14-15 gram. The total loss of fruit weight was about 13% (table 3). The largest part of this loss is caused by the fact that the larger fruits show more rot than the smaller fruits.

Green ground colour (a-value)
The mean a-value of the ground colour after storage was -10.8. Fruits stored at 0.5% CO_2 were significantly less green (-10.34) than those stored at 5.0% CO_2 (-11.34). Harvest date greatly influenced a-value (table 3). Loss of green ground colour was, generally, larger at later harvest dates.

TSS
TSS showed no dependence on %CO_2 in storage. Table 3 shows that at harvest the differences between harvest dates (1.6 □Brix) were much larger than after storage (0.7%). After all the earliest harvest still gave the lowest TSS.

Table 3. Mean fruit weight, green ground colour, and TSS in fruit from different harvest dates at harvest and after storage and rot incidence after storage.

	fruit weight		green ground colour		TSS		rot
	Harvest	stored	harvest	stored	harvest	stored	
pick 1	181 a	165 a	-13.88 a	-11.17 a	10.7 a	11.9 a	1.3 a
pick 2	193 a	182 b	-13.41 a	-11.37 a	10.9 a	12.0 a	1.7 a
pick 3	212 b	191 c	-13.58 a	-11.23 a	11.5 b	12.4 b	1.3 a
pick 4	213 b	204 d	-12.81 b	-10.26 b	12.0 c	12.6 b	2.6 a
pick 5	231 c	221 e	-13.08 b	-9.71 b	12.3 d	12.6 b	6.7 b
F test	<0.001	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001

Yellow ground colour
The later the pears were harvested, the higher the b-value (table 4). In storage the b-value increased in both storage conditions. The decrease at pick 5 in at 5 %CO_2 may be a consequence of the relative strong rot incidence in larger pears causing a change of the population.

Table 4. Yellow ground colour (b-value) at harvest and after storage at two levels of CO_2 of fruits from 5 harvest dates.

b-value	pick 1	pick 2	pick 3	pick 4	pick 5	F test
at harvest	32.16 a	32.38 a	33.76 b	35.03 c	36.00 d	< 0.001
Stored						
0.5% CO_2	32.16 a	32.63 ab	33.83 c	34.24 c	35.38 d	< 0.001
5.0% CO_2	33.52 bc	35.28 d	35.90 e	36.84 e	34.51 cd	

Firmness
Firmness decreased regularly with later harvest date (table 5). After storage the same picture was true for the condition at 5 %CO_2, but at 0.5%CO_2 the results were very 'unstable' i.d. hard to interpret. Comparing both storage conditions, at 5%CO_2 the firmness was higher in fruits from pick 1, 2 and 3 but equal or lower in fruits from pick 4 and 5.

Table 5. Firmness (kg with 7 mm plunger) at harvest and after storage at two levels of CO_2 of fruits from 5 harvest dates.

firmness	pick 1	pick 2	pick 3	pick 4	pick 5	F test
at harvest	7.2 e	6.8 d	6.5 c	6.2 b	5.9 a	< 0.001
stored						
0.5% CO_2	4.7 b	5.1 c	4.0 a	5.5 d	5.0 bc	< 0.001
5.0% CO_2	6.8 f	6.3 e	6.0 e	5.5 d	4.7 b	

(2) Comparison between CA-storage at 2, 4 and 7% O_2.

disorders

Total incidence of disorders depended strongly on % O_2 during storage (table 6). Most disorders occurred at the combination of late picking and low oxygen concentration. At optimum harvest date in combination with the higher O_2 concentrations, fruits showed no disorders at all.

Cavities

In fruits from pick 3 cavities were almost absent at all O_2 concentrations (figure 5). In $7O_2$ cavities were completely absent. In fruits from pick 5 large differences between the O_2 concentrations were observed with the same trend. i.e. lower incidence at higher O_2 concentration. All stages (1, 2, 3) were present and the relative incidence of different severity stage was more or less constant. In 34% of the pears cavities were not accompanied by symptoms of browning.

browning

As was the cas with cavities, incidence of browning was very low in fruits of pick 3 and really zero at $7\%O_2$ (figure 6). Fruits from pick 5 showed much higher sensitivity, especially at $2\%O_2$. Also at $7\%O_2$ some fruits developed internal browning. All pears showing browning also showed cavities.

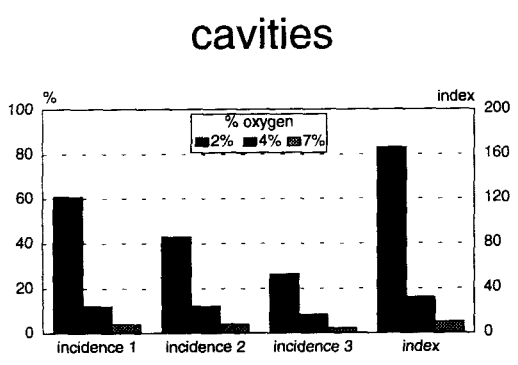


Figure 5, % op pears showing symptoms of cavities of severity classes 1, 2 or 3, and the cavity index.

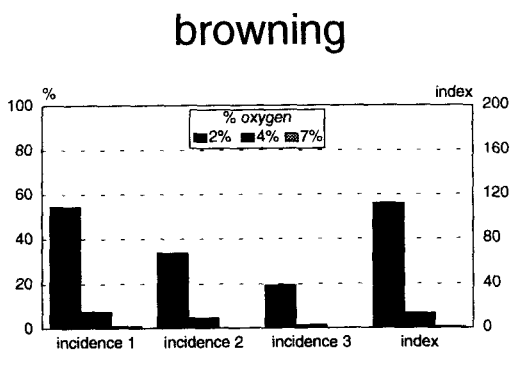


Figure 6, % op pears showing symptoms of internal browning of severity classes 1, 2 or 3, and the browning index.

rot

Fruits with rot were only found at $2\%O_2$ (table 6). The reason why at higher O_2 concentrations no rot was found are not clear and the phenomenon is unexpected.

firmness

Firmness of fruits form pick 5 was not affected by the O_2 concentration in storage, whereas in fruits from pick 3 loss of firmness was higher the lower the $\%O_2$ (table 6). Also this result is unexpected and conflicts with other data showing no influence of O_2 at all.

Table 6. Influence of % O_2 during storage on total disorders, firmness and % rotted fruits in fruits of pick 3 and pick 5.

Pick	2%O_2	4%O_2	7%O_2
	total disorders ($p < 0.001$)		
pick 3 pick 5	6.1 c 62.1 e	0.2 ab 21.3 d	0.0 a 4.7 bc
	firmness (kg) ($p < 0.001$)		
pick 3 pick 5	4.0 a 5.0 b	5.7 c 5.0 b	5.8 c 5.0 b
	rot ($p < 0.006$)		
pick 3 pick 5	1.3 b 4.0 c	0.0 a 0.0 a	0.0 a 0.0 a

loss of weight
Fruit weight decreased by 5-10% during storage, as an average of all objects (table 7). Average loss of water was 3.8%.

green ground colour (a-value)
The green ground colour decreased similarly in fruits of both picking dates. The higher the %O_2 , the higher the loss of green colour. This rise in loss was higher going from 2 to 4% than from 4 to 7 %O_2. (tables 7 and 8).

Table 7. Fruit weight and green and yellow ground colour at harvest and after storage in fruits of pick 3 and 5, as an average of 3 O_2 levels in storage.

	weight (gram)		green ground colour		yellow ground colour	
	harvest	stored	harvest	stored	harvest	stored
pick 3 pick 5	212 a 231 b	192 a 220 b	-13.6 a -13.1 b	-9.7 a -8.2 b	33.8 a 36.0 b	34.6 a 36.4 b
F test	0.007	< 0.001	0.004	< 0.001	< 0.001	< 0.001

Table 8. Influence of O_2 concentration in storage on green and yellow ground colour after storage.

% O_2	green ground colour	yellow ground colour
2 4 7	-10.1 b -8.7 a -8.1 a	34.6 a 35.5 b 36.4 c
F test	< 0.001	< 0.001

yellow ground colour (b-value)
Yellow ground colour did not change during storage in 2 %O_2 but increased at higher O_2 levels. These effects were similar in fruits from pick 3 and pick 5 (tables 7 and 8).

TSS

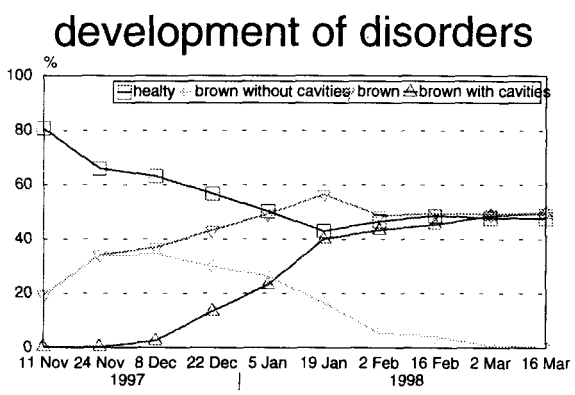
TSS, as a mean for fruits of pick 3 and 5, increased during storage from 11.9 to 12.5 % without any influence of $\%O_2$ during storage.

(3) dynamics of the disorder

Internal browning and cavities are commonly considered as two stages of the same disorder. In order to test this view, it is necessary to study the time course of symptom development.

Figure 7. shows the development averaged for all 7 orchards. Figures 8 shows only browning for separate orchards and figure 9 only cavities. A fast increase in incidence of browning and cavities was observed during the first 2 months of storage (figure 7). Browning developed within 5 weeks from start of CA in fruit samples from all 7 orchards. At that time in fruits from one orchard already 50% of the fruits showed browning symptoms. From the beginning of December (after 2 month of storage) The incidence of cavities showed a rapid rise (figure 7 and 8), followed by a further slow increase. From mid-January (15 weeks of storage) the total % of fruits with brownheart (browning plus cavities) did not increase any more. In this experiment, cavities were always accompanied by symptoms of browning.

Looking at separate orchards, three types can be discerned. (1) Two orchards were very sensitive, (2) 1 orchard had a very low sensitivity, and (3) 4 orchards had an intermediate position. These differences were the same for both types of symptom and were in accordance with experience.



Figuur 7, Development of internal browning and cavities in late picked pears of seven orchards as an mean in standard storage conditions.

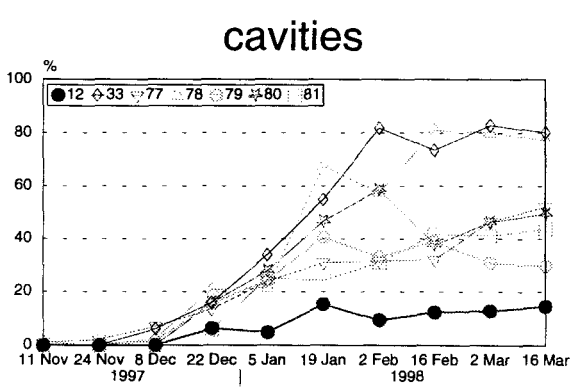


Figure 8: Development of cavities in late picked pears of seven orchards in standard storage conditions.

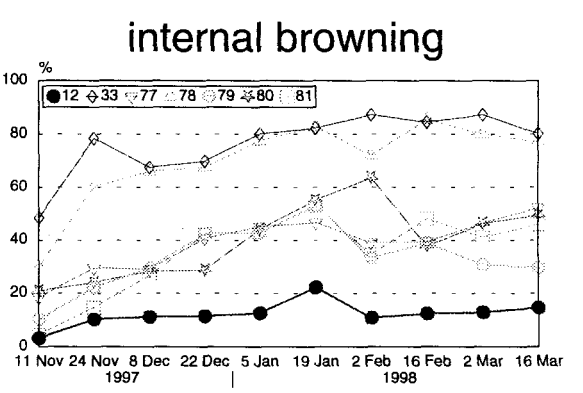


Figure 9: Development of internal browning in late picked pears of seven orchards in standard storage conditions.

Conclusions

influence of CO_2

The results show that the incidence of disorders, especially internal cavities and browning (brown heart) after storage increases with later harvest date and is much higher at 5 %CO_2 as compared to 0.5 %CO_2 during storage. Browning can be found already in the first pick (5 %CO_2) whereas cavities are found first in de second pick (7-10 days before optimum harvest date).
Rot was promoted by the high %CO_2 especially at late harvest. Fruit quality characteristics responded in the expected way except fruit firmness where at 0.5% there was no difference in firmness after storage whereas at harvest firmness was lower, the later the harvest.

influence of O_2

Oxygen had a large influence on the incidence of both cavities and internal browning at the level of 0.5% CO_2 . At 7%O_2, the level of this disorder was very low and combination with a longer cooling period before CA might completely inhibit the disorder. The influence of higher CO_2 levels on quality depended on picking date and type of quality characteristic.
Surprisingly, in this experiment, firmness of fruits picked at a normal date was higher at higher %O_2. On the other hand more green ground colour was lost.
Rot was promoted at lower %CO_2. These experiments have to be repeated

dynamics of brown heart formation

Symptoms of brown heart were observed after 5 weeks of storage. Internal browning was the first symptom to arrive and was followed by cavity formation. During a few weeks there was a rapid rise in the incidence of these symptoms, followed by slower rise. After about 15 weeks no further increases in brown heart were observed.

continuation

In the coming year emphasis should be on the effects of new combinations of CO_2 and O_2 and the length of the cooling period before CA, while repeating some experiments, especially the surprising influence of %O_2 on firmness. The results of the experiment on the dynamics of browning and cavity formation confirm earlier results and need no further repetition.

Task 4. Climate and Orchard factors

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	13.2
No. of man-month devoted already to the task:	3.3

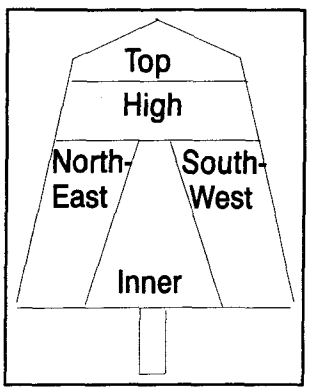
Objectives

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

Material and Methods

Climate data (temperature, rainfall and RV) from 11 orchards were either collected in the orchard itself ('Mety' station) or obtained from nearby meteorological stations.
Nutrition was varied in three orchards in the region 'Zeeland' by applying extra doses of Ca, N or K,
- K by spraying a solution of 10 gr/l potassium sulphate, 11 times between June 4 and August 9,
- Ca by spraying a solution of 7 gr/l calciumchloride id., and
- N by broadcasting two times 60 kg/ka, a total of 120 kg/ha

Fruit load was varied in three orchards in the region 'Zeeland' either by removing after the regular June drop 1/3 or 2/3 of all pears in normal bearing trees, or by selecting trees naturally showing a bearing of 2/3 or 1/3 of normal level.
Fruit position was studied by sampling five different positions ('top'(15%), 'high' (15-30%), 'inner', 'north-east' and 'south-west') according to figure 10 at three orchards in the region 'Zeeland'.



For picking and storage see tasks 2 and 3.

Results and Discussion

Climate data

These data were sent to one of the partners with responsibility for the modelling task: ATO-DLO

Nutrition

Extra nutrition with K, Ca or N did not result in significant increases in nutrient levels in the fruit one month before harvest or at harvest (pick 3) (appendix C table 4.2.a). The treatments had no effect on the incidence of brown heart.

Firmness

After storage fruits of the high N treatment showed significantly lower firmness than the control (5.7 resp. 6.0 kg) and the high Ca treatment (also 6.0 kg). Fruits of the high K treatment had at the same moment a mean firmness of 5.8 kg. At harvest the differences in firmness between the treatments were of the same magnitude and significance. Firmness in high N and control were 6.3 and 6.5 respectively (significant difference at p=0.05).
Data are shown in appendix C (table 4.2.b).
Continued treatment in 1998 may increase differences in internal concentration and induce larger effects.

Fruit load

Pears are normally picked in one run. If differences in fruit load would initiate differences in sensitivity to brown heart, this would have major consequences for the whole picking strategy.

Diseases and disorders

Brown heart was the most occurring disorder. Brown heart incidence was least in normal bearing trees. Removing 2/3 of the fruits resulted in a clear increase in brown heart incidence, especially in the late pickings. On an average less than 2% of the fruits were attacked by rot.
Incidence of rot was slightly higher in later picked fruit, but was not influenced by degree of bearing.

Table 9. Incidence of cavities in relation to fruit load either using natural variation or induced by hand thinning; cavities scored in 3 classes of severity and transformed into the cavity index.

Fruit load	cavities class 1	cavities class 2	cavities class 3	cavity index
Control	(6.4) 2.3 a	(4.2) 1.6 a	(1.6) 0.5	(19.4) 7.0 a
-1/3 natural	(22.9)	(15.7)	(10.2)	(84.8)
-2/3 natural	(15.3)	(10.2)	(5.2)	(51.5)
-1/3 thinning	(11.4) 4.3 a	(6.0) 2.5 a	(3.4) 1.2	(33.6) 12.9 ab
-2/3 thinning	(22.0) 10.6 b	(15.4) 7.0 b	(7.9) 3.3	(76.5) 34.5 b
F test	0.02	0.02	N.S.	0.03

(between brackets data of the only orchard with natural variation)

Cavities

The occurrence of cavities depended strongly on degree of bearing (table 9) and picking date (figure 11). In the control and at 1/3 of the fruits removed, cavities occurred after storage in pick 3 and later picks. Removing 2/3 of the fruits clearly increased the incidence of cavities. Low degree of bearing by natural processes (flowering, fruit set, June drop) seems to stimulate the incidence of cavities even more but in the absence of replication (this year results of only one orchard) this conclusion is not yet safe.

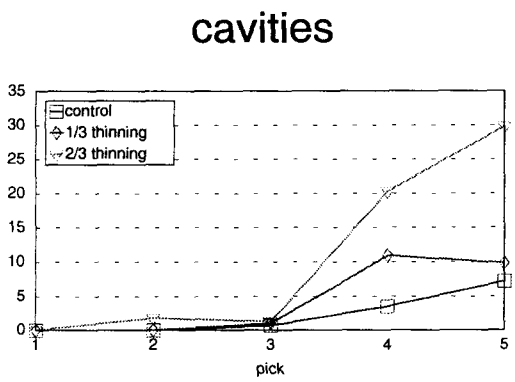


Figure 11. Incidence of cavities in relation to fruit load varied by hand thinning.

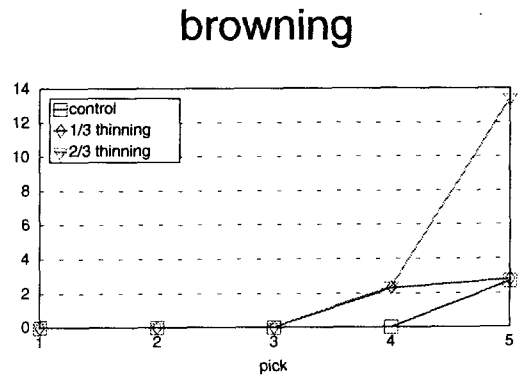


Figure 12. Incidence of browning in relation to fruit load varied by hand thinning.

Browning

The incidence of internal browning was small in this experiment (table 10 and figure 12). Browning was always associated with cavities. In the hand thinned treatments brown was found first in the fourth pick. The highest thinning treatment gave clearly more browning than the other objects. High degree of natural thinning gave more brown than hand thinning.

Table 10. Incidence of internal browning in relation to fruit load either using natural variation or induced by hand thinning; browning scored in 3 classes of severity and transformed into the browning index.

fruit load	browning class 1	browning class 2	browning class 3	browning index
Control	(1.6) 0.5 a	(0.2) 0.1	(0.0) 0.0	(2.1) 0.7
-1/3 natural	(12.2)	(5.4)	(0.8)	(25.2)
-2/3 natural	(3.2)	(1.5)	(0.0)	(6.1)
-1/3 thinning	(2.5) 1.1 ab	(1.1) 0.5	(0.0) 0.1	(4.7) 2.2
-2/3 thinning	(6.5) 3.2 b	(2.7) 1.1	(0.3) 0.1	(12.7) 5.7
F test	0.004	N.S.	N.S.	N.S.

(Between brackets date of the only orchard with natural variation in fruit load)

Fruit weight and loss of weight

Fruit weight increased clearly by thinning (table 11). Natural variation in fruit load did not influence fruit weight. Average weight loss during storage was 3.9% and did not depend on any treatment.

TSS

TSS in fruits after storage was highest at the lowest fruit load (table 11) by hand thinning. At harvest differences were somewhat smaller. Increase in TSS was 1% on an average and was highest in the treatments with lowest fruit load (both natural and hand thinned). Very interestingly, these objects showed at harvest least advanced starch decoloration stage ('least ripe').

Table 11. Fruit weight and TSS at harvest and after storage for 5 picking dates; data between brackets refer to one orchard showing natural variation in fruit load.

Fruit load	fruit weight		TSS (□Brix)	
	at harvest	after storage	at harvest	after storage
Control	(158) 167 a	(154) 163 a	(11.3) 11.5 a	(12.4) 12.6 a
-1/3 natural	(162)	(158)	(11.1)	(12.2)
-2/3 natural	(167)	(154)	(11.2)	(12.5)
-1/3 thinning	(173) 186 b	(156) 174 ab	(11.6) 11.9 b	(12.5) 12.9 ab
-2/3 thinning	(178) 194 b	(161) 189 b	(11.9) 12.2 c	(13.5) 13.5 b
F test	0.002	<0.001	< 0.001	<0.001

Ground colour and firmness
Green and yellow ground colour (a- and b-value in L*a*b system) were not affected by fruit load (appendix D table 4.2.c). Firmness effects were detected when measured in the axial plane: firmness increased with decreasing fruit load from 8.1 to 8.4 (1/3 thinning) and 8.6 kg (2/3 thinning).

Fruit position
Normal procedure for picking pears implies picking all pears at the same time. If fruits at different positions in the tree differ with respect to quality and to risk of disorders, this procedure creates heterogeneous lots of fruit.

Sum of disorders
Sum of disorders (table 12) was highest in fruits from the position 'high' and, somewhat less, from the position 'top'. Between the positions in the lower part of the tree no differences were found. The number of disorders, especially brown heart, increased clearly from pick 4 on.

Table 12. Sum of disorders (% of counts) in 5 different positions (see also figure 10) at 5 harvest dates.

Position	pick 1	pick 2	pick 3	pick 4	pick 5	mean
Top	3.7	0.0	4.1	17.6	37.4	12.5 b
High	0.0	0.0	4.6	21.8	53.0	15.9 b
North-east	0.7	0.0	0.4	0.7	17.7	3.9 a
South-west	1.4	1.5	0.5	5.4	23.6	6.5 ab
Inner	0.0	0.0	0.9	14.3	4.8	4.0 a
mean	1.1 a	0.3 a	2.1 a	12.0 b	27.3 c	8.6

Rot
No significant differences in fruit rot incidence were found between the positions though the figures were higher for the higher positions (3.7-3.8%) than for the lower positions (1.3-1.7%). Fruit rot incidence after storage clearly increased with picking date, but there was a minimum at pick 3! (see appendix E table 4.4.c.)

Cavities
In two of the three orchards involved in this experiment fruits with cavities were found (after storage) from pick 3 at all positions (figure 13). In one orchard fruit from the lower positions showed cavities in a later pick (pick 4) than fruit from the higher positions.
There was no clear difference between the inner and outer position in the lower part of the tree.

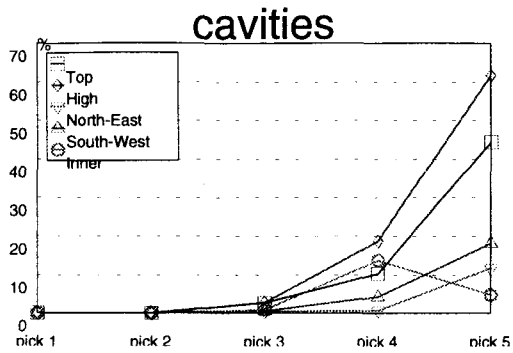


Figure 13. Percentage of fruits from different positions and at different harvest dates showing cavities after storage.

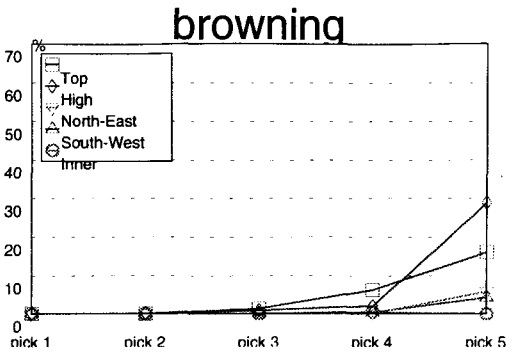


Figure 14. Percentage of fruits from different positions and at different harvest dates showing internal browning after storage.

Internal browning

Incidence of internal browning was relatively low in this experiment. At the positions 'top' and 'high' it appeared from the third pick on and at the lower positions from the fourth pick on (figure 14). Results of pick 5 indicate that fruits form the inner part of the tree are less sensitive to browning than fruits from the outside. Browning was always accompanied by cavities.

