Quality improvement of pears by predictive and adaptive technology

A shared-cost project

Individual progress reports of the first half of the second year (June 1998- December 1998). Presented at the meeting in Wageningen, The Netherlands, January 1999.

EUROPEAR RESEARCH PROGRESS REPORT

3

EC-FAIR1-CT96-1803

Quality improvement of pears by predictive and adaptive technology

Content

General outline of the project

Practical problem

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

Goal of the research

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

Short description of the approach

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

Project participants

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

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Agenda of the 4th Europear meeting Wageningen, The Netherlands

18 January 1998

09.00 Arrival
09.10 Welcome and outline of the 4th meeting
09.20 Presentation of ATO-DLO
10.30 Coffee/tea break
10.45 Presentation of IRTA
11.45 Presentation of IVTPA
12.45 Lunch
14.00 Presentation of FPO
15.00 Coffee/tea break
15.15 Presentation of VCBT
16.15 Presentation of UHOH
17.15 End of the meeting
19.00 Diner

19 January 1998

09.00 Arrival
09.10 Summary and conclusions of the first day
09.30 Discussion on tasks 1, 2 and 4: Orchard treatments and harvest
10.00 Discussion on tasks 3 and 5 : Storage and post-harvest treatments
10.30 Coffee/tea break
10.45 Discussion on task 6: Gas exchange
11.15 Discussion on task 7: Biochemical measurements
11.45 Discussion on task 9 and 10: Modelling and validation
12.30 Lunch
13.30 Excursion to research facilities of ATO
15.15 Coffee/Tea break
15.30 Cooperation: laser device (Huug de Vries)
16.00 Discussion on task 11: Dissemination (coöperation and publications)
16.10 Planning for the next year
16.30 Mr. Hardwick, EU commission
17.00 Closure of the meeting

Schedule of meetings

Number	Day *	Month	Year	Host	Location
1	3	6	1997	VCBT	Leuven (B)
2	12/13	1	1998	UHOH	Weingarten (D)
3	15/16	6	1998	IVTPA	Milan (I)
4	11/12	1	1999	ATO	Wageningen (NL)
5	14/15	6	1999	IRTA	Llerida (E)
6	10/11	1	2000	FPO	Wilhelminadorp (NL)
7	14/15	6	2000	?	?
8	10/11	1	2001	?	?
9	?**	7	2001	ATO	Wageningen (NL)

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

Schedule of deliverables

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

Brief Progress Report of Partner 1: ATO

Scientific team

- Dr. H.W. Peppelenbos	coordinator
- Ing. E.C. Otma	task 6
- Ing. A.C.R. van Schaik	tasks 2, 3, 7, 8
- Dr H.A.G.M. van den Boogaard	task 9, 10
- Drs. R.H. Veltman	task 7
- Ir. J.P.J. de Wild	task 6

Results

Task 6

Harvest

Gas exchange characteristics were measured directly after harvest at 5 different harvest dates (see table 1). Remarkably no differences were found in the model parameters, where a rise in VmO2 was expected. Possible explanation is that in 1998 the optimal harvest date was hard to estimate, since the measured parameters almost did not change for a long period. The absolute values in respiration and fermentation are comparable to the measurements of 1997. Also resistance to gas diffusion did not change with the harvest date. The values found, however, were remarkably lower than the values found in 1997. The calculated internal gas concentrations were comparable for every harvest date (graph 1).

Table 1 Results of the 'harvest date' experiment of 1998.

	Harvest date					
	1997 optimal	31-08-98	07-09-98	14-09-98 optimal	23-09-98	30-09-98
R^2	83.5	80.6	88.8	83.6	88.8	79.0
Vm_{O2}	20.8	18.0	19.3	17.1	17.4	14.5
Km_{O2}	0.885	0.312	0.430	0.329	0.792	0.744
Km_{CO2}	11.7	23.8	18.4	19.3	21.4	26.0
R^2	80.7	42.8	60.4	57.8	62.8	37.9
Vmf_{CO2}	28.1	23.4	25.1	19.5	14.8	22.9
Kmf_{O2}	0.042	0.075	0.082	0.068	0.116	0.035
Rqox	0.890	0.858	0.781	0.832	0.722	0.970
Resist	823	774	549	637	640	649

Statistical parameters of Table 1 and 2: R^2 = percentage of explained variance, Vm_{O2} = maximum O_2 uptake rate (nmol/kg.s), Km_{O2} = Michaelis-Menten constant for O2 influence on O2 uptake (kPa), KmCO2 = Michaelis-