Loss of weight

The average weight loss was rather high in this experiment (about 4%). Differences between objects were not systematic. Generally, weight loss decreased with later picking whereas pick 5 showed a rise again (appendix E table 4.4.d). The latter might be caused by fruit rot.

Fruit weight

Fruit weight at the positions 'top' and 'high' was larger than at the other positions (table 13). Fruit weight at the position 'inner' was lower than at the outer positions.

Table 13. The influence of position in the tree on fruit weight, firmness and TSS at harvest and after storage.

	weight		firmness		TSS	
	harvest	storage	harvest	storage	harvest	storage
Top	204 bc	206 b	6.6	5.9	11.6 b	12.7
High	193 bc	191 b	6.8	6.0	11.3 ab	12.4
North-east	169 a	165 a	6.8	6.2	11.4 b	12.4
South-west	179 ab	168 a	6.8	6.2	11.3 ab	12.5
Inner	164 a	149 a	6.9	6.2	11.0 a	12.0
F test	< 0.001	< 0.001	N.S.	N.S.	0.01	N.S.

Firmness

Firmness of fruits from the position 'top' was somewhat lower than from the other positions, both at harvest and after storage (table 13) but these difference were not significant.

TSS

TSS was highest in fruits from the position 'top' and lowest in fruits form the position 'inner' (table 13). These differences were significant at harvest but not after storage though differences were larger.

Green ground colour
Fruits from the position 'top' had the lowest a-value (greenest), those from the outside the highest (less green). It is possible that differences in fruit russeting overrule the real differences in green ground colour. Existing differences at harvest were more pronounced after storage (table 14).

Yellow ground colour
The ground colour was most yellow (highest b-value) in fruits from the positions 'top'and 'high'. B-value increased during storage, except for the position 'inner', and existing differences were more pronounced after storage (table 14).

Table 14. Green and yellow ground colour as measured by a- and b-value (L*a*b with Minolta chromameter) for fruits from 5 different positions at harvest and after storage.

	green ground colour		yellow ground colour	
	at harvest	Stored	at harvest	stored
'top'	-13.5	-11.7 b	34.2 b	34.7 b
'high'	-12.9	-10.6 ab	33.2 ab	34.1 b
'north-east'	-12.5	- 9.8 a	32.4 a	32.7 a
'south-west'	-12.7	- 9.7 a	32.4 a	33.0 a
'inner'	-13.1	-10.2 ab	33.0 a	32.8 a
F test	N.S.	0.047	0.004	<0.001

Conclusions
Both fruit load and position of the fruit in the tree affected the incidence of brown heart after storage. When these effects are consistent in following years, this understanding might lead to a different view on picking strategies in order to increase the homogeneity of the lot of fruit picked from one orchard. Effects of mineral treatments are yet inconclusive. Only a tendency was observe to lower firmness following high N applications

continuation
All experiments, including the collection of meteorological data, have to be replicated in the coming year in order to affirm the position and fruit load effects and in order increase the chance of nutrition effects by repeated treatments at the same trees

Task 5. Postharvest treatments

Duration: 48 months
Current Status: 36 months to completion
Total estimated Man-month: 6.0
No. of man-month devoted already to the task: 1.5

Objectives
The objectives of this task are
(1) to develop storage scenario's that minimize the risk of brown heart and
(2) to find treatments that give an early indication of the sensitivity of pears to the development of brown heart in storage.

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

(1) At all harvest dates, after 7 days cooling at -0.5 □C, storage was continued at -0.5 □C, 2% O_2 and 0.5 or 5.0% CO_2. Fruits from pick 3, 4 and 5 was placed at cooling conditions for 0, 2, 7, 21 or 50 days before being moved to -0.5 □C, 2% O_2 or 0.5% CO_2. Fruits were stored till March 30 1998. Incidence of brown heart, and fruit quality were measured after warming-up during one day.
(2) Pears from pick 2 (insensitive) and pick 4 (sensitive) from 7 orchards were placed at combinations of the following conditions: 2, 5 or 9 days at 18°C and 17, 14 or 9 days at 4 □C, 2 % O_2 and 0.5 or 5% CO_2. The total duration of the test was always 19 days.

Results and Discussion

mild scenarios (minimized risk)

Earlier data already indicated that postponing the start of CA conditions could reduce the incidence of brown heart in storage considerably without affecting general quality negatively.

disorders

The level of disorders depended very much on harvest date and length of cooling period before start of CA conditions (table 15). In fruits of pick 3 (optimum harvest date) differences between treatments were small and not significant but in fruits of pick 4 and 5 cooling before start of CA reduced the level of disorders significantly.

Table 15. Influence of length of cooling period before starting CA-conditions on the level of disorders (% weight) at three harvest dates (normal, late and very late).

Cooling days	pick 3	pick 4	pick 5	average
0	1.6 a	23.9 c	78.9 d	34.8
2	1.8 a	26.3 c	64.6 c	30.9
7	6.1 a	19.6 bc	60.9 c	28.9
21	1.1 a	10.4 ab	27.5 b	13.0
50	0.2 a	2.4 a	2.1 a	1.6
Average	2.2	16.5	46.8	21.8

$P < 0.001$

Cavities

Figure 15 clearly shows the dependence of the incidence of cavities on picking date and the length of the cooling period before start of CA. There is a tendency for an optimum curve with maximum sensitivity at a shorter cooling period at later harvest date, 0 days in fruits from pick 5 and 7 days in fruits from pick 3. In earlier research a cooling period of 10 days was found as the maximum. The development of cavities at the three different degrees (a, b, c) and of that of the cavity index (d), were very much identical in form.

Cavities
storage scenarios / harvest date

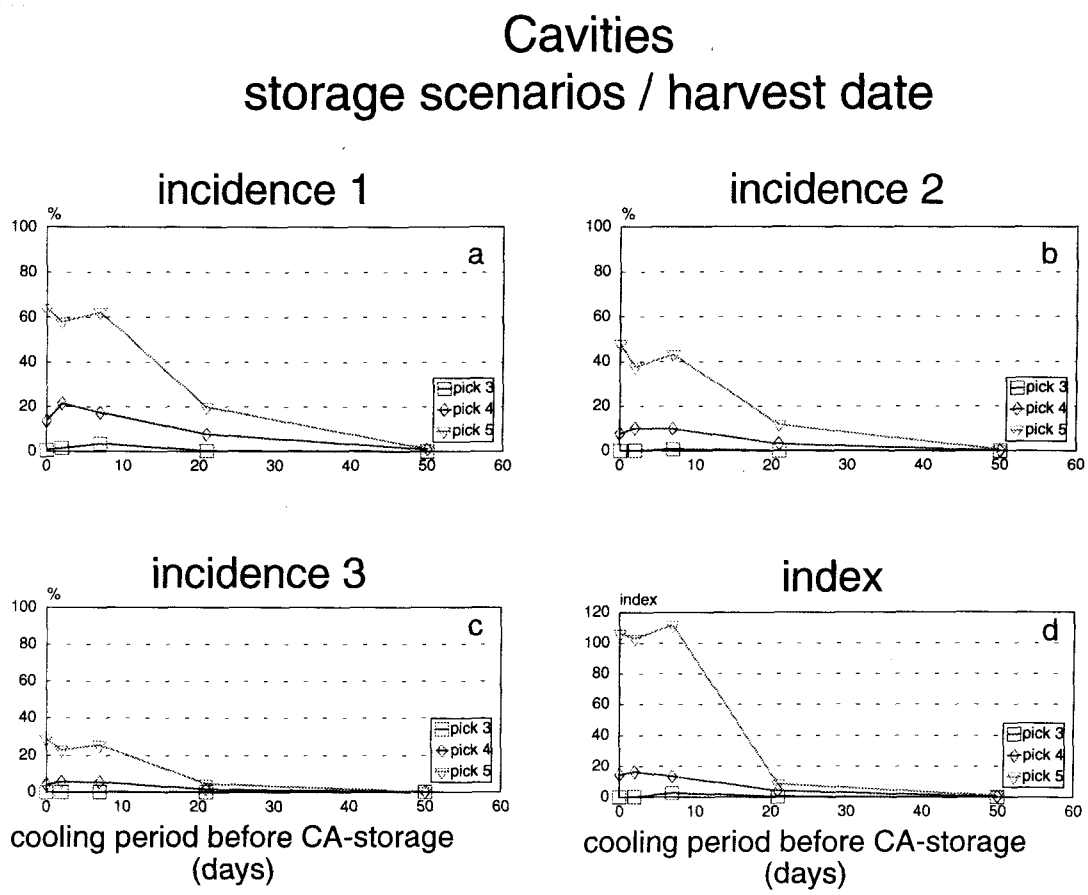


Figure 15. Incidence of cavities of three severity classes and cavity index as a function of length of cooling period before start of CA-storage for fruits from pick 3, 4 and 5.

Internal browning

The development of internal browning in response to length of cooling period before CA (figure 16) is very similar to that of cavities (figure 15), also considering the absolute level. in this particular experiment both symptoms were coupled in 63% of the pears, while 11% showed only browning and 26% only cavities. Contrary to what was observed on cavities, browning at severity of level 2 and 3 only occurred in pick 5 and also a constant ratio between the severity classes was not found.

Fruit weight

Fruit weight increase with harvest date and averaged 219 gram over pick 3, 4 and 5 (table 16). After storage mean fruit weight was 205 gram. The longer the period of cooling before start of CA, the lower mean fruit weight after storage (table 17).

Green ground colour (a-value)

Mean a-value at harvest was -13.2 (table 16). Loss of ground colour (increase in a-value) during storage increased with length of the period of cooling before start of CA (table 17).

TSS

TSS was not influenced by length of the period of cooling before start of CA. Differences in TSS between picking dates at harvest were larger than after storage (table 17).

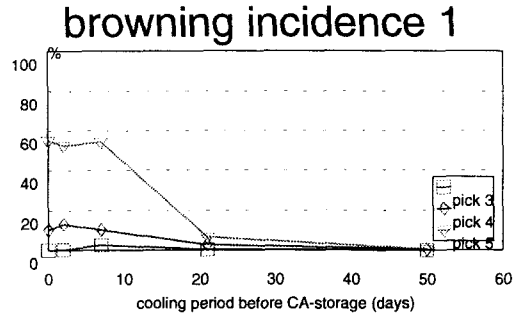


Figure 16. Browning as a function of length of cooling period before start of CA-storage for fruits from pick 3, 4 and 5.

Rot

The average incidence of rot was 2.2% of total weight (table 16) and was not influenced by length of the period of cooling before start of CA. Incidence of rot after storage increased with harvest date.

Loss of fruit weight

Mean loss of fruit weight during storage was 3.8% (table 17). There was no systematic relation with length of the period of cooling before start of CA though loss was highest at the longest cooling period. Loss of water may be a little higher than normal because of uneconomic filling of the stores during the cooling period before CA-storage in the containers.

Table 16. Mean fruit weight, green ground colour (a-value) and TSS at harvest and after storage and rot after storage in fruits of pick 3, 4 and 5.

	weight		a-value		TSS		rot
	harvest	stored	harvest	Stored	harvest	stored	
pick 3	212 a	191 a	-13.6 b	-10.6 c	11.54 a	12.35 a	1.5 a
pick 4	213 a	203 b	-12.8 a	-9.9 b	11.96 b	12.63 ab	1.7 a
pick 5	231 b	221 c	-13.1 ab	-9.2 a	12.30 c	12.69 b	3.6 b
F test	0.016	< 0.001	0.013	< 0.001	<0.001	0	0.004

Table 17. Green ground colour (a-value), mean fruit weight and loss of fruit weight as a function of the length of cooling period before start of CA conditions.

days cooling before CA	a-value	Fruit weight (gram)	loss of weight %
0	-10.9 c	205 ab	3.7 ab
2	-10.0 b	206 b	4.1 bc
7	-10.0 b	206 b	3.1 a
21	-9.6 b	208 b	3.6 ab
50	-8.9 a	201 a	4.8 c
F test	< 0.001	0.04	0.004

Table 18. Yellow ground colour (b-value) of fruits from pick 3, 4 and 5 at harvest and after storage, the latter as a function of length of cooling period before CA.

	pick 3	pick 4	pick 5	
	b-value at harvest			
days cooling before starting CA	338	35	36	(l.s.d. = 0.7)
	b-value after storage			mean
0	37.4 c	38.0 b	35.9 a	37.1
2	33.3 a	34.4 a	35.6 a	34.5
7	33.8 ab	34.2 a	35.4 a	34.5
21	34.0 ab	34.2 a	35.5 a	34.5
50	34.3 b	34.3 a	35.7 a	34.8
mean	34.6	35	35.6	35.1

P (<0.001)

Yellow ground colour (b-value)
Changes in b-value during storage depended much on harvest date and length of the period of cooling before start of CA. Generally, changes were only small. Fruits from pick 3 and 4 tended to show larger increases in b-value during storage as compared to pick 5 (table 18).

Firmness
The mean loss of firmness during the storage period was about 0.9 kg. The influence of the length of cooling period before start of CA conditions was rather irregular (table 19). The reason for that is not understood. Surprisingly, 50 days of cooling before start of CA conditions gave the smallest loss of firmness

Table 19. Firmness (kg 7 mm plunger) of fruits from pick 3, 4 and 5 at harvest and after storage, the latter as a function of length of cooling period before CA.

	pick 3	pick 4	pick 5	
	at harvest			
	6.5	6.2	5.9	l.s.d = 0.2
days cooling before starting CA	after storage			mean
0	5.7 b	5.5 ab	4.9 a	5.4
2	5.7 b	5.3 a	5.0 ab	5.3
7	4.0 a	5.5 ab	5.0 ab	4.8
21	5.7 b	5.3 a	5.3 b	5.4
50	6.0 b	5.7 b	5.3 b	3.7
mean	5.4	5.4	5	5.3

F test P (<0.001)

Stress test
The aim of a stress test is to indicate sensitive lots of fruit before in a normal scenario CA conditions are started i.e. within 21 days (according to our results). Possible factors that could induce early browning in sensitive lots are temperature, O_2 and CO_2. The effect of these factors is combined with a preceding period at room temperature.
Table 20 shows the combinations applied in this experiment. Stress tests resulted within the short period of the test only in internal browning except for the treatments starting with 9 days at 18 □C. In these objects also cavities were observed.
The other experiments already showed that pears of pick 2 did not show brown heart symptoms in normal storage conditions, whereas in most orchards pears of pick 4 did show such symptoms. Therefore, we are looking for methods
(1) that induce symptoms in pears of pick 4 and not in those of pick 2 where
(2) the incidence is correlated with the incidence after storage in pick 4.
The results of this test have been compared to ('correlated with') results of storage experiment in the scenario of 7 days cooling before establishment of CA since of other scenarios no results were available of pears of pick 2.
The results of two tests showed a very good correlation with the storage results i.e. the following test scenarios:
(1) 5 days at 18□C followed by 14 days at 4□C, 2% O_2 and 0.5% CO_2 and
(2) 2 days at 18□C followed by 17 days at 4□C, 2% O_2 and 5% CO_2
The two models show correlation coefficients of 0.91 and 0.92 respectively and yield models with 81.7% and 82.6% explanation of variation respectively.

Table 20. Brown heart after stress tests and standard storage.

pick	days 18 □C	condition during stress test	orchard (code)							corr. coeff
			12	33	77	78	79	80	81	
2	2	4 □C, 2% O_2, 0.5% CO_2	0	0	0	0	0	0	0	0.00
	5	4 □C, 2% O_2, 0.5% CO_2	0	3	0	0	0	0	0	0.91
	9	4 □C, 2% O_2, 0.5% CO_2	22	60	46	69	38	51	35	0.39
	2	4 □C, 2% O_2, 5.0% CO_2	0	0	0	1	0	0	0	0.92
	5	4 □C, 2% O_2, 5.0% CO_2	2	0	0	15	1	1	3	-0.15
	9	4 □C, 2% O_2, 5.0% CO_2	79	90	84	99	78	99	93	0.10
	incidence after storage		*0*	*0*	*0*	*0*	*0*	*0*	*0*	
4	2	4 □C, 2% O_2, 0.5% CO_2	0	0	0	0	0	0	0	
	5	4 □C, 2% O_2, 0.5% CO_2	0	9	27	14	4	13	15	
	9	4 □C, 2% O_2, 0.5% CO_2	5	65	47	65	6	60	63	
	2	4 □C, 2% O_2, 5.0% CO_2	0	6	10	2	2	0	3	
	5	4 □C, 2% O_2, 5.0% CO_2	0	2	1	2	0	1	0	
	9	4 □C, 2% O_2, 5.0% CO_2	42	85	78	87	36	96	92	
	incidence after storage		*0*	*31*	*43*	*21*	*3*	*8*	*26*	

CC = correlation coefficient (of both pick's)

Conclusions

The results of these experiments show

(1) that a rather long cooling period before establishment of CA can prevent the occurrence of brown heart in pears picked at normal harvest date, without large effects on quality

(2) that there are perspectives of developing tests that can discriminate between sensitive and insensitive lots of fruit within the optimum cooling period of 21, before starting CA conditions.

continuation

The effect of length of cooling period should be further explored in the 'region' between 20 and 50 days, especially in combination with a variation of O_2 concentration in the store. Further testing the found models for prediction of sensitive lots of fruit is very important and deserves relative much attention.

Task 7: Destructive measurements

Duration: 48 months
Current Status: 36 months to completion
Total estimated Man-month: 7.6
No. of man-month devoted already to the task: 1.9

Task 7a. Quality evaluation

Quality data have been given in all chapters and sections along with the effects of treatments with respect to brown heart.

Task 7c. Mineral composition

Objectives

The aim of determining the nutrient status of the fruits is to detect any possible relation of concentration of a particular nutrient and the incidence of brown heart. In order to detect any such relation this has to be repeated a number of years in this project.

Material and methods

Fruits from 11 orchards were sampled one month before actual optimum harvest date and at optimum harvest date (pick 3) for analysis of Ca, K, Mg, N and P.

Results

Table 21 shows the concentration in the fruit one month before optimum harvest date (pick 3) in relation to the incidence of brown heart in pick 5 in a normal storage procedure (7 days cooling before CA at -0.5□C, 2% O_2 and 0.5% CO_2. All nutrients showed a correlation (0.5 - 0.7) between the incidence of cavities and nutrients 1 month before optimum harvest. No correlation (> 0.5) was found between the incidence of internal browning and nutrients 1 month before optimum harvest.
Only a weak correlation (-0.47) was found between magnesium concentration at optimum harvest date and the incidence of internal browning. At that sample date no correlation was found with the incidence of cavities.

Table 21 Concentration of nutrients (mg/100 gram fresh weight) at 2 sample dates of 11 orchards and incidence of cavities (cav.) and internal browning (i.b.) in fruits from pick 3 after standard CA-storage.

orchard	1 month before optimum harvest					at optimum harvest (pick 3)					after storage	
	Ca	K	Mg	N	P	Ca	K	Mg	N	P	cav.	i.b.
12	11.65	150.48	7.56	58.65	13.58	7.86	146.49	7.07	55.02	12.79	0.0	0.0
33	16.08	158.80	9.19	81.36	15.75	9.18	141.37	7.07	65.73	13.01	4.2	1.1
77	11.32	146.41	6.78	51.57	12.19	7.73	131.85	6.62	55.86	10.39	1.0	1.0
78	13.04	152.63	7.71	61.29	12.48	6.88	133.98	6.23	53.45	10.28	0.0	0.0
79	14.39	154.82	8.28	61.68	13.98	9.37	136.64	6.67	52.83	11.84	0.0	0.0
80	13.91	151.09	8.08	65.07	14.14	7.86	131.30	6.15	55.53	11.28	0.0	4.2
81	9.68	153.63	6.56	63.75	13.54	6.23	141.46	6.59	59.79	11.06	0.0	0.0
88	10.33	147.74	7.46	69.78	12.98	7.93	151.93	7.33	67.80	11.71	0.0	0.0
167	-	-	-	-	-	7.15	148.04	6.51	54.78	12.09	15.0	11.7
177	-	-	-	-	-	6.80	134.70	6.03	57.60	11.29	10.3	8.8
178	-	-	-	-	-	6.68	148.94	7.45	71.68	11.76	4.0	1.4

- = not observed

Continuation

To be continued in the next three years.

Task 8. Non-destructive measurements

Duration: 48 months
Current Status: 36 months to completion
Total estimated Man-month: 2.0
No. of man-month devoted already to the task: 0.5

Objectives

Lots containing pears with brown heart have lost there economic value. Sorting might be a method to get rid of pears with brown heart and preserve the value of the pears. This requires the application of non-destructive detection techniques. Sorting through water is used in practice, but laborious. A rapid technique that could be built in a sorting line would be an important improvement. The objective of this experiment is to test a so-called acoustic technique (Firmalon, Eshet Eilon, Israel) for this

purpose. Results of test of de Baerdemaker (Leuven, Belgium) with a similar apparatus, produced by himself, indicated that this might be a promising technique.

Material and Method

Fruits from one orchard (code 78) from all harvest dates and scenarios and CA-conditions, and fruits from 7 orchards and one standard scenario and CA-condition have been measured with the Firmalon prior to inspection for internal browning and cavities.

Table 22, Firmalon index values of pears with or without cavities and with or without internal browning; incidence of cavities and internal browning scored in 3 classes of severity.

	cavities	internal browning
healty	10.28	10.41 a
class 1	12.91	9.03 a
class 2	12.73	13.25 b
class 3	10.96	8.15 a
F test	N.S.	0.02

The data in table 22 show clearly that there is no significant relation between the average Firmalon index values and cavities. The significant difference of the mean Firmanlonindex values between different severities of internal browning can not be interpreted and are probably meaningless.
In order to further analyse the Firmalon data, a reverse analysis was done. Figure 17 shows that the percentage of disorders, cavities and internal browning is only slightly depending on the Firmalon index value, So the Firmalon can not discriminate between healthy fruits and fruits with brownheart.

Conclusions

These first results indicate that the Firmalon is not a usefull tool for non-destructive measurement of pears for the selection of healthy pears or pears with a brownheart from a mixed group. These results are opposite to conclusions of De Baerdemaker.

Continuation

The used testing method will be compared with the one of De Baerdemaker. Work will be continued if the comparison gives new testable ideas.

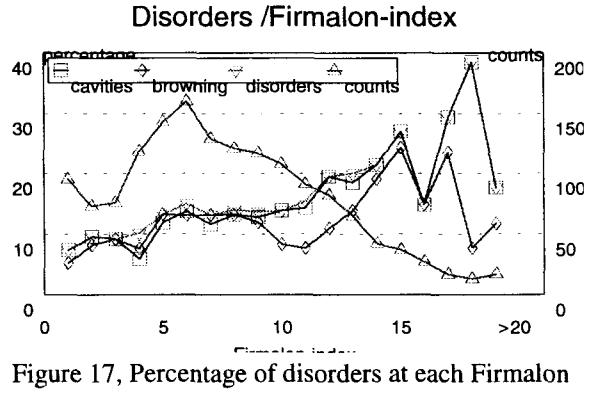


Figure 17, Percentage of disorders at each Firmalon index value.

Appendix A: Tables of task 3 (1) Influence of CO_2 on brown heart.

Table 3.1.a The effect of increasing CO_2 levels and of harvest date on the incidence of cavities scored in 3 classes of severity and on the cavity index, calculated from the incidence in the severity classes.

CO_2 and pick	cavity class 1	cavity class 2	cavity class 3	cavity index
0.5%CO_2				
pluk 1	0.0 a	0.0 a	0.0 a	0.0 a
pick 2	0.1 a	0.0 a	0.0 a	0.1 a
pick 3	3.5 a	0.8 a	0.3 a	6.0 b
pick 4	17.3 b	9.9 b	5.4 b	53.1 c
pick 5	59.7 c	42.7 c	28.0 c	229.1 d
5.0% CO_2				
pick 1	0.0 a	0.0 a	0.0 a	0.0 a
pick 2	0.9 a	0.3 a	0.0 a	1.5 ab
pick 3	32.9 b	11.0 b	5.2 b	70.6 c
pick 4	79.6 d	49.9 c	30.1 c	269.6 d
pick 5	54.7 c	38.0 c	23.2 c	200.4 d
F test	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.1.b. The effect of increasing CO_2 levels and of harvest date on the incidence of internal browning scored in 3 classes of severity and on the browning index, calculated from the incidence in the severity classes.

CO_2 and pick	brown class 1	brown class 2	brown class 3	browning index
0.5%CO_2				
pick 1	0.0 a	0.0 a	0.0 a	0.0 a
pick 2	0.1 a	0.0 a	0.0 a	0.1 ab
pick 3	2.6 ab	0.0 a	0.0 a	2.6 b
pick 4	10.3 b	2.2 ab	0.9 a	17.2 c
pick 5	54.3 d	33.8 c	19.4 b	180.0 e
5.0% CO_2				
pick 1	0.1 a	0.0 a	0.0 a	0.1 ab
pick 2	0.9 a	0.0 a	0.0 a	0.9 ab
pick 3	29.4 c	5.1 b	2.6 a	47.3 d
pick 4	82.3 e	62.6 d	45.1 c	342.9 f
pick 5	99.1 f	97.7 e	95.2 d	580.0 f
F test	< 0.001	< 0.001	< 0.001	< 0.001

Appendix B: Tables of task 3 (2) Influence of O_2 on brown heart.

Table 3.2.a. The effect of increasing O_2 levels and of harvest date on the incidence of cavities scored in 3 classes of severity and on the cavity index, calculated from the incidence in the severity classes.

% O_2 and pick	cavity class 1	cavity class 2	cavity class 3	cavity index
pick 3				
2	3.5 bc	0.8 ab	0.3 a	6.0 b
4	0.1 ab	0.1 a	0.0 a	0.3 a
7	0.0 a	0.0 a	0.0 a	0.0 a
pick 5				
2	60.7 e	42.9 d	26.3 c	225.4 d
4	11.9 d	11.9 c	8.1 b	60.1 c
7	3.8 c	3.8 b	2.1 a	17.8 b
F test	<0.001	< 0.001	< 0.001	< 0.001

Table 3.2.b. The effect of increasing CO_2 levels and of harvest date on the incidence of internal browning scored in 3 classes of severity and on the browning index, calculated from the incidence in the severity classes.

%O_2 and pick	brown class 1	brown class 2	brown class 3	browning index
pick 3				
2	2.6 ab	0.0 a	0.0 a	2.6 a
4	0.1 a	0.0 a	0.0 a	0.1 a
7	0.0 a	0.0 a	0.0 a	0.0 a
pick 5				
2	54.3 c	33.8 c	19.4 b	180.0 c
4	7.5 b	4.6 ab	1.3 a	20.4 b
7	0.9 a	0.3 a	0.1 a	1.9 a
F test	< 0.001	< 0.001	< 0.001	< 0.001

Appendix C: Tables of task 4 (2) Influence of nutrition on brown heart.

Table 4.2.a. Concentration of nutrients in mg/100 gram and % dry matter (dm) of fresh fruit weight at 2 sample dates of orchards with additional nutrient supply.

Object	1 month before estimated optimum harvest						at optimum harvest (pick 3)					
	CA	K	Mg	N	P	dm	CA	K	Mg	N	P	dm
contr.	11.4	155.4	7.88	63.2	12.31	15.4	7.39	119.5	5.90	49.5	9.11	15.1
+ K	10.4	154.4	7.39	63.6	11.30	15.7	6.23	135.1	6.33	58.2	9.75	14.4
+ Ca	10.9	153.4	7.15	62.0	11.14	14.9	7.36	127.9	5.87	47.5	9.38	14.4
+ N	10.2	148.5	6.77	60.0	10.74	14.8	6.10	129.8	6.22	55.1	9.40	14.7
F pr	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Mean	1064	1529	7.30	622	11.37	152	677	1281	6.08	52.6	941	146

Table 4.2.b. Fruit weight (FW) (gram), ground colour (L*a*b), firmness (radial (FR) and axial (FA) (kg), starch breakdown pattern, TSS (°Brix), acidity (%malic acid) and harvest index (HI) (Firmness/(starch*TSS)) of different harvest dates at harvest.

	FW	a	b	L	FR	FA	starch	TSS	acid	HI
pick 1	172	-13.3	32.6	54.4	7.3	9.9	2.3	11.3	0.16	0.34
pick 2	189	-14.2	33.6	55.1	6.8	9.6	2.7	11.4	0.13	0.25
pick 3	198	-13.8	33.9	55.4	6.5	8.3	3.8	11.6	0.13	0.18
pick 4	211	-13.2	35.2	57.4	6.1	7.4	4.7	12.5	0.12	0.12
pick 5	197	-12.7	36.3	58.5	5.7	6.8	6.0	12.7	0.10	0.08
F	***	**	***	***	***	***	***	***	***	***
l.s.d.	11	0.6	0.8	1.0	0.1	0.3	0.4	0.2	0.01	0.03
mean.	193	-13.4	34.32	56.17	6.5	8.4	3.9	11.9	0.13	0.19

Appendix D: Tables of task 4 (3) Influence of cropload on brown heart.

Table 4.3.a. The incidence of cavities (after storage) in fruits from 5 harvest dates; cavities scored in 3 classes of severity and on the cavity index, calculated from the incidence in the severity classes.
(not including objects variation in natural bearing)

cavity class	cavity class 1	cavity class 2	cavity class 3	index
pick 1	0.0 a	0.0 a	0.0 a	0.0 a
pick 2	0.6 a	0.2 a	0.0 a	1.1 a
pick 3	1.0 a	0.8 a	0.0 a	2.6 a
pick 4	11.6 b	5.9 b	2.2 a	30.1 b
pick 5	15.6 c	11.4 c	6.2 b	56.8 b
F test	< 0.001	< 0.001	< 0.001	< 0.001

Table 4.3.b. The incidence of internal browning (after storage) in fruits from 5 harvest dates; internal browning scored in 3 classes of severity and on the browning index, calculated from the incidence in the severity classes.
(not including objects variation in natural bearing)

	brown class 1	brown class 2	brown class 3	index
pick 1	0.0 a	0.0 a	0.0	0.0 a
pick 2	0.0 a	0.0 a	0.0	0.0 a
pick 3	0.0 a	0.0 a	0.0	0.0 a
pick 4	1.6 a	1.3 ab	0.0	2.2 a
pick 5	6.3 b	1.5 b	0.3	5.7 b
F test	< 0.001	0.01	N.S.	< 0.001

Table 4.3.c. Fruit weight, ground colour, firmness and TSS of different harvest dates at harvest (1) and after storage (2).

pick	fruit weight (gram)		green ground colour (a)		yellow ground colour (b)		firmness (kg)		TSS (°Brix)	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1	164a	149 a	-12.0ab	- 8.8 a	31.9a	30.9a	7.6e	6.7c	11.1a	12.5a
2	173ab	164 b	-13.0c	-10.6 b	32.6ab	33.0b	6.9d	6.0b	11.4b	13.1b
3	183b	172 b	-12.3bc	-10.0ab	32.7b	32.8b	6.7c	6.1b	11.8c	13.1b
4	194bc	195bc	-11.3a	- 8.2 a	33.5c	33.1b	6.3b	5.8b	12.5d	13.1b
5	199bc	196 c	-11.7ab	- 8.6 a	34.6d	35.9c	5.9a	5.3a	12.5d	13.3b
F test	0.004	<.001	0.001	0.005	<.001	< .001	< .001	< .001	< .001	0.006

Appendix E: Tables of task 4 (4) Influence of position on brown heart.

Table 4.4.a. The incidence of cavities after storage of fruits picked from 5 positions.

cavity class 1	pick 1	pick 2	pick 3	pick 4	pick 5	mean
'top'	0.0 a	0.0 a	2.7 a	10.4 ab	44.5 c	11.5
'high'	0.0 a	0.0 a	2.8 a	18.8 b	61.7 c	16.7
'north-east'	0.0 a	0.0 a	0.4 a	0.7 a	11.8 b	2.6
'south-west'	0.0 a	0.0 a	0.5 a	4.3 a	18.2 b	4.6
'inner	0.0 a	0.0 a	0.9 a	13.9 b	4.8 ab	3.9
Mean	0.0	0.0	1.5	9.6	28.2	3.9

Table 4.4.b. The incidence of internal browning after storage of fruits picked from 5 positions.

brown class 1	pick 1	pick 2	pick 3	pick 4	pick 5	mean
'top'	0.0 a	0.0 a	1.4 ab	6.3 b	16.7 c	4.9
'high'	0.0 a	0.0 a	0.9 ab	2.1 ab	28.8 d	6.4
'north-east'	0.0 a	0.0 a	0.0 a	0.0 a	5.9 ab	1.2
'south-west'	0.0 a	0.0 a	0.0 a	0.4 a	4.3 ab	0.9
'inner'	0.0 a	0.0 a	0.0 a	0.4 a	0.0 a	0.1
Mean	0.0	0.0	0.5	1.8	11.1	2.7

Table 4.4.c. Rot incidence after storage of different harvest dates.

rot	pick 1	pick 2	pick 3	pick 4	pick 5
F test 0.046	1.8 ab	1.6 a	0.8 a	2.4 ab	5.3 b

Table 4.4.d. Weight loss (%) after storage of fruits from different positions of different harvest dates.

position	pick 1	pick 2	pick 3	pick 4	pick 5	mean
'top'	5.8	4.0	5.2	1.2	3.8	4.0
'high'	6.3	4.9	2.0	2.1	1.7	3.4
'north-east'	4.1	2.8	4.8	3.6	2.4	3.5
'south-west'	3.3	3.5	2.0	4.0	5.7	3.7
'inner	6.0	8.2	3.2	3.3	4.7	5.0
Mean	5.1 (b)	4.7 (b)	3.4 (a)	2.9 (a)	3.7 (a)	4.0

Appendix F: Tables of task 5 (1) Minimizing brown heart.

Table 5.1.a. The incidence of length of cooling period before CA-storage on cavities after storage of fruits from 5 harvest dates; cavities scored in 3 classes of severity and on the cavity index, calculated from the incidence in the severity classes.

cooling period before CA-storage	pick 3	pick 4	pick 5	mean	F test
cavity class 1					
0	0.7 ab	13.7 bc	63.8 c	26.1	< 0.001
2	1.5 ab	21.4 c	57.8 c	26.9	
7	3.5 b	17.3 c	62.2 c	27.7	
21	0.3 ab	7.6 b	19.8 b	9.2	
50	0.0 a	0.8 a	1.2 a	0.7	
mean	1.2	12.16	41.0	18.1	
cavity class 2					
0	0.1 a	7.7 c	47.8 d	18.6	< 0.001
2	0.2 a	10.0 c	37.8 c	16.0	
7	0.8 a	9.9 c	43.3 cd	18.0	
21	0.1 a	3.2 b	11.7 b	5.0	
50	0.0 a	0.0 a	0.5 a	0.2	
mean	0.3	6.1	28.2	11.5	
cavity class 3					
0	0.0 a	4.2 bc	28.9 c	11.0	< 0.001
2	0.0 a	5.6 c	22.9 c	9.5	
7	0.3 a	5.4 c	25.7 c	10.5	
21	0.0 a	1.6 ab	4.5 b	2.1	
50	0.0 a	0.0 a	0.2 a	0.1	
mean	0.1	3.4	16.4	6.6	
cavity index					
0	0.9 ab	41.7 e	246.1 f	96.2	< 0.001
2	2.0 abc	58.0 e	202.1 f	87.3	
7	6.0 c	53.1 e	226.1 f	95.1	
21	0.5 ab	18.9 d	56.6 e	25.3	
50	0.0 a	0.8 abc	2.8 bc	1.2	
mean	1.9	34.5	146.7	61.0	

Table 5.1.b. The incidence of length of cooling period before CA-storage on internal browning after storage of fruits from 5 harvest dates; internal browning scored in 3 classes of severity and on the browning index, calculated from the incidence in the severity classes.

cooling period before CA-storage	pick 3	pick 4	pick 5	mean	F test
browning class 1					
0	0.0 a	10.2 b	55.0 c	21.7	< 0.001
2	0.2 a	13.1 b	52.4 c	21.9	
7	2.6 a	10.3 ab	54.3 c	22.4	
21	0.3 a	2.8 a	6.6 b	3.2	
50	0.0 a	0.3 a	0.4 a	0.2	
mean	0.6	7.3	33.7	13.9	
browning class 2					
0	0.0 a	2.7 b	30.7 b	11.1	< 0.001
2	0.0 a	2.2 ab	29.6 b	10.6	
7	0.0 a	2.2 ab	33.8 b	12.0	
21	0.0 a	0.8 ab	1.5 a	0.8	
50	0.0 a	0.0 a	0.2 a	0.1	
mean	0.0	1.6	19.2	6.9	
browning class 3					
0	0.0 a	1.6 a	17.1 b	6.2	< 0.001
2	0.0 a	1.0 a	19.8 b	6.9	
7	0.0 a	0.9 a	19.4 b	6.8	
21	0.0 a	0.7 a	0.4 a	0.4	
50	0.0 a	0.0 a	0.2 a	0.1	
mean	0.0	0.8	11.4	4.1	
browning index					
0	0.0 a	20.4 de	167.7 f	62.7	< 0.001
2	0.2 ab	20.6 de	170.9 f	63.9	
7	2.6 bc	17.2 de	180.0 f	66.6	
21	0.3 ab	6.4 c	11.0 d	5.9	
50	0.0 a	0.3 ab	1.5 ab	0.6	
mean	0.6	13.0	106.2	39.9	

Appendix G: Parameters at harvest task 3.

Quality- and maturity parameters

pick	weigth	a	b	L	Firm	Firm[1]	starch	TSS	acid	index
1	181	-13.9	32.2	52.9	7.2	10.4	2.4	10.7	0.16	0.31
2	193	-13.4	32.4	53.1	6.8	9.4	2.9	10.9	0.14	0.25
3	212	-13.6	33.8	54.9	6.5	8.4	4.3	11.5	0.13	0.16
4	213	-12.8	35.0	56.6	6.2	7.8	4.3	12.0	0.12	0.14
5	231	-13.1	36.0	57.6	5.9	7.1	6.4	12.3	0.12	0.09
F	***	***	***	***	***	***	***	***	***	***
l.s.d.	13	0.5	0.6	0.9	0.2	0.3	0.6	0.2	0.01	0.02
mean	206	-13.4	33.9	55.0	6.5	8.6	4.1	11.5	0.13	0.19

Internal volume e.d. (not from orchards 167, 177 and 178)

	product volume	specific weight	internal volume	relative internal volume
pick 1	0.00019	986.8	0.0092	4854
pick 2	0.00019	987.1	0.0091	4900
pick 3	0.00021	987.3	0.0103	4888
pick 4	0.00020	989.5	0.0093	4674
pick 5	0.00022	987.4	0.0107	4872
F test	***	*	***	*
l.s.d.	0.00001	1.7	0.0006	163
mean	0.00020	987.6	0.0097	4854

* = P <0.05; ** = P <0.01; *** = P < 0.001

Concentration of nutrients in mg/100 gram and % dry matter (dm) of fresh fruit at 2 sample dates.

orchard	1 month before estimated optimum harvest						at optimum harvest (pick 3)					
	Ca	K	Mg	N	P	dm	Ca	K	Mg	N	P	dm
12	11.65	150.48	7.56	58.65	13.58	15.3	7.86	146.49	7.07	55.02	12.79	13.4
33	16.08	158.80	9.19	81.36	15.75	13.4	9.18	141.37	7.07	65.73	13.01	13.1
77	11.32	146.41	6.78	51.57	12.19	14.3	7.73	131.85	6.62	55.86	10.39	12.9
78	13.04	152.63	7.71	61.29	12.48	15.7	6.88	133.98	6.23	53.45	10.28	14.3
79	14.39	154.82	8.28	61.68	13.98	16.6	9.37	136.64	6.67	52.83	11.84	15.7
80	13.91	151.09	8.08	65.07	14.14	14.8	7.86	131.30	6.15	55.53	11.28	13.5
81	9.68	153.63	6.56	63.75	13.54	13.4	6.23	141.46	6.59	59.79	11.06	12.0
88	10.33	147.74	7.46	69.78	12.98	14.2	7.93	151.93	7.33	67.80	11.71	11.9
167	-	-	-	-	-	-	7.15	148.04	6.51	54.78	12.09	14.2
177	-	-	-	-	-	-	6.80	134.70	6.03	57.60	11.29	12.7
178	-	-	-	-	-	-	6.68	148.94	7.45	71.68	11.76	13.8

- = not observed

Appendix H: Parameters at harvest task 4 (Fruit load).

Quality- and maturity parameters

	weigth	a	b	L	Firm	Firm[1]	starch	TSS	acid	index
norm	167	-11.9	32.8	54.7	6.7	8.2	3.9	11.5	0.12	0.20
-1/3 th	186	-12.2	32.9	54.7	6.6	8.4	3.8	11.9	0.13	0.19
-2/3 th	194	-12.1	33.1	55.0	6.7	8.6	3.3	12.2	0.15	0.22
F	**	N.S.	N.S.	N.S.	N.S.	**	*	***	***	N.S.
l.s.d.	11					0.3	0.4	0.2	0.01	
mean	182	-12.1	32.9	54.8	6.7	8.4	3.7	11.9	0.13	0.21

Relative internal volume (only orchard 78)

		product volume	specific weigth	internal volume	relative internal volume
pick 2	normal	0.0001368	994.225	0.0058277	4217.20
pick 3		0.0001441	993.834	0.0060750	4254.90
pick 4		0.0001733	1001.395	0.0062814	3526.48
pick 5		0.0001967	990.571	0.0090309	4569.23
pick 2	-1/3 th	0.0001673	989.735	0.0077114	4649.84
pick 3		0.0001703	994.408	0.0071626	4199.58
pick 4		0.0002262	1002.854	0.0073692	3385.98
pick 5		0.0001785	993.506	0.0075258	4286.47
pick 2	-2/3 th	0.0001738	995.879	0.0070530	4057.93
pick 3		0.0002122	998.361	0.0079016	3818.76
pick 4		0.0001851	1003.610	0.0064288	3313.15
pick 5		0.0001856	993.736	0.0078611	4264.32

FPO

Concentration of nutrients in mg/100 gram and % dry matter (dm) of fresh fruit at 2 sample dates.

Object	1 month before estimated optimum harvest						at optimum harvest (pick 3)					
	Ca	K	Mg	N	P	dm	Ca	K	Mg	N	P	dm
33												
norm	13.3	156.6	9.04	72.4	12.86	15.4	7.84	131.38	6.81	56.06	10.52	13.6
-1/3 na	13.4	157.4	8.15	79.8	14.33	13.9	8.61	129.17	7.37	63.86	11.90	14.5
-2/3 na	13.7	163.2	9.35	82.2	15.28	14.7	8.03	127.20	6.75	62.44	11.05	12.7
-1/3 th	12.7	153.2	8.90	78.6	13.96	14.9	7.50	121.16	6.75	61.54	10.88	13.6
-2/3 th	15.0	162.0	8.33	67.8	13.42	15.3	10.11	128.00	7.35	64.51	12.44	14.4
all												
norm	11.8	149.6	8.41	68.6	11.95	15.3	7.15	129.9	6.71	56.2	10.06	14.6
-1/3 th	11.1	155.3	8.52	72.4	12.41	15.2	7.71	122.1	6.38	52.1	9.90	14.9
-2/3 th	12.2	159.6	7.62	64.6	12.38	15.1	8.24	136.2	6.91	58.7	11.34	15.3
F pr.	N.S.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	*
l.s.d.												0.5
mean	11.7	154.8	8.18	68.5	12.24	15.2	7.70	129.4	6.67	55.7	10.4	14.9

Quality- and maturity parameters

pick	weigth	a	b	L	Firm	Firm[1]	starch	TSS	acid	index
1	164	-12.0	31.2	53.4	7.6	10.1	2.4	11.1	0.16	0.33
2	173	-13.0	32.6	54.5	6.9	9.5	2.5	11.4	0.14	0.28
3	183	-12.3	32.7	54.0	6.7	8.3	3.4	11.8	0.14	0.19
4	194	-11.3	33.5	55.5	6.3	7.3	4.0	12.5	0.12	0.14
5	199	-11.7	34.6	56.7	5.9	6.7	6.0	12.5	0.11	0.09
F	**	*	***	***	***	***	***	***	***	***
l.s.d.	19	0.9	0.7	0.9	0.1	0.3	0.5	0.2	0.01	0.04
mean	182	-12.1	32.9	54.8	6.7	8.4	3.7	11.9	0.13	0.21

Fruit position

Quality- and maturity parameters.

Object	weigth	a	b	L	Firm	Firm[1]	starch	TSS	acid	index
Top	204	-13.5	34.2	55.7	6.6	8.4	4.3	11.6	0.13	0.17
High	193	-12.9	33.2	54.8	6.8	8.7	4.0	11.3	0.14	0.19
N-E	169	-12.5	32.4	54.6	6.8	8.3	3.6	11.4	0.14	0.23
S-W	179	-12.7	32.4	54.6	6.8	8.4	3.6	11.3	0.13	0.23
inner	164	-13.1	33.0	54.7	6.9	8.7	3.8	11.0	0.13	0.22
F	***	N.S.	**	N.S.	N.S.	N.S.	N.S.	*	N.S.	***
l.s.d.	20		1.0					0.4		0.03
mean	182	-12.9	33.0	54.9	6.8	8.5	3.9	11.3	0.13	0.21

FPO

Concentration of nutrients in mg/100 gram and % dry matter (dm) of fresh fruit at 2 sample dates.

Object	1 month before estimated optimum harvest						at optimum harvest (pick 3)					
	Ca	K	Mg	N	P	dm	Ca	K	Mg	N	P	dm
Top	9.93	154.2	7.67	69.3	13.25	15.0	7.31	134.9	6.82	57.2	11.51	14.2
High	11.54	157.1	8.00	68.6	13.82	14.8	7.75	142.1	6.98	58.6	11.97	13.8
N-E	12.23	160.2	8.40	68.8	14.06	15.0	8.18	141.1	7.26	58.5	11.97	14.2
S-W	12.51	160.1	8.20	69.6	14.16	14.4	8.01	149.5	7.50	62.9	12.37	14.1
inner	12.85	172.7	9.47	80.9	15.91	14.3	8.91	154.9	7.95	70.7	13.51	13.9
F pr	N.S.	N.S.	*	N.S.	*	N.S.	N.S.	N.S.	*	*	N.S.	N.S.
			1.13		1.58				0.64	7.3		
Gem	11.81	160.9	8.35	71.4	14.24	14.7	8.03	144.6	7.30	61.6	12.27	14.1

Quality- and maturity parameters.

pick	weigth	a	b	L	Firm	Firm[1]	starch	TSS	acid	index
1	165	-12.6	31.4	53.7	7.6	9.9	2.4	10.5	0.17	0.36
2	165	-13.2	32.3	53.6	7.1	9.6	2.8	11.0	0.14	0.26
3	195	-13.1	32.4	54.0	6.8	8.3	3.6	11.1	0.14	0.19
4	189	-13.0	33.9	55.9	6.4	7.5	4.2	12.2	0.12	0.14
5	196	-12.8	35.2	57.2	6.1	7.1	6.3	11.8	0.10	0.09
F	**	N.S.	***	***	***	***	***	***	***	***
l.s.d.	20		1.0	1.5	0.2	0.4	0.6	0.4	0.01	0.03
mean	182	-12.9	33.0	54.9	6.8	8.5	3.9	11.3	0.13	0.21

Relative internal volume (only orchard 33)

		product volume	specific weigth	internal volume	relative internal volume
pick 1	Top	0.0001935	989.874	0.0089342	4636.42
pick 2		0.0001588	992.632	0.0069880	4370.74
pick 3		0.0001703	994.635	0.0071393	4177.73
pick 4		0.0001754	1009.289	0.0048820	2765.99
pick 5		0.0002228	988.829	0.0105662	4737.05
pick 1	High	0.0001515	986.858	0.0073928	4926.94
pick 2		0.0001631	990.178	0.0075356	4607.12
pick 3		0.0001981	993.272	0.0085546	4309.05
pick 4		0.0001885	1006.877	0.0058779	2998.34
pick 5		0.0002159	987.455	0.0106540	4869.47
pick 1	N-E	0.0001129	984.959	0.0057480	5109.89
pick 2		0.0001054	989.759	0.0049194	4647.53
pick 3		0.0001611	987.390	0.0078238	4875.71
pick 4		0.0001460	1010.173	0.0040799	2680.81
pick 5		0.0001662	989.316	0.0078431	4690.22
pick 1	S-W	0.0001182	984.726	0.0060394	5132.41
pick 2		0.0001335	991.475	0.0060190	4482.14
pick 3		0.0001796	990.834	0.0081907	4543.92
pick 4		0.0001699	1004.938	0.0056023	3185.14
pick 5		0.0001935	988.160	0.0092235	4801.58
pick 1	inner	0.0001127	984.179	0.0058104	5185.04
pick 2		0.0001308	984.543	0.0067474	5150.05
pick 3		0.0001576	990.559	0.0072859	4570.42
pick 4		0.0001814	1005.572	0.0057231	3124.07
pick 5		0.0001648	987.291	0.0081031	4885.25

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

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

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Participant no.5: Flanders Centre for Postharvest Technology (VCBT)
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A. PARTNER INFORMATION

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

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

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

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

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

Actions in the project
Task 1: Cultivation of pears
Task 2: Harvest of pears
Task 3: Storage of pears
Task 4: Variation in climate and orchard characteristics
Task 6: Measuring gas exchange and diffusion rates
Task 7a: Destructive measurements, Quality evaluation

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

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	1
No. of man-month devoted already to the task:	0,25

Objectives
Produce pears grown in different climate conditions, in orchards with varying susceptibility for Brown Heart.

Material and methods
3 orchards are used located in Velm, Rillaar and Zellik. The orchard of Zellik is known to be more susceptible. However this is more an opinion from the owner than it is a scientifically based fact. The locations of the orchards are given in Figure 1. Each orchard had 4 subsets of 10 trees each.

Results and Discussion
In the season '97-'98 the production of pears suffered from frost damage of the blossoms. The orchard at Velm had a water spraying system to prevent frost damage. The spraying dates are shown in Table 1. The other two orchards were treated with gibberilic acid to force the development of fruits on the frozen blossom.

Task 2: Harvest of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	4
No. of man-month devoted already to the task:	1

Objectives
Investigate the influence of picking date on the development of Brown Heart.

Material and methods
Optimal picking date was determined by the Streif index. Pears are picked at the optimal picking date, 1 week before and 1 week after the optimal picking date.

Results and Discussion
Starting from 3/8/97, each week pears were picked at the orchard of Velm. Firmness (Figure 2), soluble solids content (Figure 3) and starch index (Figure 4) are measured. From these measurements the Streif index is calculated. The results are shown in Figure 5. With this data and data from previous years the optimal picking period for long time storage was determined as being from 8 till 20/9/97.

Conclusions
The pears for the project are picked at the following dates:
Early picking: 8-9/9/97
Optimal picking: 15-16/9/97
Late picking: 22-23/9/97

Task 3: Storage of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	4
No. of man-month devoted already to the task:	1

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

Material and methods
After picking the pears were stored for 1 week at -0.5°C, 95%RH in air. Then, half of the pears was stored at 2% O_2 and 0.7% CO_2 (control storage conditions) and the other half at 2% O_2, 5% CO_2 (Brown Heart inducing condition).

Results and Discussion
There have been some technical difficulties with the storage facilities in the beginning of the storage period. Figure 6 shows the temperature profile during the whole storage period of one of the storage containers.

Conclusions
Probably the high temperatures had a certain effect on the pears. However, it is believed that the overall effect on the project results is small and that it will not effect the final results of the project. To which extend we can use the data has to be discussed.

Task 4: Variation in climate and orchard characteristics

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	1
No. of man-month devoted already to the task:	0,25

Objectives
Relate the meteorological data with the pear characteristics and occurrence of Brown Heart.

Material and methods
The orchards at the locations Velm and Rillaar have their own weather stations. For the orchard of Zellik data of a nearby meteorological station was used.

Results and Discussion
In the orchard of Velm temperature, relative humidity, radiation and rainfall were measured and logged every hour. In the orchard of Zellik temperature, relative humidity and rainfall were measured and logged every hour. Since june '98 there are also radiation measurements. In the orchard of Rillaar the maximum and minimum temperature and rainfall were measured daily.
Charts have been drawn for the different parameters. The temperature (Figure 7) follows the same pattern for all three orchards. The radiation profile from Velm (Figure 8) shows a distinct day to day variation. In the beginning of august it was cloudy but the second half was very sunny. In the rainfall pattern (Figure 9) we can clearly see the difference in location of the three orchards. In the humidity chart (Figure 10) we see a big variation between days.

Conclusions
The data are available for Task 9 Modelling and have been sent to the ATO-DLO. The variation in climate characteristics is clear.

Task 6: Measuring gas exchange and diffusion rates

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	7
No. of man-month devoted already to the task:	2

Objectives
Determination of respiration rates under normal and Brown Heart inducing storage conditions.

Material and methods
The respiration rate of the pears was measured under several gas conditions as shown in Table 2. The experiment was carried out at - 0.5°C. The pears were weighed and put in respiration containers of 1.2 litre. The pears were allowed to equilibrate with the atmosphere for 48 hours during which the respiration container was flushed with the particular gas mixture. Then the respiration container was closed and the concentrations of O_2 and CO_2 were measured using a microGC, type 2002 from Chrompack. After about 48 hours the concentrations of O_2 and CO_2 were measured again.
The method for the diffusion measurements is being refined.

Results and Discussion
The results of the respiration measurements are shown in Table 3.
A gas-mixing device is being constructed which will facilitate the work in the future. In this way it will be possible to do more measurements as well.

Conclusions
The respiration rates are lowest with low O_2 and high CO_2, while they are highest for high O_2 and low CO_2 conditions.

Task 7a: Destructive measurements, quality evaluation

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-months:	4
No. of man-month devoted already to the task:	1

Objectives
To evaluate the quality changes after storage followed by a post storage shelf life simulation.

Material and methods

Selection of pears
From each tree (See Task 1) 10 pears were picked at each of the 3 picking dates (See Task 2). From these 10 pears, 5 were stored in control conditions and 5 under Brown Heart inducing conditions (See Task 3). From each group of five pears, 1 pear was evaluated in evaluation 1, 1 in evaluation 2 and so on (Figure 11).

Quality evaluation method
Ground colour: with Eurofru colour chart.
Firmness: with penetrometer
Brown Heart: browning and cavities with picture chart delivered by partner 4. The pear was sliced in two from stem to blossom end and compared with a range of pictures. Each pear was classified on a scale from 0 to 4.
Picture charts to evaluate open style channel were developed and are shown in Figure 12.

Results and Discussion

At this date, all four quality-evaluations were carried out. Evaluation 1 was carried out on 6-7/11/97, evaluation 2 on 22-23/12/97, evaluation 3 on 4-6/02/98 and evaluation 4 on 11-12/03/98.
The results of ground colour and firmness are summarised in Table 4.
The results of the Brown Heart (brown spots) evaluation are shown in Figures 13 and 14. The effect of picking date is evident. The late picked pears show the severest occurrence of brown spots. The same was observed for the Brown Heart cavities shown in Figures 15 and 16. The effect of storage conditions is also very clear. The Brown Heart inducing storage conditions (2% O_2, 5% CO_2) have clearly more browning and cavities then the control storage conditions. Further, there were differences between the orchards. We can see that Rillaar is most sensitive, followed by Zellik and Velm has the lowest Brown Heart occurrence. There are little differences between evaluation 1 and evaluation 2 for 0.7% CO_2 conditions but for 5% CO_2 conditions the differences were more pronounced especially at the late picking date. The difference between evaluations 2 and 3 are substantial while the differences between evaluations 3 and 4 are small for both storage-conditions. Cavities increased from evaluation 1 to evaluation 2.
The pears from Zellik and Rillaar had almost all open style channels while pears from Velm which were not treated with gibberilic acid had significantly less open style channels. This is shown in Figures 17 and 18. There is no difference between the two storage-conditions.

Conclusions

Picking date, location and CO_2 conditions are the determining factors in the occurrence of Brown Heart. Seen the fact that Velm came out best and in Velm no gibberilic acid was used we can conclude that this use is also an important factor.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997 TO 31-05-1998

The storage of the pears was done in a new experimental storage facility. Because of the complexity of the system there have been some technical difficulties in the beginning of the storage period. The temperature of -0.5°C was not reached the whole time. The problems have been solved and everything is working as planned.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

Tasks 1 to 4 will be continued in a similar way as in the period 01-03-1997 to 31-05-1998.
Tasks 6 and 7a will be continued. Details of the work to be performed need to be discussed on the next meeting 15-16/06/1998. In addition to the respiration rate measurements, diffusion measurements will be carried out in Task 6.
Task 9: Data of 1 year is now available. Detailed statistical analysis will be carried out and model-activities will be started.

E. DISSEMINATION

No action so far.

F. ANNEX

Tables

Table 1: Frost damage prevention spraying at the orchard of Velm

date	Spaying period (Hr)	Minimum temperature (°C)	Freezing period (Hr)
22/03/97	3.15 - 10.00		
07/04/97	3.30 - 11.00	-1.8	4.00 - 9.00
08/04/97	3.45 - 10.15	-1.5	6.00 - 8.00
13/04/97	5.30 - 9.00	-1	6.00 - 8.00
17/04/97	5.00 - 8.00	0	6.00
18/04/97	4.45 - 10.00	-1.1	5.00 - 8.00
21/04/97	23.45 - 12.00	-5	00.00 - 8.00
23/04/97	2.30 - 10.15	-3.3	2.00 - 8.00

Table 2: Atmospheric conditions for which respiration measurements were carried out

% O2	0,5				0				2				5	8	21			
% CO2	0,7			5	0			5	0,7			5	5	0,7	0			5
Temp	-0,5	1	-0,5	1	-0,5	1	-0,5	1	-0,5	1	-0,5	1	-0,5 1	-0,5	1	-0,5	1	-0,5 1

Table 3: Results of the respiration measurements

Respiration conditions			Respiration rate					
O_2 [%]	CO_2 [%]	temp. [°C]	O_2 [mmol O_2/kg.u]			CO_2 [mmol CO_2/kg.u]		
			mean	st.dev	No of obs.	mean	st.dev	No of obs.
0	0	-0.5	0.0012	0.0003	3	0.0495	0.0118	3
0	0	1	0.0129	0.0136	3	0.0687	0.0110	3
0	5	-0.5	0.0034	0.0005	4	0.0292	0.0130	4
0	5	1	0.0029	0.0007	4	0.0244	0.0070	4
0.5	0.7	-0.5	0.0280	0.0028	4	0.0224	0.0030	4
0.5	0.7	1	0.0341	0.0159	4	0.0419	0.0113	4
0.5	5	-0.5	0.0381	0.0048	3	0.0296	0.0062	4
0.5	5	1	0.0249	0.0156	4	0.0189	0.0186	4
2	0.7	-0.5	0.0786	0.0098	4	0.0725	0.0123	3
2	0.7	1	0.0518	0.0030	4	0.0449	0.0050	4
2	5	-0.5	0.0614	0.0100	4	0.0475	0.0117	4
2	5	1	0.0414	0.0023	4	0.0341	0.0053	4
5	5	-0.5	0.0703	0.0220	4	0.0130	0.0076	4
5	5	1	0.0400	0.0103		0.0349	0.0033	4
8	0.7	-0.5	0.0477	0.0035	4	0.0463	0.0032	4
8	0.7	1	0.0447	0.0058	4	0.0424	0.0053	4
21	0	-0.5	0.0511	0.0075	3	0.0396	0.0039	4
21	0	1	0.0594	0.0423	4	0.0525	0.0244	3
21	5	-0.5	0.0726	0.0290	4	0.0616	0.0089	4
21	5	1	0.0462	0.0061	4	0.0480	0.0024	4

Table 4: Quality evaluation of the stored pears in normal and Brown Heart inducing conditions

	Conservation at 2 % O_2 and 0.7 % CO_2									Conservation at 2 % O_2 and 5 % CO_2								
	8-9/9/97			15-16/9/97			22-23/9/97			8-9/9/97			15-16/9/97			22-23/9/97		
	Velm	Zellik	Rillaar	Velm	Zellik	Rillaar	Velm	Zellik	Rillaar	Velm	Zellik	Rillaar	Velm	Zellik	Rillaar	Velm	Zellik	Rillaar
Evaluation 1																		
Background Colour	3.20	-	3.80	4.60	-	5.40	4.40	-	5.20	2.75	3.00	4.40	3.60	2.50	4.60	4.60	3.00	4.60
Firmness	1.68	1.34	2.04	1.74	1.40	1.98	1.62	1.16	1.58	1.70	1.54	1.88	1.72	1.50	1.84	2.48	1.50	1.64
Evaluation 2																		
Background Colour	1.88	1.25	2.88	1.88	1.50	2.38	2.00	1.88	3.38	1.25	1.00	1.88	1.13	1.00	2.63	2.71	1.25	2.63
Firmness	2.51	2.44	2.93	2.36	2.34	3.00	2.28	2.06	2.99	2.98	3.66	4.26	2.83	2.41	2.80	2.78	2.26	2.88
Evaluation 3																		
Background Colour	4.0	3.0	5.0	2.8	1.4	2.6	3.6	2.6	4.8	1.8	1.5	2.8	1.9	1.4	2.4	4.6	1.4	5.1
Firmness	2.0	1.71	1.89	2.50	2.31	2.56	2.34	1.99	2.24	2.79	1.90	2.41	2.60	2.13	2.39	1.83	2.29	1.89
Evaluation 4																		
Background Colour	4.1	3.0	5.1	3.6	2.4	3.8	4.3	2.1	4.8	1.6	1.8	2.5	3.1	2.1	4.1	3.9	2.1	4.0
Firmness	2.5	2.18	2.67	2.65	2.06	2.55	2.69	2.44	2.71	3.03	2.38	2.60	2.26	2.38	3.20	2.64	2.00	2.58

Figures

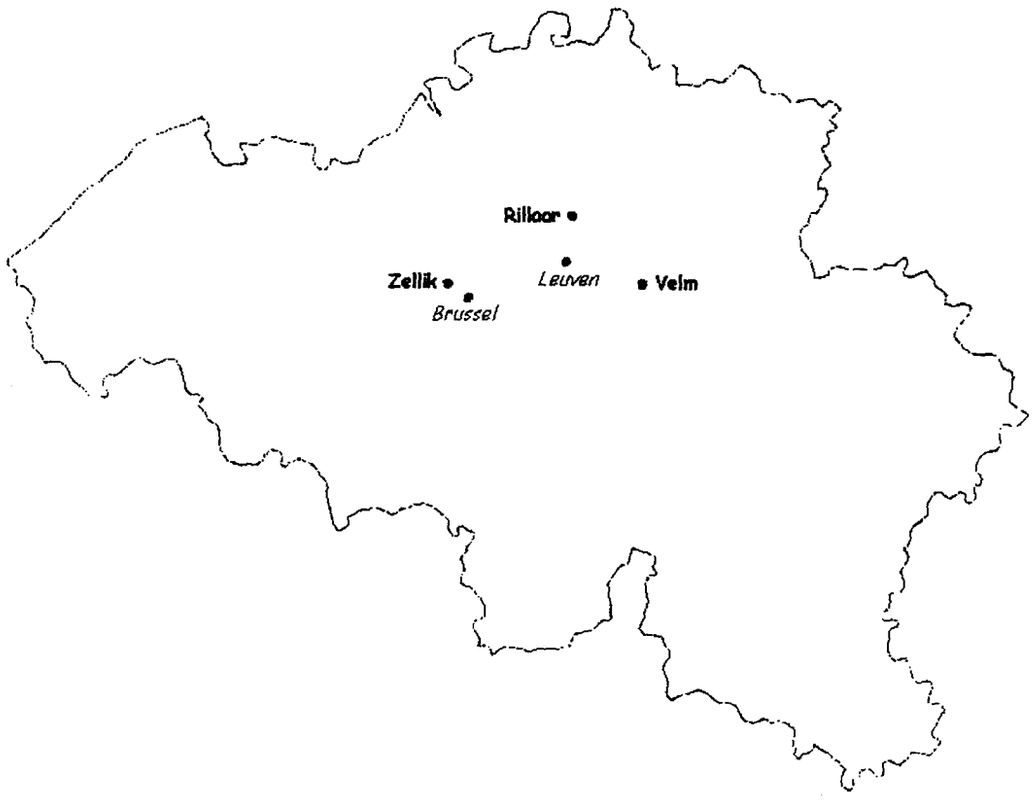


Figure 1: Locations of orchards

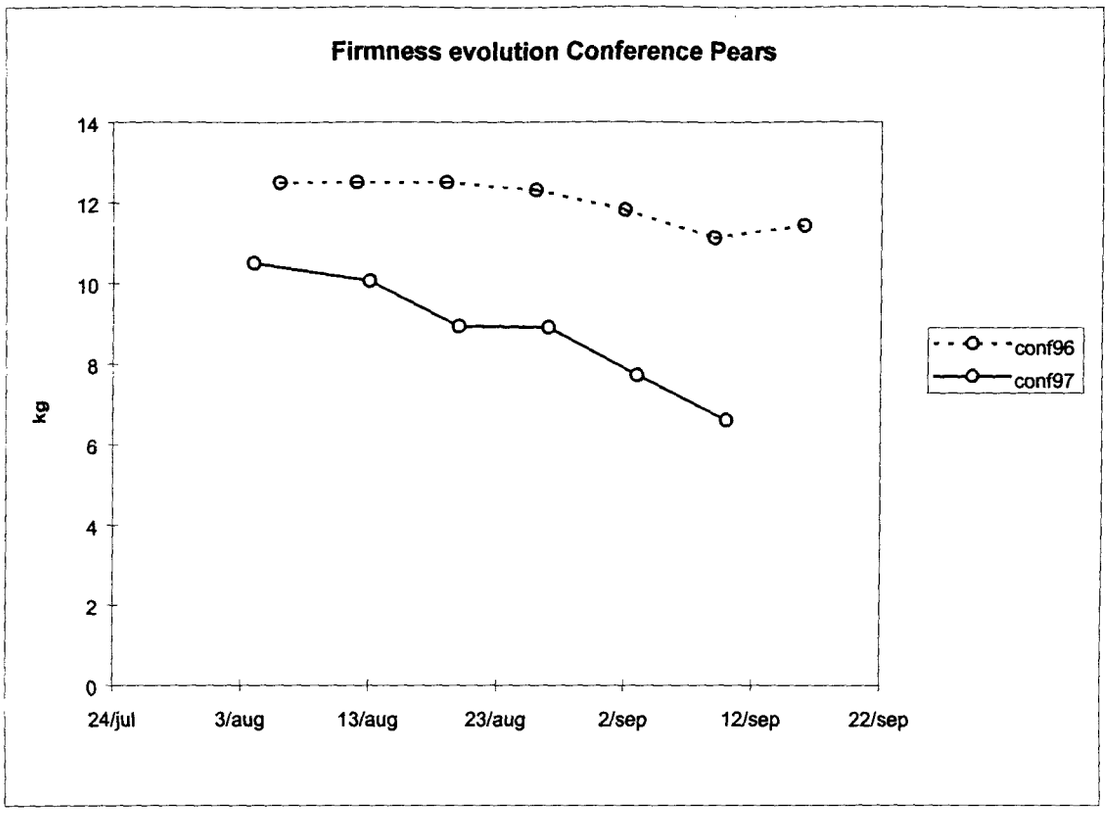


Figure 2: Firmness evolution of Conference pears during growth

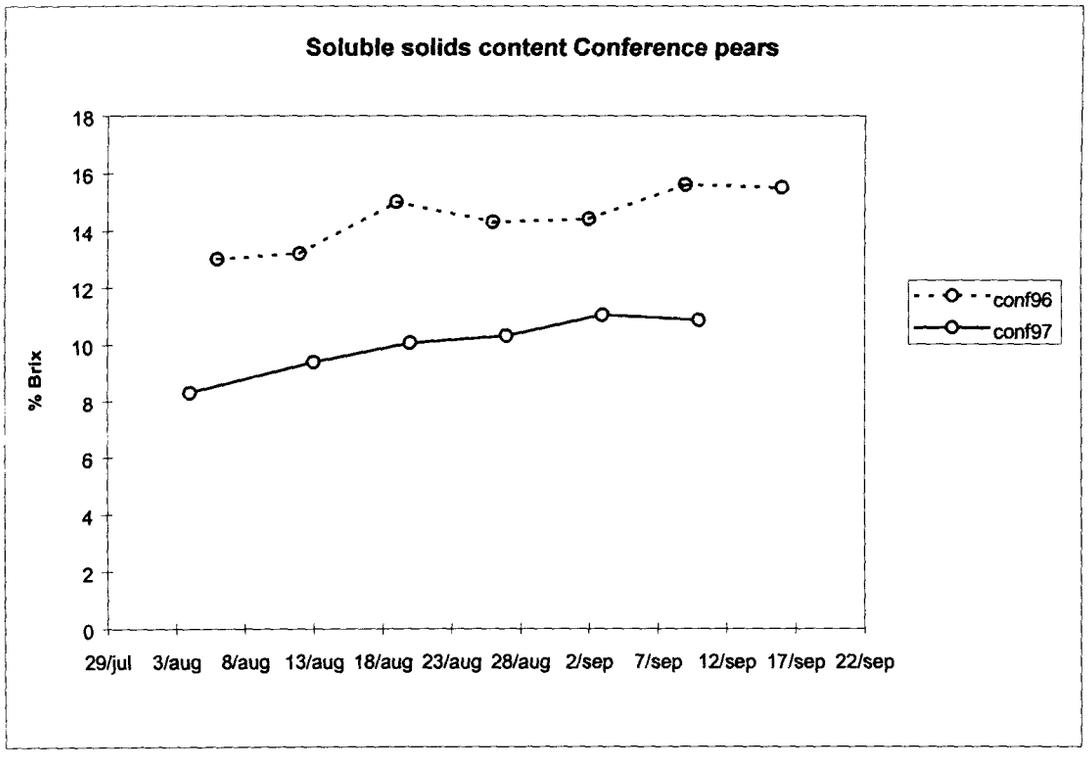


Figure 3: Soluble solids content evolution of Conference pears during growth

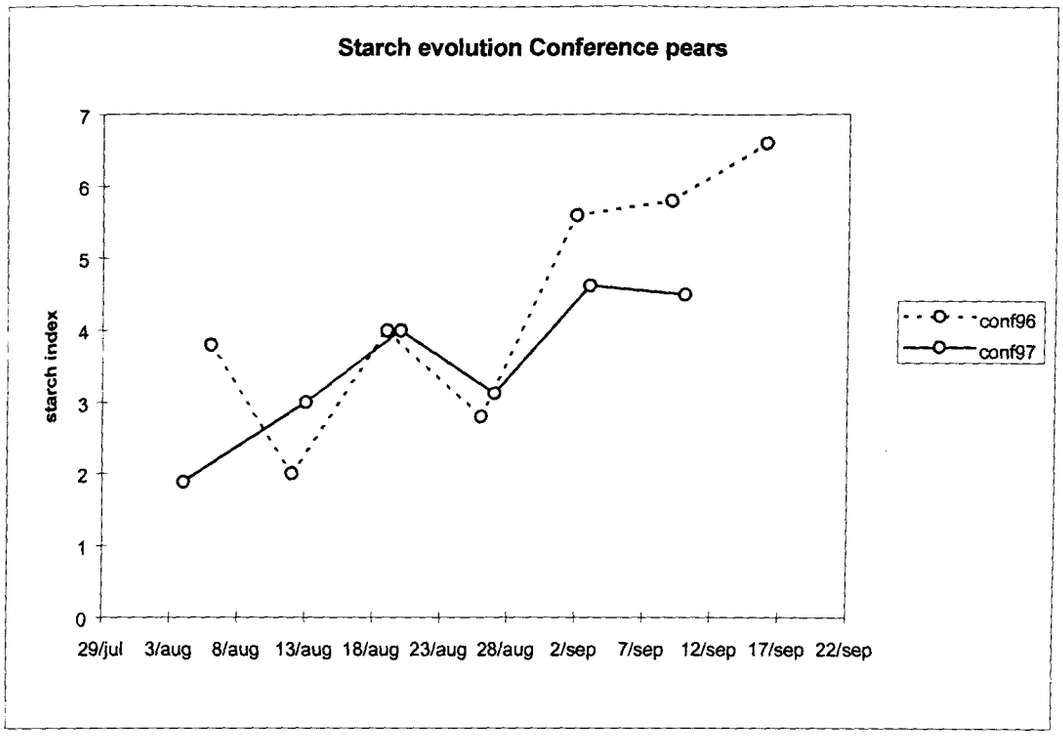


Figure 4: Starch index evolution of Conference pears during growth

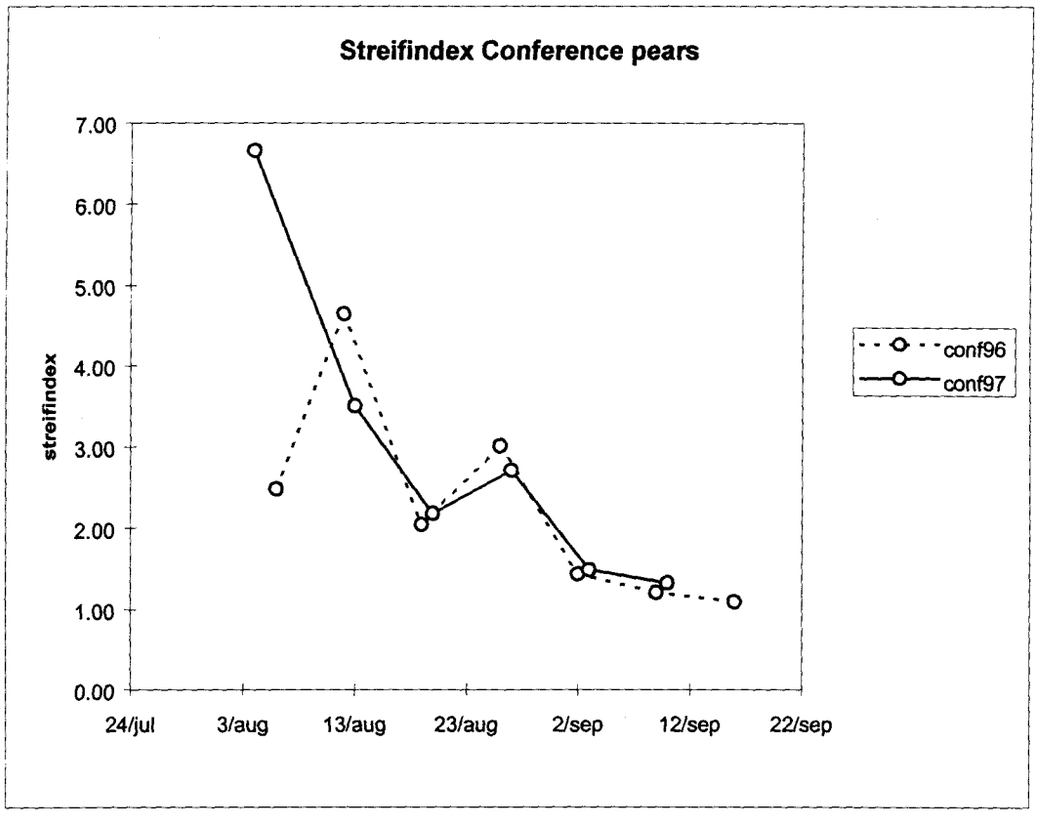
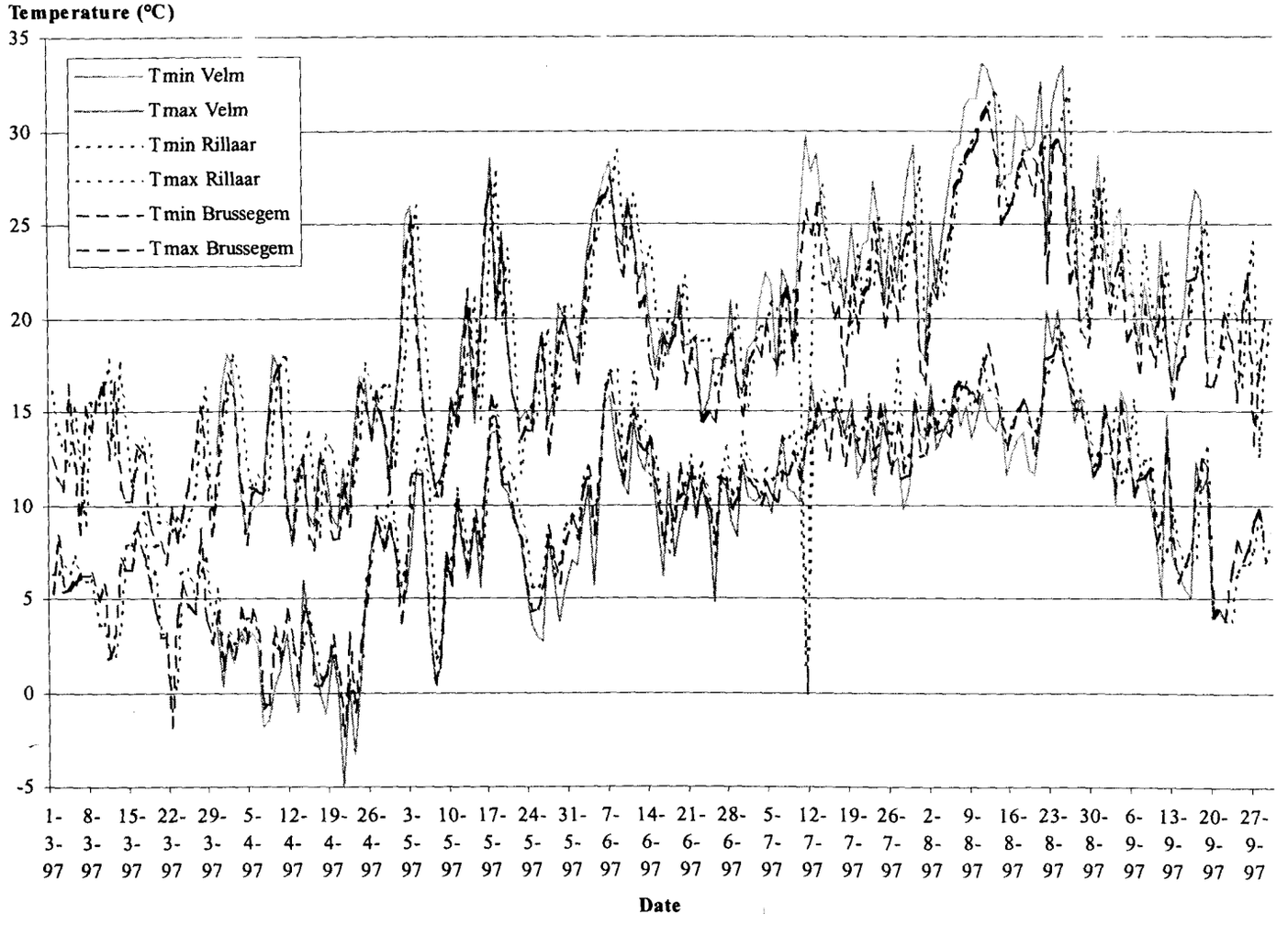


Figure 5: Evolution of the Streif index of Conference pears during growth

Figure 6: Temperature during storage of pears



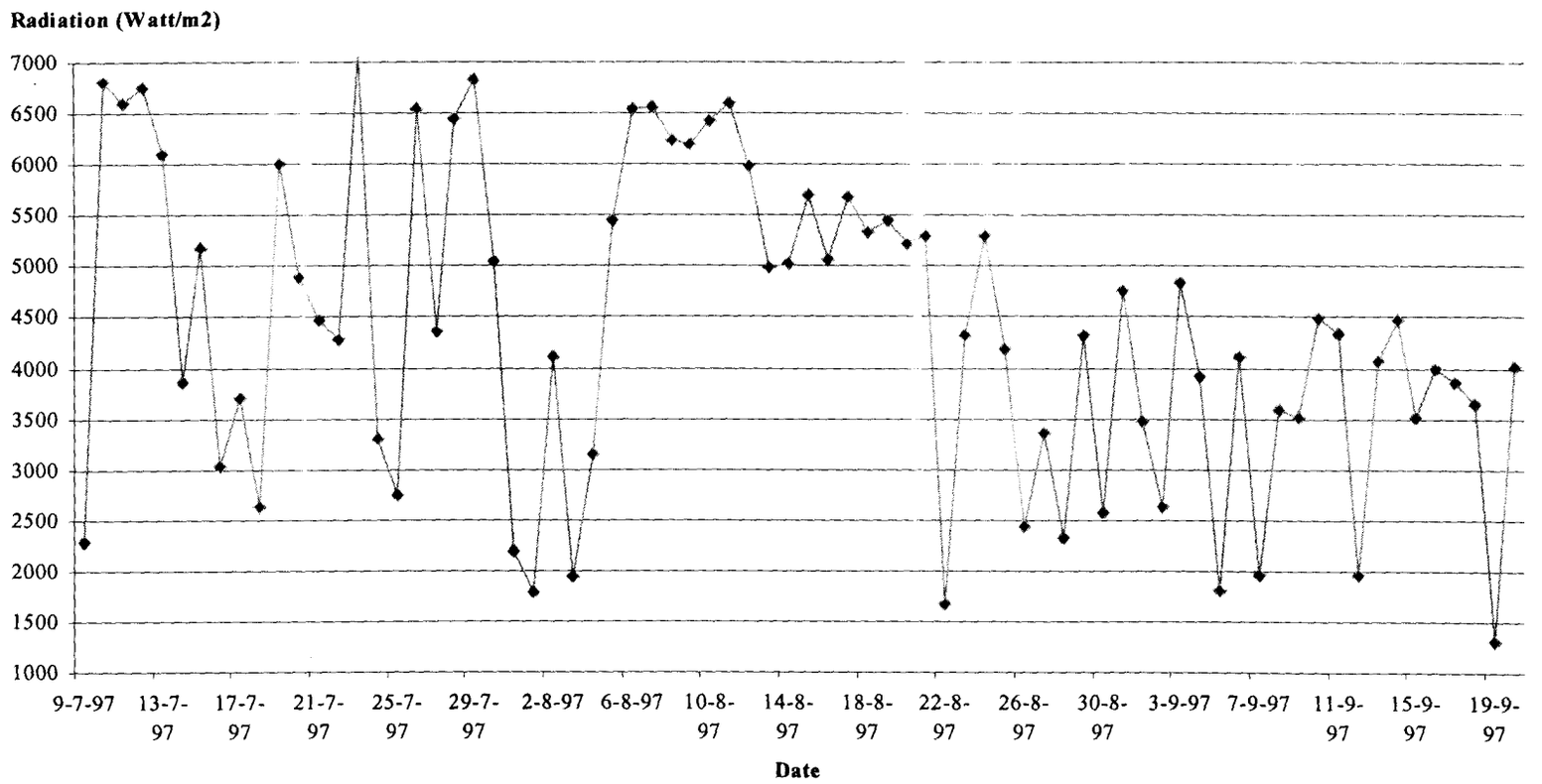


Figure 8: Radiation

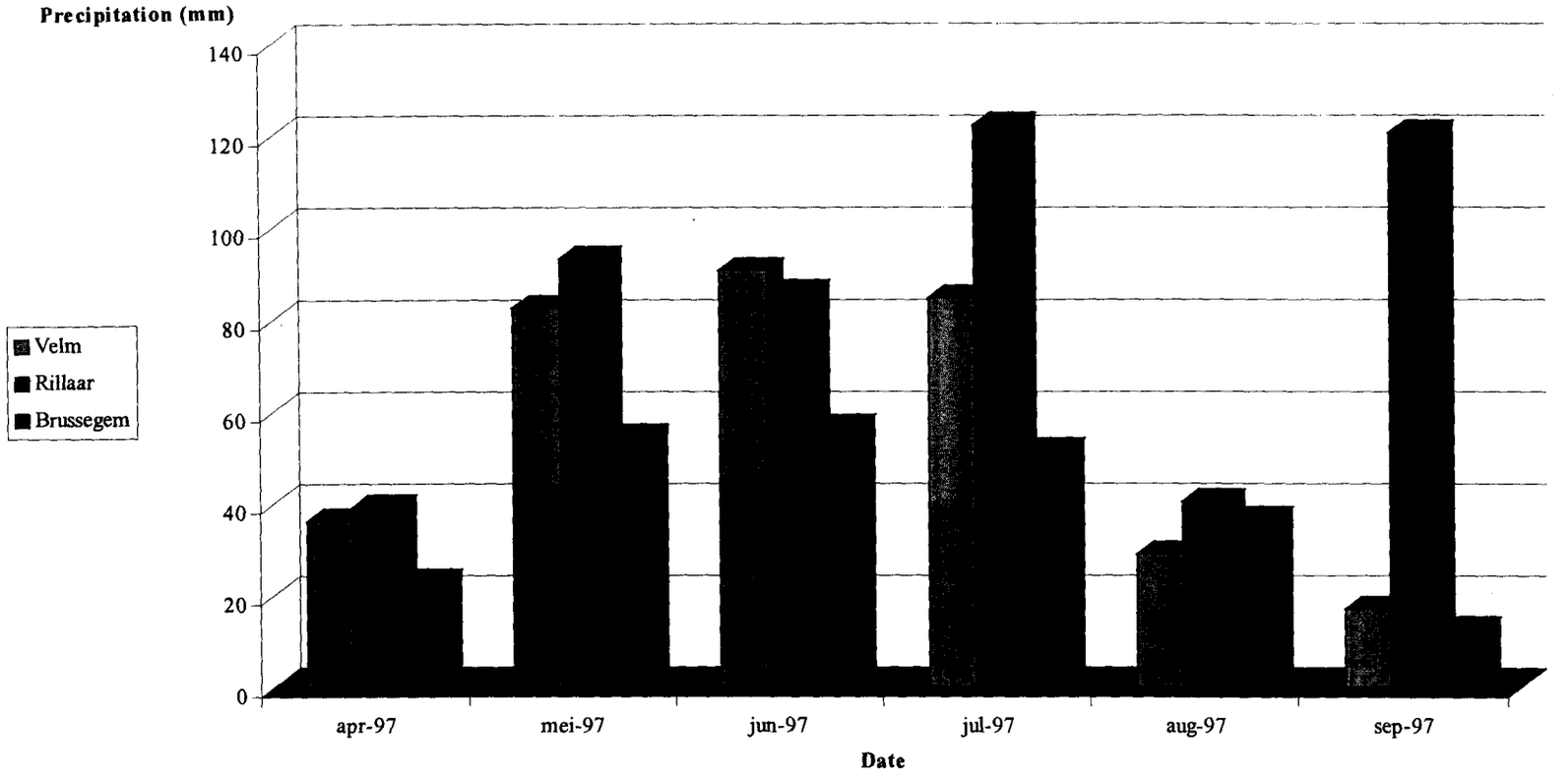


Figure 9: Precipitation

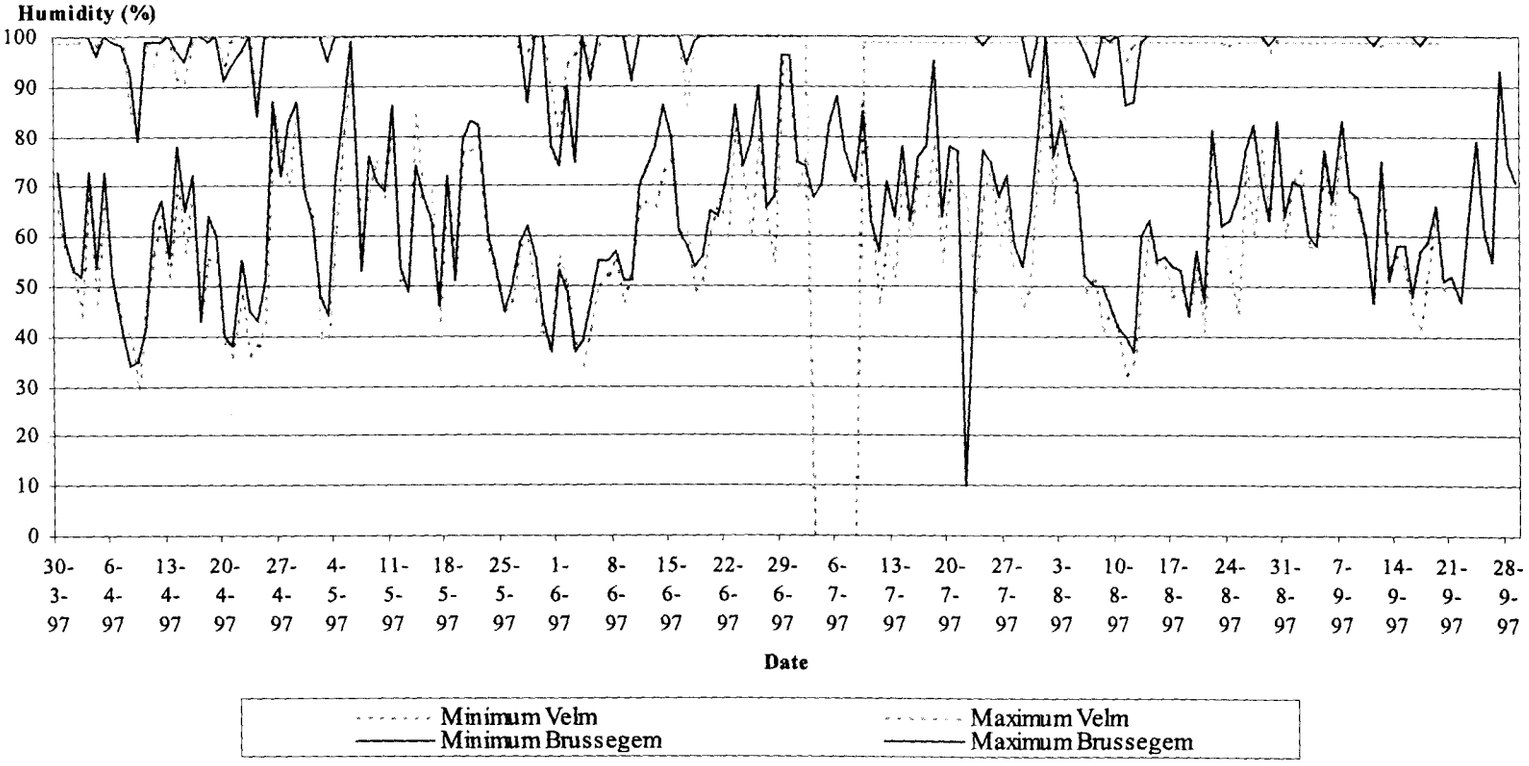


Figure 10: Relative Humidity

Figure 11: Production storage and quality evaluation of pears

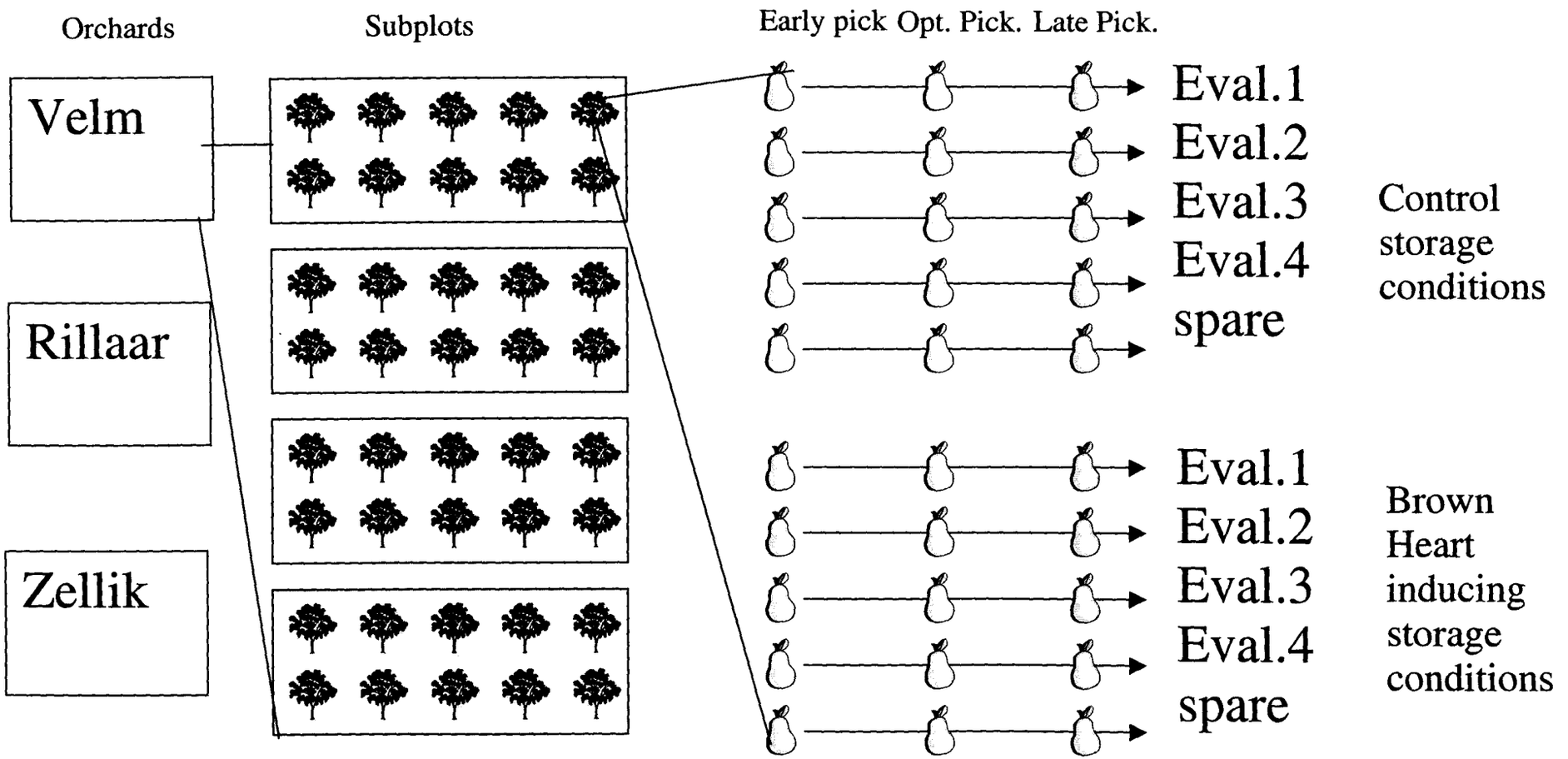
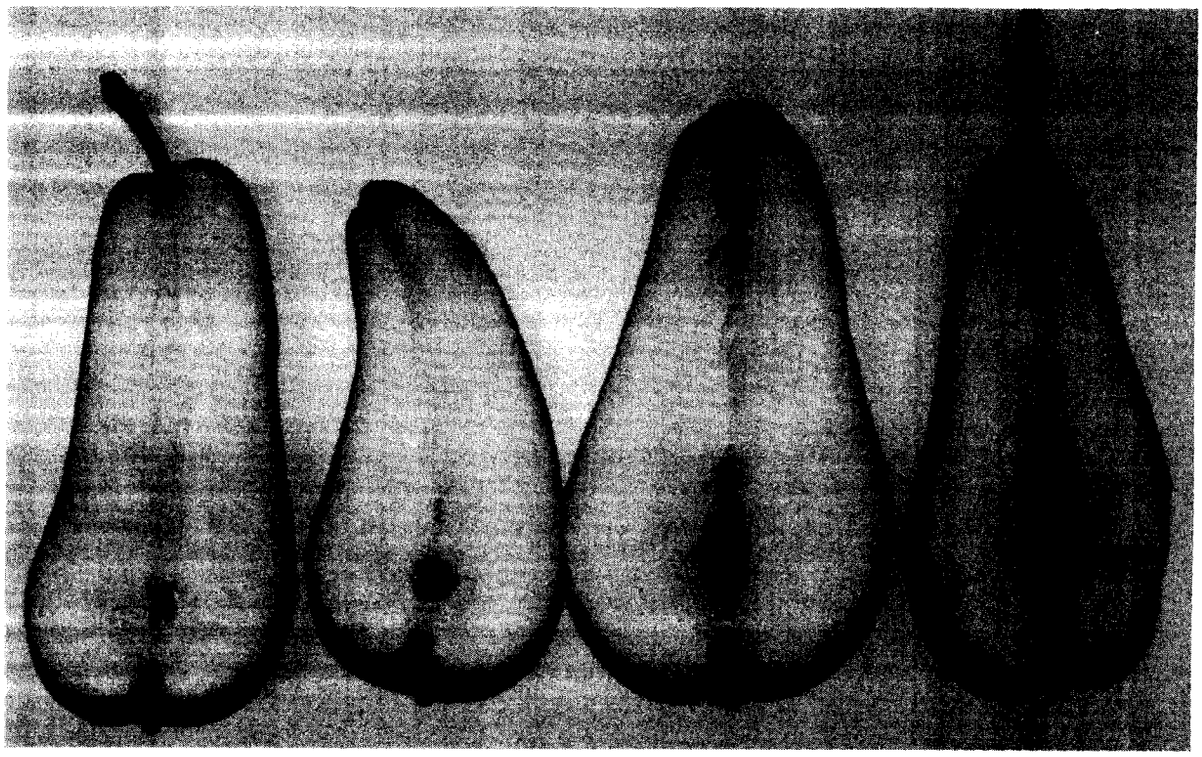


Figure 12: Picture chart to evaluate open style channel



Figures on next pages:

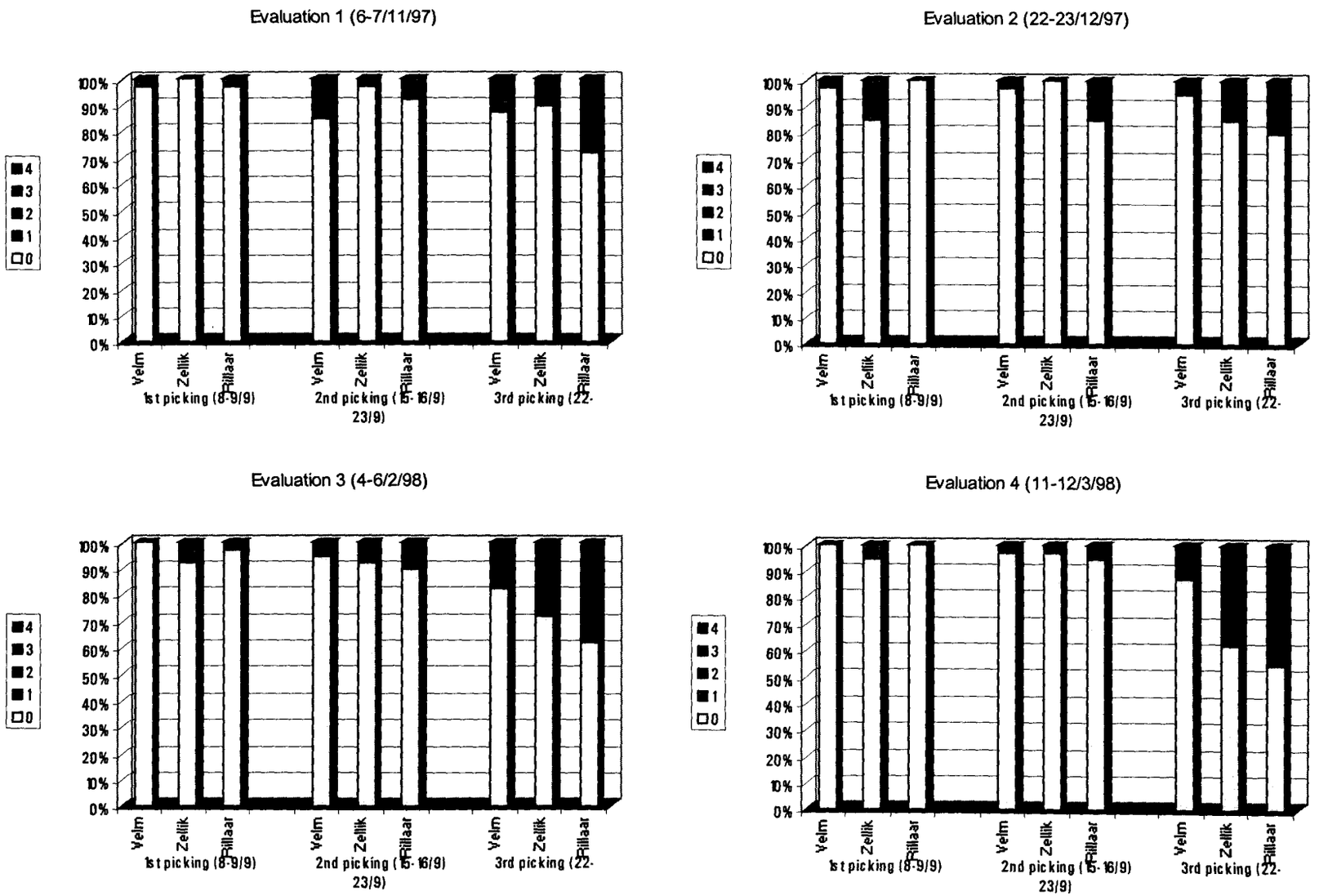
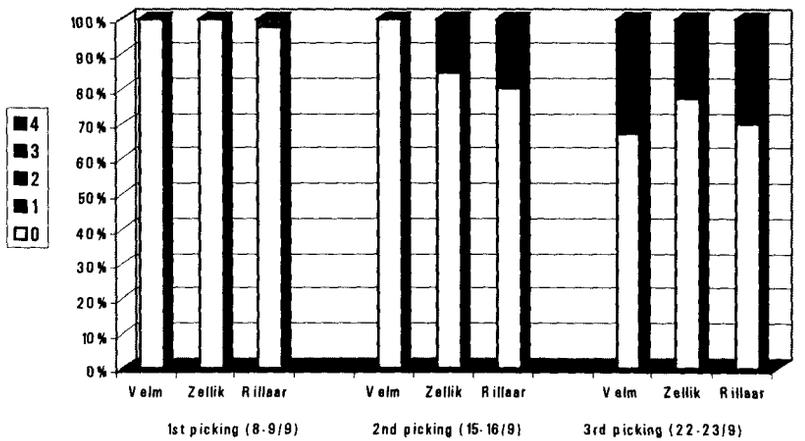
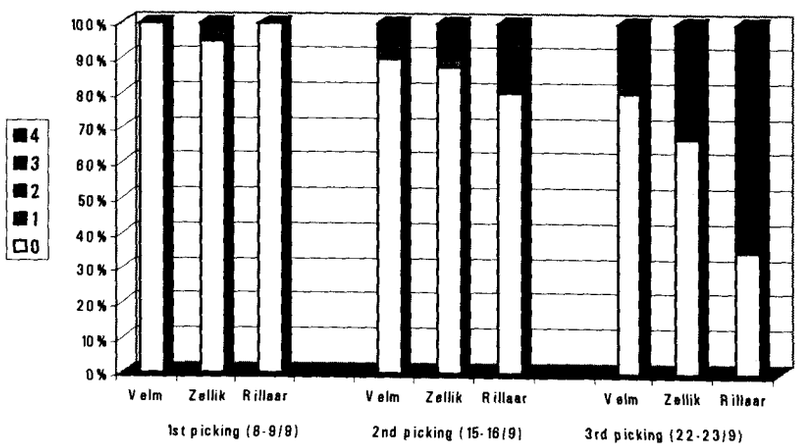


Figure 13: Production storage and quality evaluation of pears

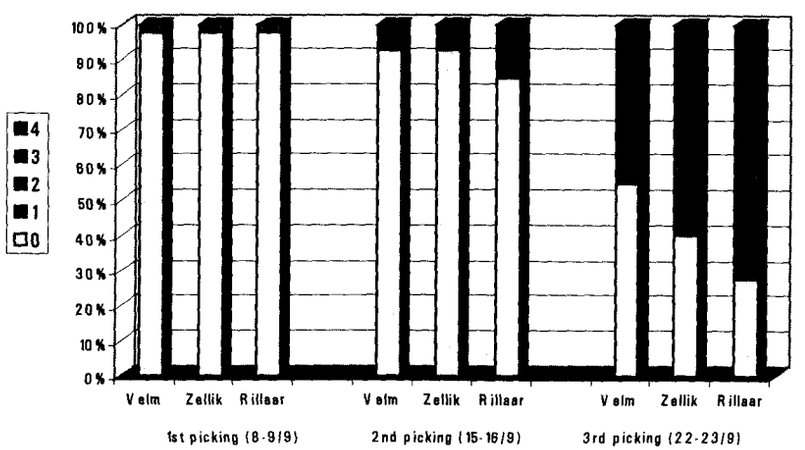
Evaluation 1 (6-7/11/97)



Evaluation 2 (22-23/12/97)



Evaluation 3 (4-6/2/98)



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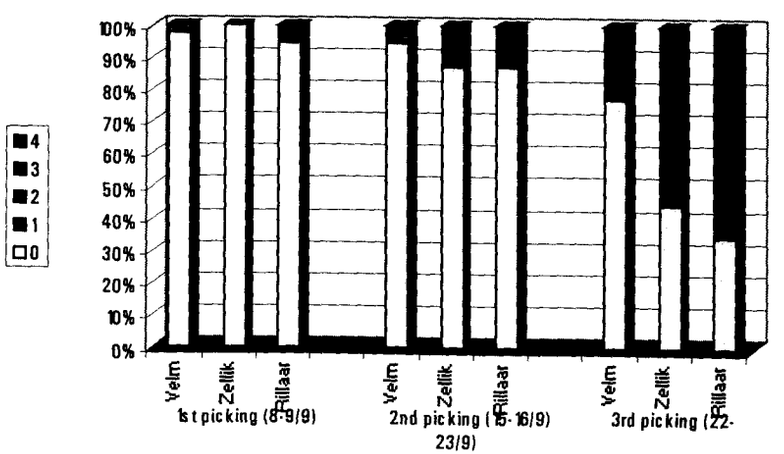

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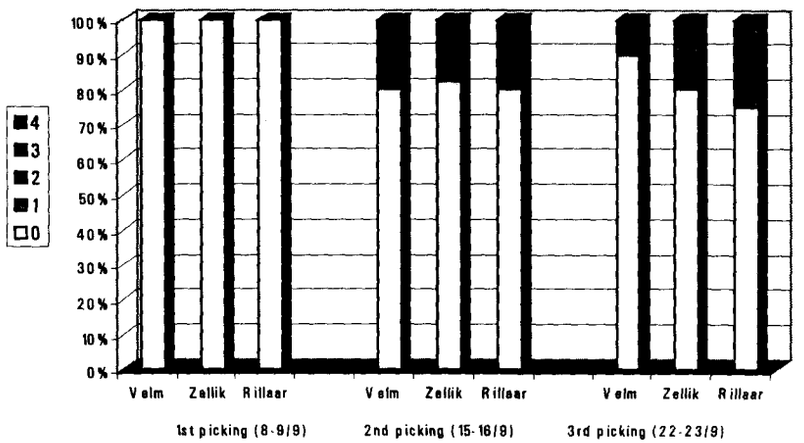

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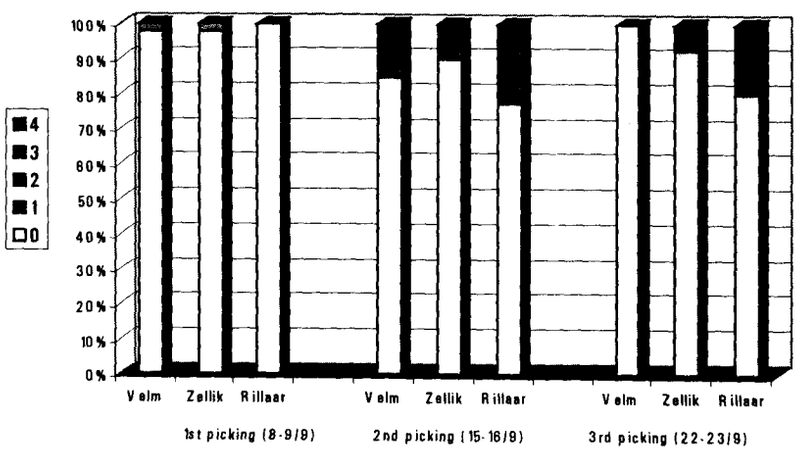

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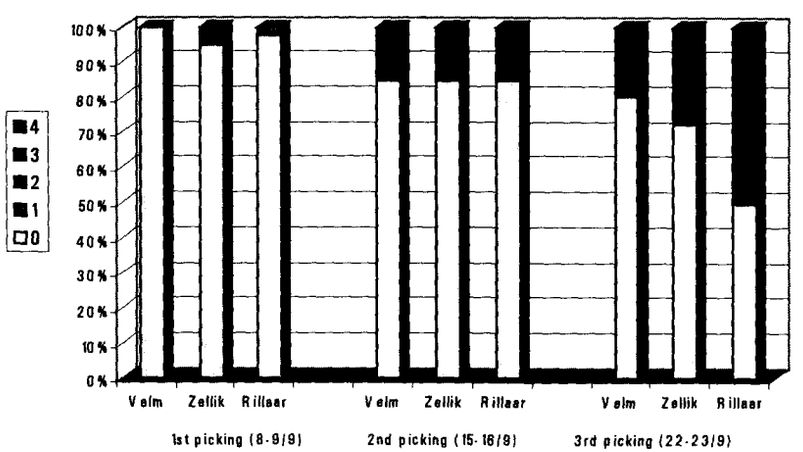

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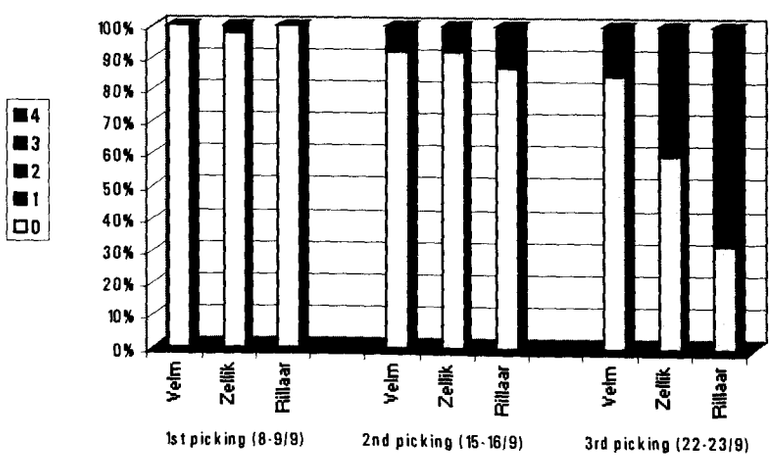

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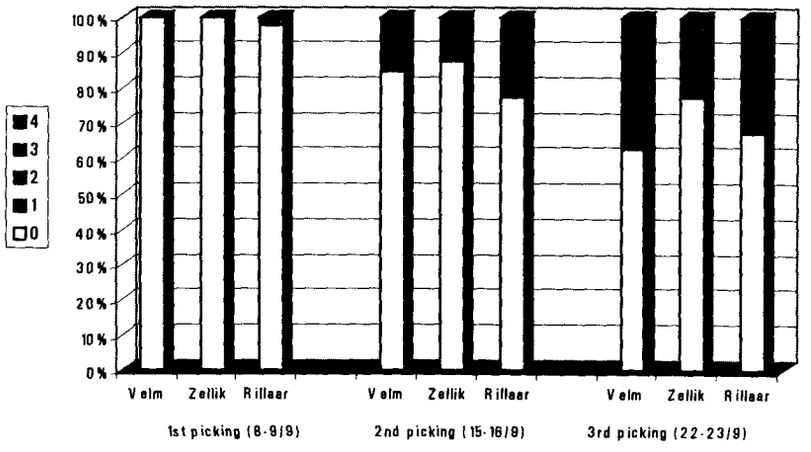

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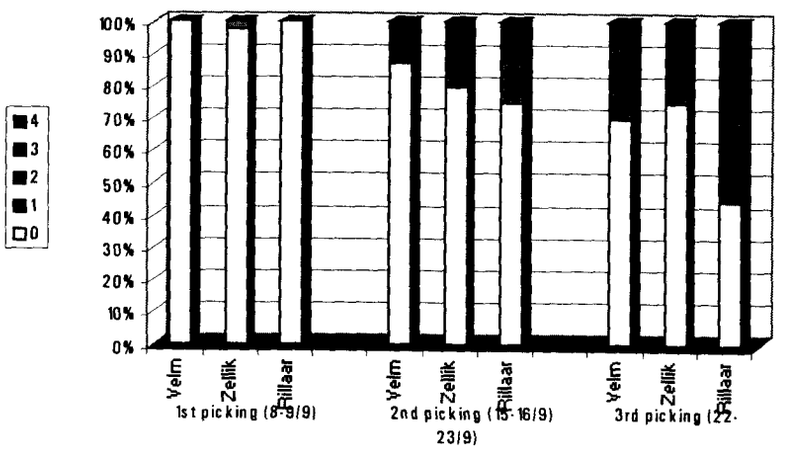

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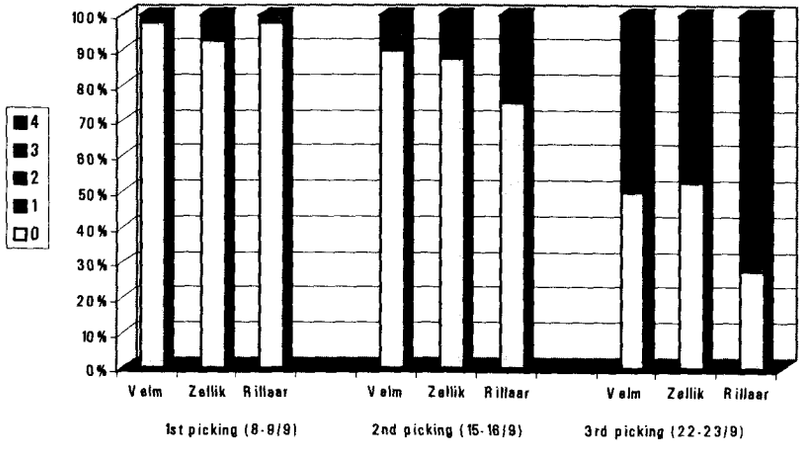

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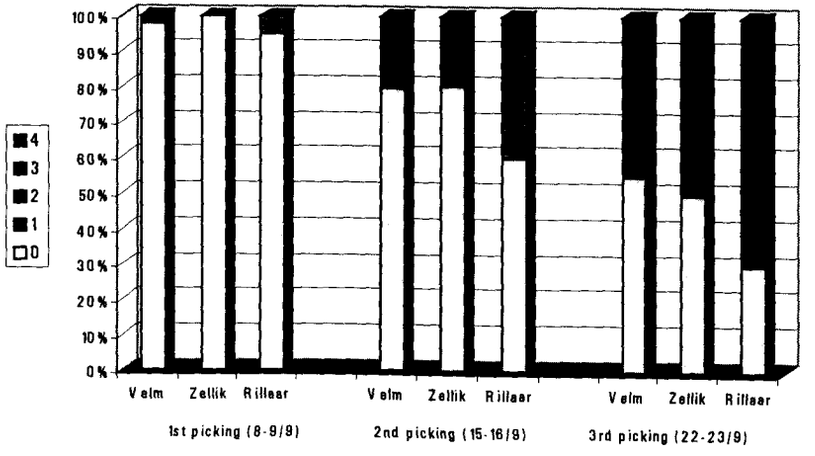

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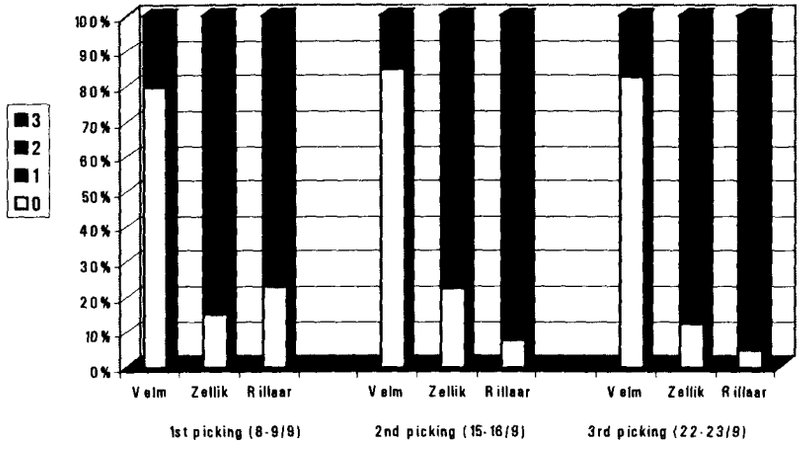

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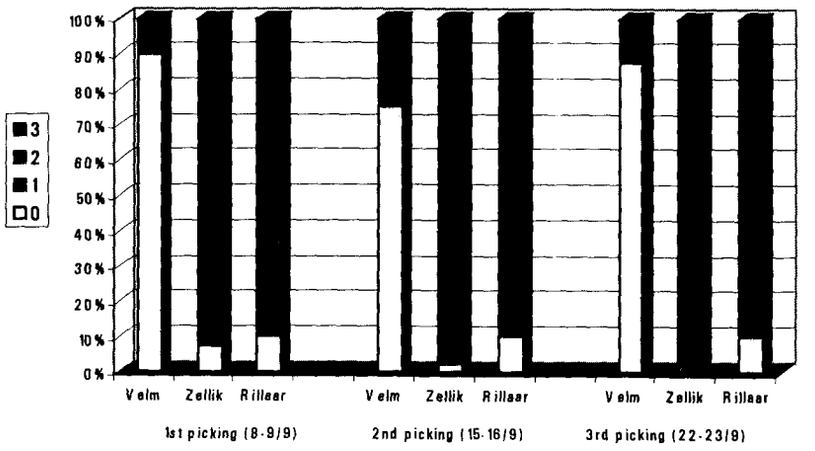

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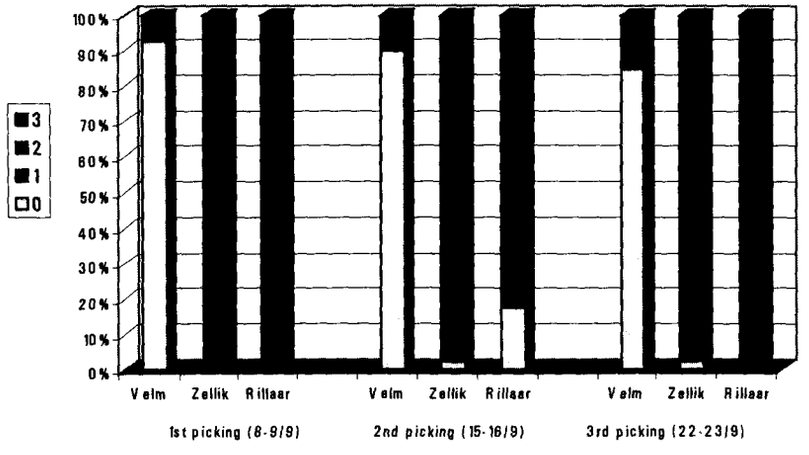

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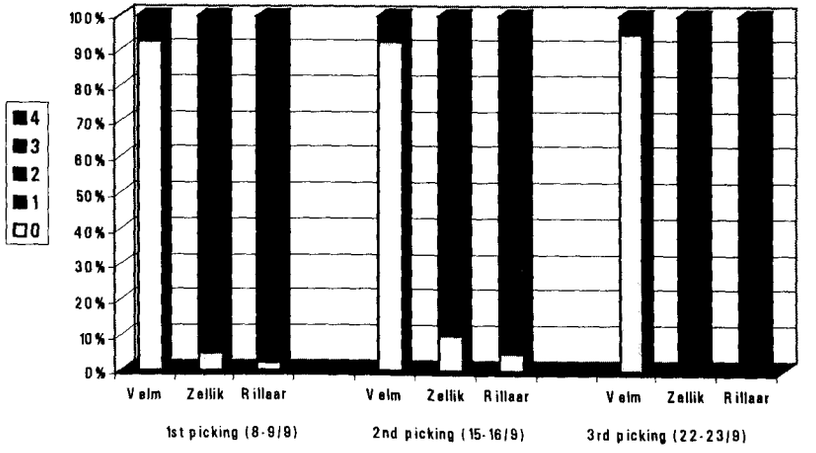

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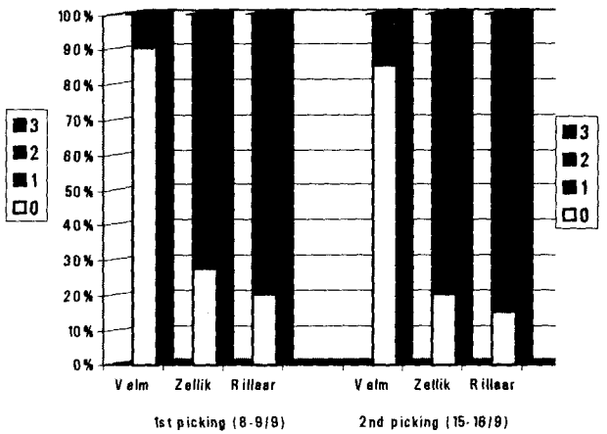

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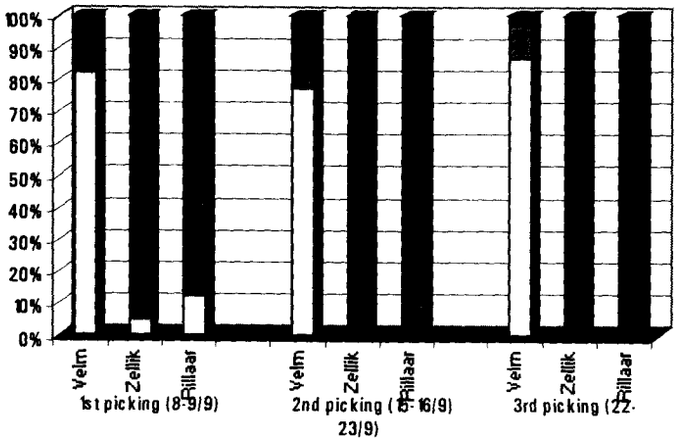

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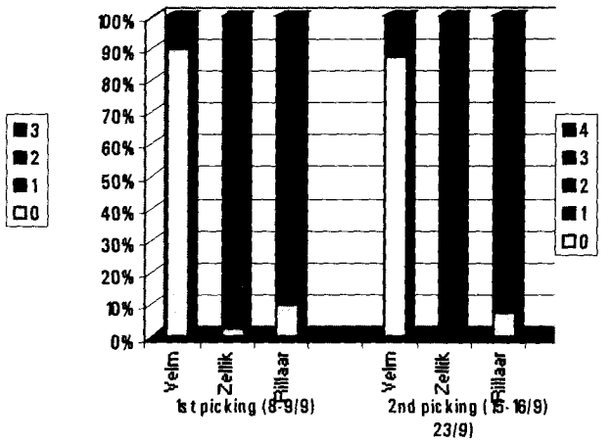

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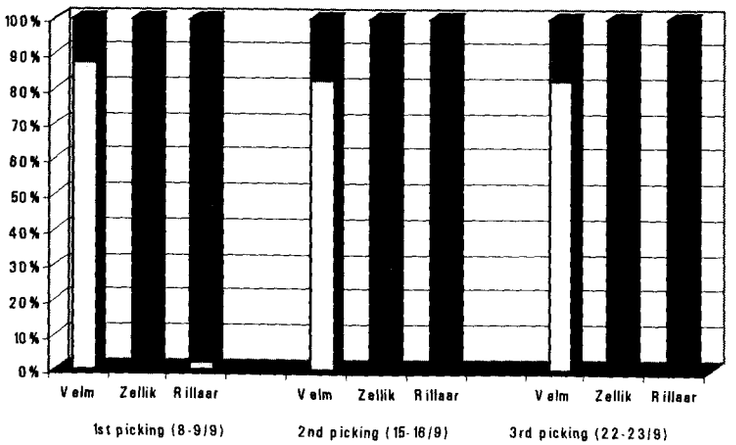

FAIR CT96 1803

"Quality improvement of pears by predictive and adaptive technology"

Individual Progress Report for the period

from 01-06-97 to 31-05-98

Type of contract:	Shared-cost research project			
Total cost: (65,3%)	1600,3 kECU	*EC contribution:*	1045	kECU
Participant no. 6 total cost: (100%)	196,2 kECU	*EC contribution to partner no. 2:*	197,1	kECU
Commencement date:	01-06-1997	*Duration:*	4 years	
Completion date:	31-05-2001			
EC contact: Fax: +32 - 2 296 3029	DG VI/F.II.3			

Coordinator: Dr. H.W. Peppelenbos
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Participant no.6: Contractor
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Fax: +49 - 751-7903322
e-mail: Streif@Uni-Hohenheim.de

A. PARTNER INFORMATION

Name and address

Name of the institute : University Hohenheim, Institut für Obstbau, Bavendorf
Address: D 88213 Ravensburg
country: Germany

Scientific team

Team leader: Dr. Josef Streif
Other scientific staff: Ing. Claudia Rabus, Ing. Haibo Xuan

Objectives

Quality improvement of pears by predictive and adaptive technology
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 climatic and ecological conditions in the Lake of Constance area, South-West Germany.
After different harvest dates the pears were stored under various CA conditions. During the storage period the change of fruit quality and incidence of core browning, mineral composition, biochemical changes as well as gas exchange characteristics were evaluated in order to find physiological explanations and practical decisions to prevent core browning disorders.
An other objective in the first year of the project was the development of equipment and methodology for gas exchange and diffusion measurement and enzyme analysis.

Actions in the project

The following tasks were carried out by partner 6 in the described period:

Task 1 Cultivation of pears
Task 2 Harvest of pears
Task 3 Storage of pears
Task 4 Registration of climate and orchard factors
Task 5 Postharvest treatments
Task 6 Gas exchange and diffusion measurement
Task 7 a: Fruit quality evaluation
b: Biochemical analysis
c: Mineral analysis

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

Task 1: Cultivation of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	0,5

Objectives

Testing of the influence of growing conditions such as climate, site conditions and crop load for susceptibility of Conference pears under CA storage

Material and methods

Conference pears were grown in 2 orchards

Orchard I:

Experimental Station, Bavendorf,
3 subsets of 12 trees each
Orchard characteristics:

Tree age: 18 years, rootstock: quince A, formation type: central axis
planting system: 4x2 m, area: 0,3 ha,

Special treatments:

Spray of growth regulators (Berelex, GA_3 +Promalin, GA3+Bencyladenine) after spring frost in April 97 for improving fruit set
The fruits from this orchard were all parthenocarpic without any seeds.
Hand thinning for regulation of crop load (23, 24, 27 calendar week)

Orchard II:

Fruit farm Hans Knäple, Salem-Mittelstenweiler
3 subsets of 4 trees each
Orchard characteristics:

Tree age: 31 years, rootstock: seedling, formation type: central axis
planting system: 4x6m, area: 0,30 ha
Special treatments:
The same as in orchard I, but not sprayed with growth regulators

Results and Discussion

The pear production in orchard I was substantial reduced by severe bloom frost during several night mid of April. Treatments with growth regulators improved fruit set but only 25-30% of pears developed normal seeds. Orchard II had not such big frost damages and nearly a normal crop. Nevertheless, variation in crop load of the trees were only possible in a limited scale.

Conclusions

The cultivation of pears for the different storage experiments has suffered this year from frost damages in April. Therefore differentiation in crop load could be only partly achieved.

Task 2: Harvest of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	1

Objectives

The date of harvest seems strongly related to the occurrence of brown heart in 'Conference' pears. Fruits must be picked at different dates in order to get a variation in physiological and biochemical fruit characteristics such as flesh firmness, soluble solids and starch content, respiration, ethylene production etc.

Material and methods

Samples of Conference pears to estimate the optimum picking date of two different orchards were taken 3 times in weekly intervals. The second pick should be at the estimated optimum date. Progress in maturity was monitored observing changes in firmness (hand-penetrometer), starch conversion (scale 1-10) and soluble solid content (hand-refractometer). Special interest was given to firmness change which reached in former experiments values between 6-6.5 kg/0.5 cm^2 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)

Results and Discussion

The changes in some fruit characteristics used for ripening determination during three different picking dates are shown in Figure 1. From these measurements the Streif index is calculated (figure 1). According to these results the difference in ripening development between orchard I and orchard II was about 1 week.. For Conference pears an optimum index for the beginning of picking was assumed between 0.12 and 0.14. This means that September 17th (pick 3) for orchard I and September 10th (pick 3) for orchard II should result in higher amounts of healthy fruits at the end of storage period. But this could not be verified by lower incidence of browning disorders in pears (see results task 3).

Conclusions

By the use of fruit firmness alone or the calculated index of firmness, starch conversion and soluble solids content, it was possible to characterise the picking dates.

Task 3: Storage of pears

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	4
No. of man-month devoted already to the task:	1

Objectives

Pears from different orchards and picking dates were stored under various CA storage conditions in order to study the influence of CO_2 and O_2 on the occurrence of core browning in the course of storage period.

Material and methods

Storage devices:
Pear fruits were stored in an experimental CA storage equipment consisting of 15 gas tight containers with 540 l volume (≅120 kg of fruits). The temperature of the containers was 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 chambers was constantly controlled and regulated by a computerised system connected to an infrared CO_2 and paramagnetic O_2 analyser.
The following 3 CA-storage conditions were used at temperature of -0.5 to -1 °C:

2.0 % 0_2 plus 0.7 % CO_2 (conditions avoiding core browning)
2.0 % 0_2 plus 5.0 % CO_2 (conditions provoking core browning)
4.0 % 0_2 plus 2.0 % CO_2 (conditions advised in practise for the storage of Conference pears)

Fruit material:
Fruits of 2 orchards with variations in crop load and picking dates were harvested, selected for homogenous size, colour and maturity and brought as soon as possible under the adopted CA-conditions. For 3 different CA-concentrations, 3 replications and 4 sampling dates one box with 15 kg fruits each were stored.
Fruit samples were taken from storage after 2, 4 and 7 months. 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
The duration of storage period in total was 7 months. After this time core browning disorders were developed mainly under high CO_2 condition in combinations with pick 3. Already after 2 months of storage this treatment have shown fruits with a high index for core browning and cavities. In CA-treatment with higher O_2 concentration together with 2 % CO_2 browning disorders didn't occur (Figure 2). In all treatments fruits were infected with fungi at 5 to 10 percent. In general, the development of disorders was relative low. Fore all 3 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, but the ripening process was accelerated (see task 7a).

Conclusions
There exist a strong relationship between the incidence of the browning disorders and the picking date and the amount of CO_2 concentration. The symptoms of disorders already appear during the first storage period.

Task 4: Registration of climate and orchard factors

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	0,5

Objectives
Site conditions and climate variation may influence the occurrence of brown heart The objective of this task is to collect meteorological data of various pear orchards and to relate them with incidence of disorders of pears from trees with different crop load.

Material and methods

The climate characteristics: temperature, rel. humidity, rainfall, radiation and hours of sunshine were monitored. In the orchard I (Experimental Station Bavendorf) the data were logged every ten minutes by an automatic equipment. For orchard II the climatic data of a meteorological station in the neighbourhood (2 km) were used.

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 crop weight of the pears is related to the area of the trunk in 30 cm height above ground. In our case we tried to regulate the crop load for:

high crop:	≅ 0,25 kg pears /cm^2
normal crop:	≅ 0,18 kg pears /cm^2
low crop:	≅ 0,12 kg pears /cm^2

Results and Discussion

The climate characteristics as means of the months from May to August are seen in Table 1. Orchard II is more favoured in temperature and shows also higher rel. humidity, whereas rainfall was higher in orchard I.

The effects of various crop loads in respect to the occurrence of disorders were very indifferent (Figure 3). The main reason for this was to get enough trees with distinct high crop load, because of bloom frost. This experiment has to be repeated in the following year.

Conclusions

The meteorological data as means of days for each orchard will be used also for network modelling together with harvest dates and storage disorders.

Task 5: Postharvest treatments

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	2
No. of man-month devoted already to the task:	0,5

Objectives

The aims of this task is

- to determine the preventive effect of various prestorage treatments
- to develop a rapid test in order to determine the susceptibility of pears to browning disorders before storage.

Our special aim was to examine which CA regime and which time of storage period is most favourable for the induction of disorders.

Material and methods

Storage regimes

During 6 months pear fruits were kept under 3 different storage regimes: O_2 concentration was 2 % in each case. The CO_2 concentration was 3 % in the average of the whole storage period but with an continuous increase from 1,5 % at the beginning up to 4,5 % CO_2 at the end of the 6 months storage

period. Storage regime 2 was the opposite, a decrease from 4,5 % to 1,2 % CO_2 during the storage period and regime 3 was constant 3 % CO_2. Fruit samples of the different treatments were taken each 2 months from the store and inspected for disorders.

Results and Discussion

The storage results as occurrence of core browning and cavities after different periods are shown in Figure 4. Although the CO_2 concentration of all 3 storage treatments was the same on an average, the initial low CO_2 concentration resulted in lower amounts of damaged fruits during all sampling dates compared with the other treatments.

Conclusions

Special attention for the appearance of first symptoms of browning must be paid to the conditions during the early storage period.

Task 6: Gas exchange and diffusion measurement

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	16
No. of man-month devoted already to the task:	4

Objectives

The measurement of gas exchange rates includes both O_2 uptake and CO_2 production under a range of CO_2 and O_2 concentrations. The O_2 concentrations should include 0% (maximum fermentative CO_2 production) and 21 % (normal air). Measurement of gas diffusion can be used for information about the gas exchange between internal and external atmosphere and for calculation of internal gas concentrations.

Material and methods

Respiration measurements:

Respiration measurements are done by headspace GC analysis of CO_2 and O_2 at different times from the beginning to the end of the storage period of the pears. The instrumental device (Micro GC, Chrompack) and the measuring method are very similar to that described by Peppelenbos and van't Leven (1996). The average temperature to store 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 12-16 hours. The experimental setup for respiration measurement is:

Temp.	% O_2	% CO_2	nr. of fruits	days of storage
2 °C	0, 0,7, 2, 6, 21	0,7, 5	2	3, 4, 5

Ethylene production:

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

Diffusion measurement:

Gas exchange is measured in terms of diffusion of Ethylene through a disc of fruit tissue with or without skin. The disc is placed in a special device of gas-tight connected syringes. The penetration of

ethylene from one side with higher concentration to the other side of the tissue is measured periodically during 3 hours. See also Figure 5.

Results and Discussion

Respiration measurements: During the first time of respiration measurements we had to solve a lot of technical problems, regarding the tightness of the jars and the lines. Therefore we obtained reliable results only of the last sampling date at the end of storage period. Results of respiration measurement for pears of 3 picking dates which were stored during 7 months under low and high CO_2 conditions are shown in Figure 6. Obvious differences between the different treatments can't be observed and at this time it's to early to discuss the data.

Ethylene production: Ethylene was measured together with respiration and the data are shown in Figure 7.The amount of ethylene production is highly influenced by the O_2 concentration in the respiration jars. The influence of CO_2 concentration between 0.7% or 5% CO_2 is only low. Obvious effects of storage conditions which are avoiding or provoking browning disorders can't be clearly seen.

Gas diffusion: Gas diffusion measurements were done by monitoring the penetration of ethylene through the fruit tissue from the side with high to the side with low concentration. In Fig 8 you can see differences in gas diffusion after 150 minutes between earlier and later picked fruits and between 'low CO_2' and 'high CO_2' stored fruits as well. The lowest diffusion rate was measured in fruits from pick 3 and high CO_2, which corresponds with the highest susceptibility of pears against Brown Heart. But this first results must be confirmed by more data.

Conclusions

Measurement of respiration and gas diffusion must be performed during the whole storage period to get more sure information on the effect of browning disorders.

Task 7: Destructive measurements

Duration:	48 months
Current Status:	36 months to completion
Total estimated Man-month:	26
No. of man-month devoted already to the task:	6,5

Objectives

Destructive measurements will accompany the different measurement during storage period in order to increase essential knowledge on different aspects of quality and browning disorders.
Several biochemical pathways are expected to be involved in the development of Brown Heart in pears. The measurement of fermentative metabolites can help to understand damages of tissues by toxic substances. Different enzymes, involved in oxydation processes, are likely involved in the onset of Brown Heart

Material and methods

a: Fruit quality evaluation

Ground colour of peel: The measurement of colour changes during storage period is performed 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:
Individual 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.

b: Biochemical analysis
*Lypoxygenase activity (LOX):*Lypoxygenase activity is measured by a slighly modified polarografically method described by Feys et al. (1980). Fruit material without skin is homogenised 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.

*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: For sample preparation fruit pulp is sliced, homogenised, 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.

c: Mineral analysis
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

Results and Discussion

a: Fruit quality evaluation.
The results of quality changes in Conference pears monitored at harvest and during 7 months of CA storage are shown in Fig 9. Because of limited space of our storage facilities fruits from orchard II of different picking dates were only sampled four times during the 7 months storage period. Decrease in quality occurred mainly in flesh firmness, acidity, and ground colour whereas soluble solids increased. Preservation of quality characteristics as influenced by storage conditions was better under

higher CO_2 concentration ($2\%O_2$ +$5\%CO_2$) than under low CO_2 ($2\%O_2$+$0,7\%CO_2$) with the exception of acidity. It seems that problems with browning disorders are only reflected in a faster loss of acidity.
The quality evaluation of storage experiment with pears from trees with different crop load resulted in very similar data (not shown).

b: Biochemical analysis
Vitamin C and ascorbic acid content and Lypoxygenase-activity: Antioxidative substances like vitamin C may play an important part in the browning of fruit tissue. Pears show the browning disorders in a distinguished part of the fruit tissue. The outer part of fruit cortex, about 1 cm under the peel, remains normally free of browning disorders. Therefore we analysed separately both parts of tissue for vitamin C and ascorbate content. The results are given in Figure 10. Treatments which show higher incidence of disorders seem to be related with lower vitamin C and lower ascorbic acid content, as it can be seen in general from the CA treatment 5% CO_2 + 2 % O_2 and from the late harvest date in special. This is also true for the activity of Lypoxygenase (LOX) which seems much higher in fruits from the late picking date (see Tab. 10 also). During the storage period the loss of vitamin C, especial under high CO_2 conditions, was continued.

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.

c: Mineral analysis:
The mineral content of the pears was analysed at the end of July during the growth of the fruits and directly after the various harvest dates of both orchards. These data are shown in Table 2. It can clearly noticed 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 sampling 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 unfavourable for storage life.
Table 2 shows 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 gives a good connection to the tree load.

Conclusions
The results of quality, mineral and biochemical analysis provide some interesting relationships to browning disorders, especially decrease of vitamin C can possibly act as an indicator to predict browning disorders. Antioxidants need energy for generation. This seems to confirm that energy metabolism plays an important role in avoiding brown heart.

C. DIFFICULTIES OR DELAYS EXPERIENCED IN PERIOD 01-06-1997TO 31-05-1998

The pear production in 1997 has suffered from frost damage of the blossom set. Orchard I was treated with phytohormons for improving the fruit set. This means that more than 70 % of the harvested fruits were parthenocarpic. This could have been influenced mineral supply and storage behaviour, perhaps.
In the beginning of the storage period we also had a lot of problems with respiration measurement because of insufficient tightness of jars and connecting plastic lines. After changing the gaskets with a special plastic material and the PVC lines with PE we got dependable data for the last sampling date.
We had also methodological difficulties with the determination of LOX-enzyme. We were not able to repeat first very promising results with a polarographic method. We also have some delay with

determination of other enzymes related to browning disorders because this kind of research is new for our laboratory. Therefore a scientist of our team will visit IRTA in Lleida (partner 2) in August to learn some more about methods for enzymatic studies.

D. PLANNED RESEARCH ACTIVITIES FOR PERIOD 01-06-1998 TO 31-05-1999

In the next time period some investigations from the last year must be completed and the different tasks for the second year have to be repeated.. Collected fruit samples for acetaldehyde and ethanol determination will be analysed in the next few weeks.
In the ongoing fruit growing season the cultivation, harvest and storage of pears will be performed principally in the same way as before but with more experience and better methods. The thinning and picking date experiments will be much more reliable this year because all the trees show a much better and regular cropping behaviour than last period.. More attention will be paid also to task 5, to get more information about the first appearance of browning disorders in the very early period of storage.
Furthermore we will start with investigations about the energy metabolism of oxidative and fermentative pathways. This work will be done together with the Fruit Science Institute of Hohenheim University. Respiration measurements can be started next season immediately after harvest and will be continued each sampling date during the 6 months storage period. Diffusion resistance of pears will be measured according our own developed method which have to be further optimised and compared with other method described by Peppelenbos and Jeksrud (1997)

E. DISSEMINATION

Quality improvement of pears by predictive and adaptive technology. First progress report and 2nd meeting in Weingarten, 12-13 January, 1998
Results which concern the method of gas diffusion measurement are submitted for publication during the IHC in Bruxelles 1998.

F. ANNEX

Tables 1 - 2 and Figures 1 - 10

Tab. 2 Mineral content of Conference pears of orchard I six 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 pre harvest	1	20,5	11,2	1,6	0,7	1,4	7,1
	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 harvest date I	1	16,5	8,5	0,6	0,4	0,7	13,7
	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 harvst date II	1	16,6	7,4	0,6	0,4	0,8	13,5
	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 harvst date III	1	16,3	7,7	0,4	0,4	0,8	18,0
	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 harvest date I	1	16,0	7,1	0,5	0,4	0,9	13,2
	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 harvst date II	1	16,0	5,9	0,5	0,4	0,8	11,0
	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 harvst date III	1	16,9	6,8	0,5	0,4	0,7	15,0
	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

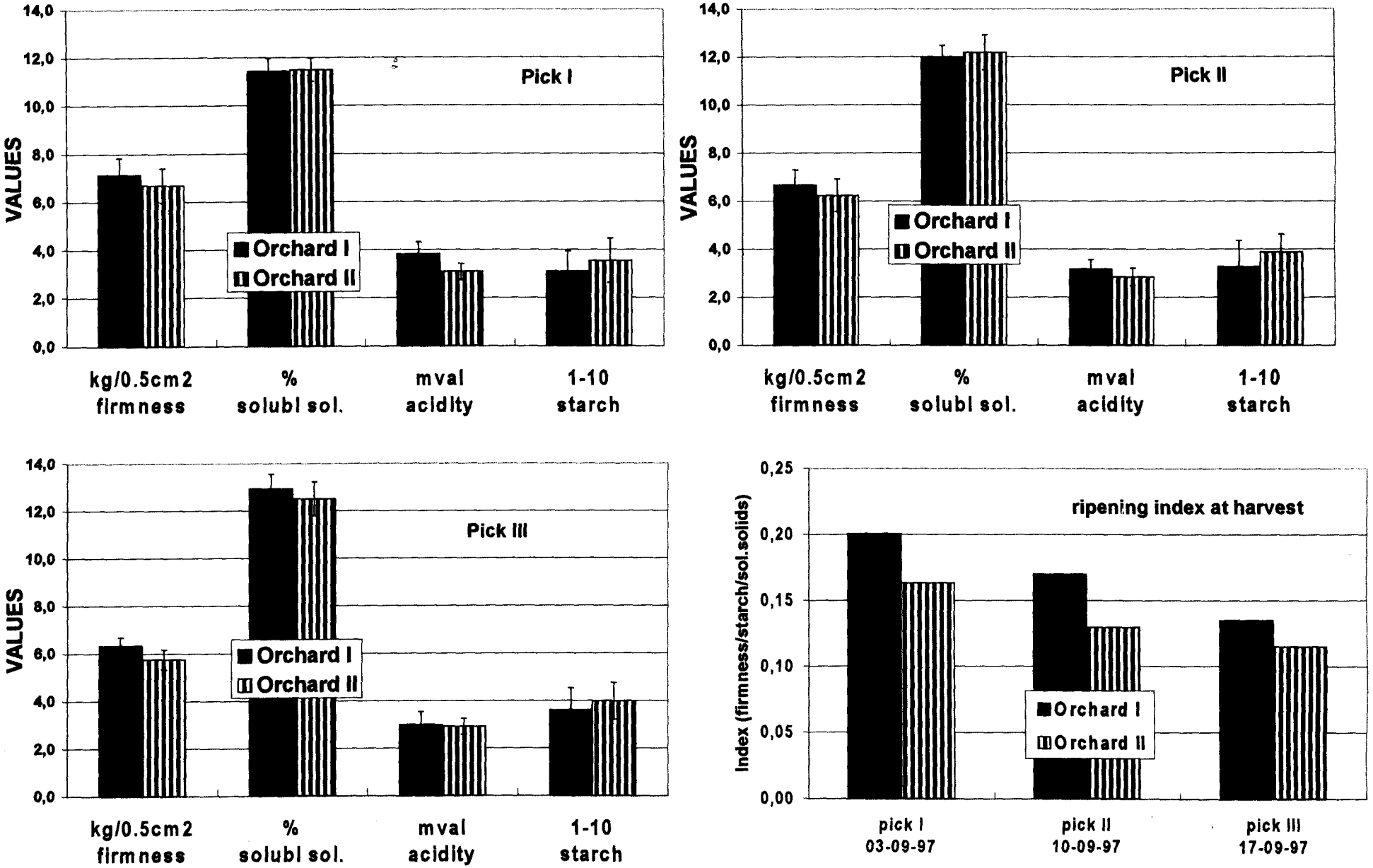

Fig 1. Quality parameters of Conference pearfruit pick I-III and ripening index at harvest of different orchards

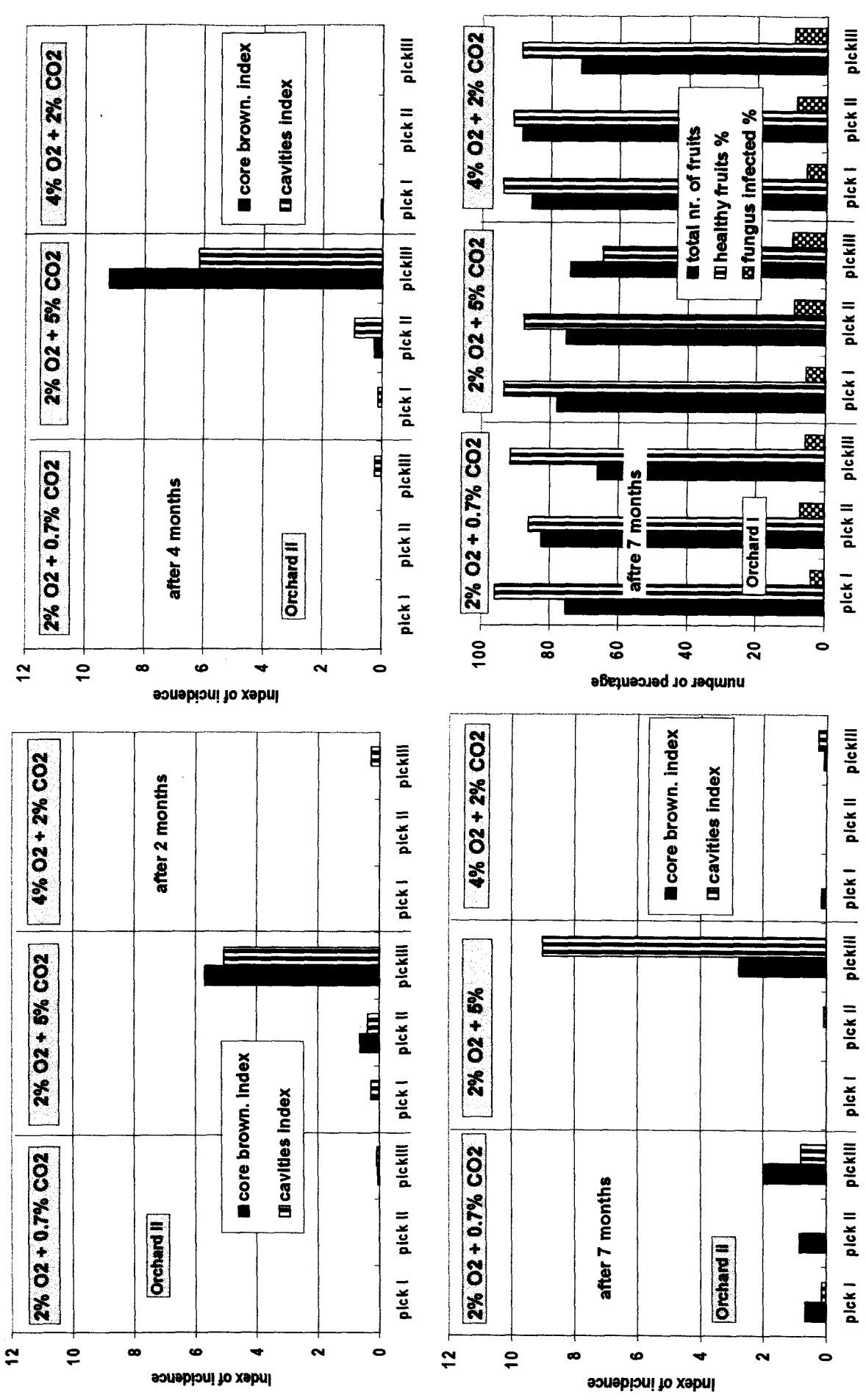

Fig. 2. Incidence of disorders and diseases in Conference pears after different periods of CA storage

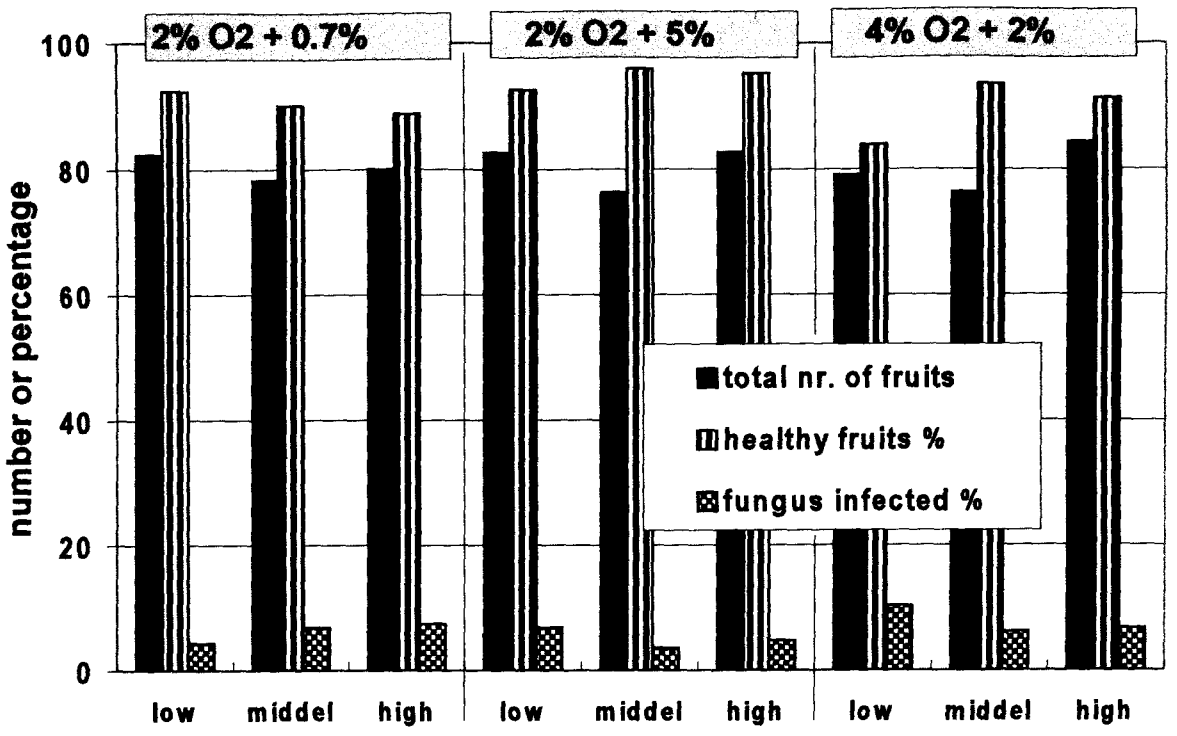

Fig. 3. Incidence of disorders and diseases in Conference pears from trees with difference fruit load after 7 months of CA storage

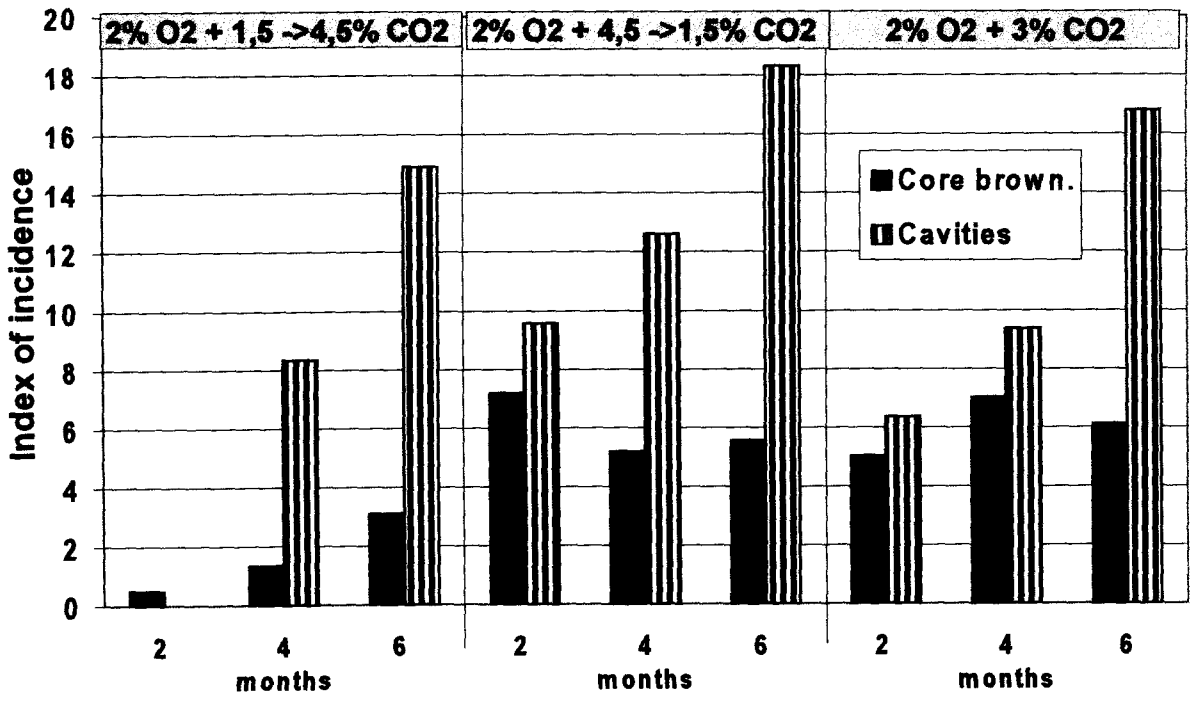

Fig. 4. Incidence of disorders and diseases in Conference pears after different periods under various CA-regimes

Fig. 5 Experimental device for measurement of gas diffusion through the fruit tissue

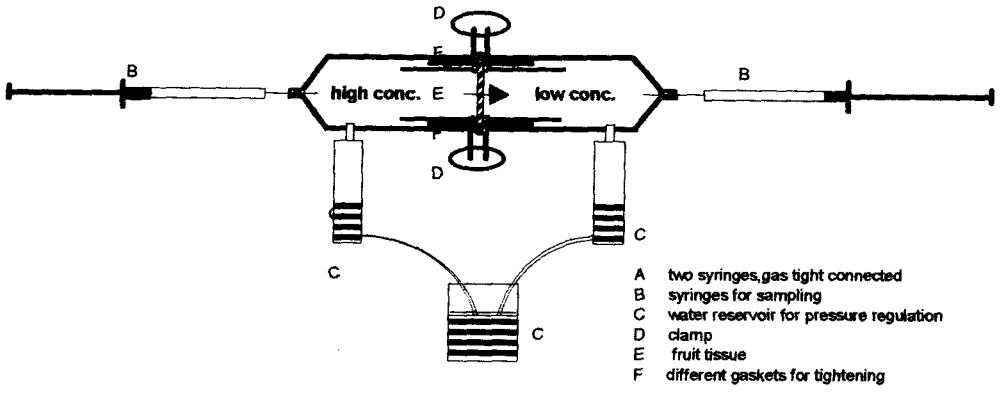

Table 1. Data of climate characteristics as means of months May -August for orchard I and II

Orchard I

	temperature °C T. mean	T. min	T. max	relativ humidity %	rainfall mm/m2	radiation W/m2	sun shine h
May	13,2	-0,4	29,7	67	54	160,3	270
June	15,7	4,9	30,1	74	136	141,8	187
July	16,1	6,9	29,1	76	107	137,7	202
August	19,0	6,0	31,0	73	73	142,2	192
mean/sum	16,15	4,35	29,98	72,5	370	582	851

Orchard II

May	13,6	-2.3	31,7	70	32
June	16,3	5.0	33.3	78	134
July	17,1	5,3	31.0	82	104
August	!9,4	6.0	35.8	79	49
mean/sum	16,6	3,5	32.95	77.25	319

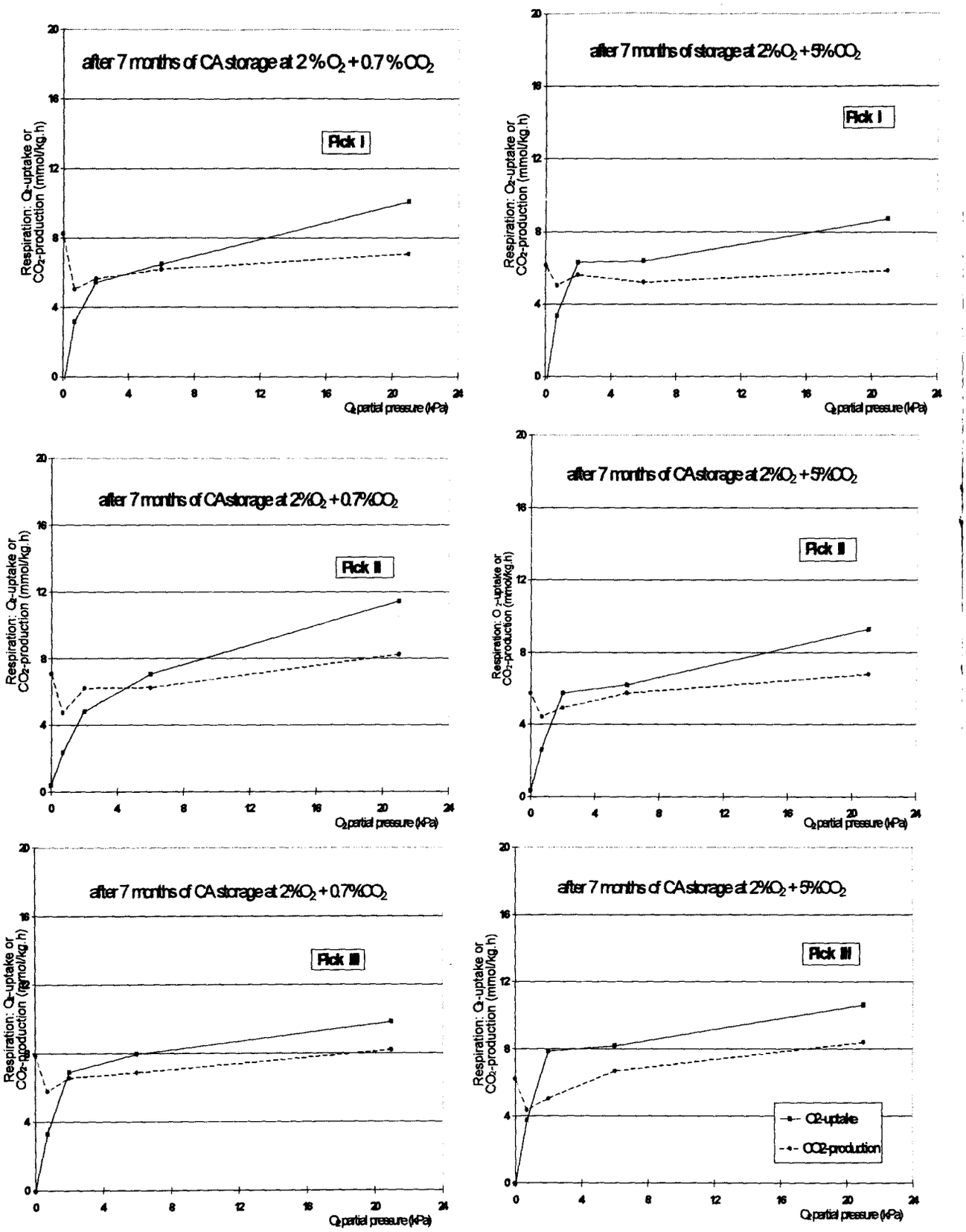

Fig.6 The O_2-uptake and CO_2-production of Conference pears from different harvest dates

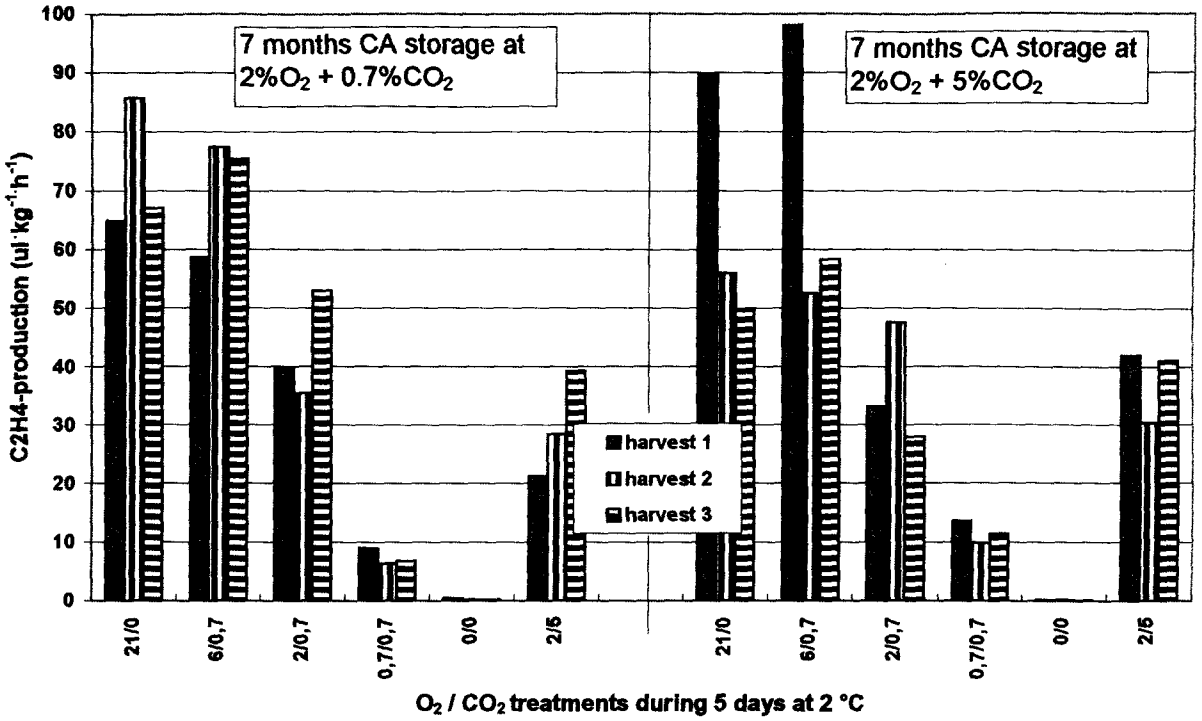

Fig. 7. Ethylene-production of Conference pears after 7 months under different CA-conditions followed by 5 days of various O_2/CO_2 treatments at 2 °C

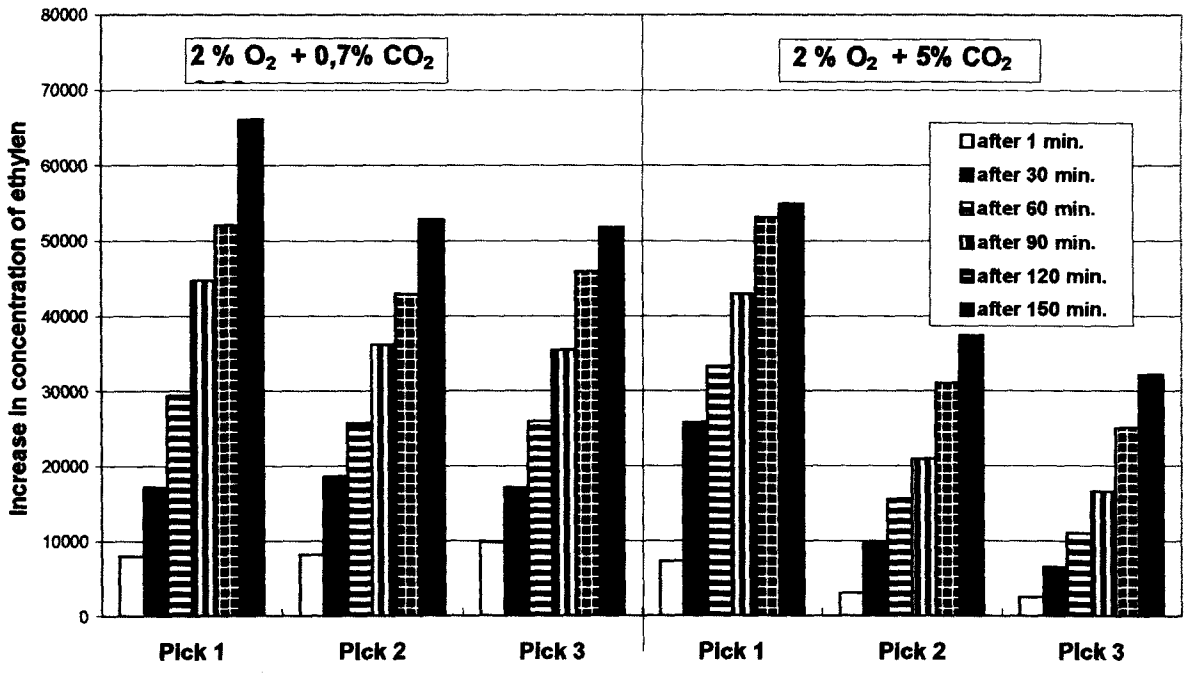

Fig. 8. Diffusion of ethylen through disks of Conference pear tissue during 150 min. The pears were stored before under different CA-conditions for 4 months.

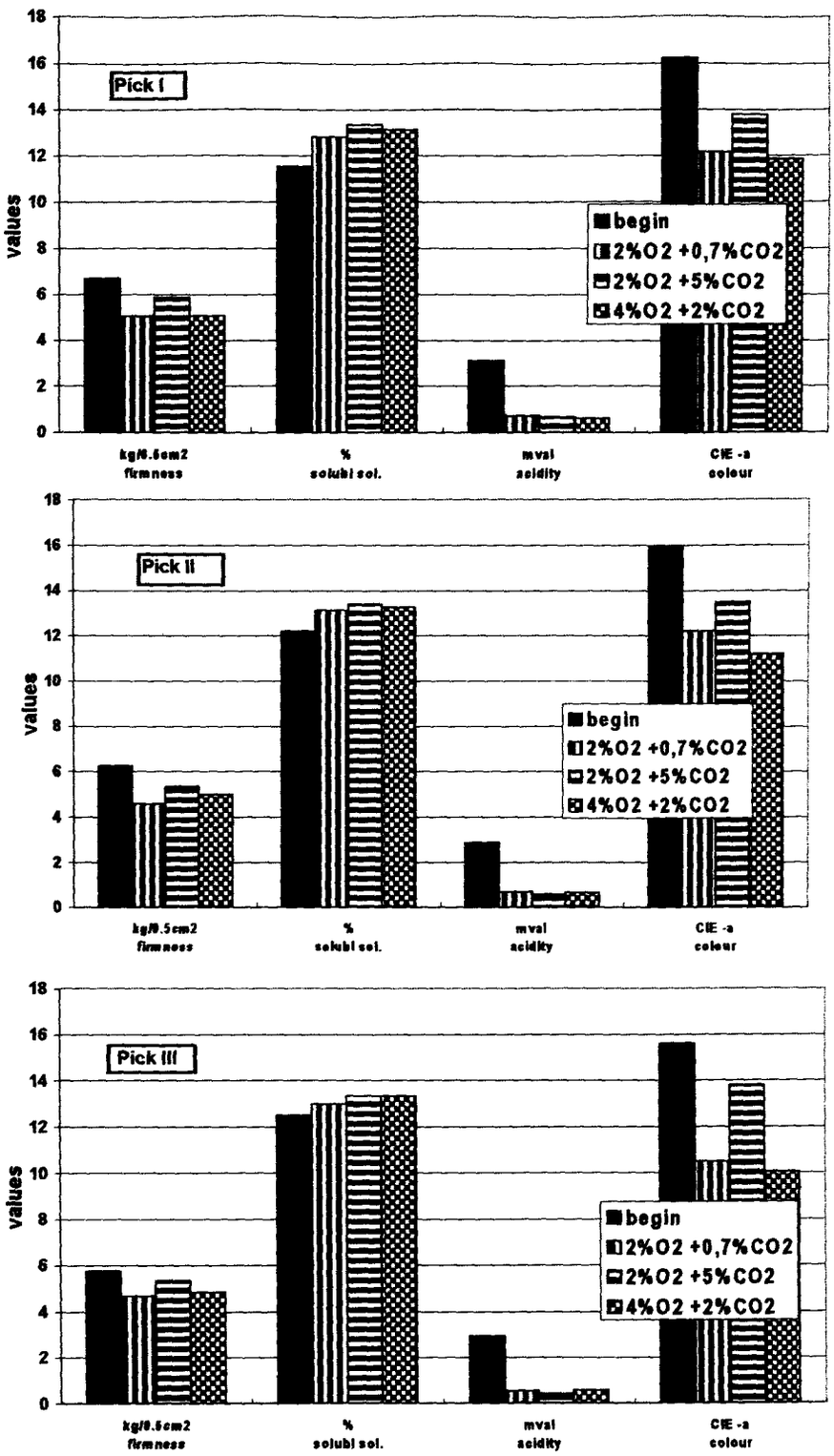

Fig. 9. Changes in quality parameters of Conference pears from different picking dates after 7 months of CA storage under various conditions

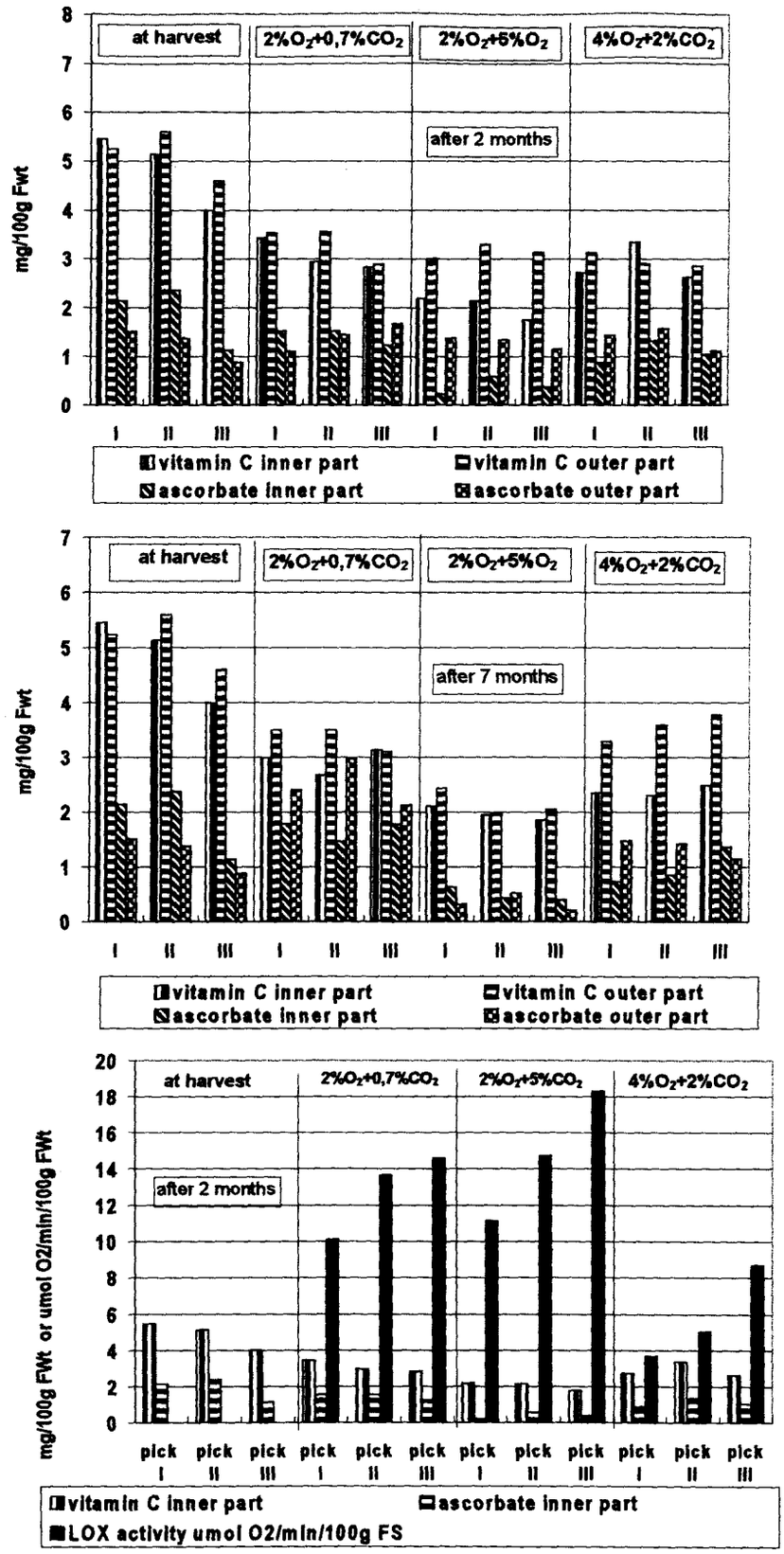

Fig. 10. Vitamin C and ascorbate content in the inner and outer part of cortex and the activity of LOX at harvest after 2 and after 7 months of CA storage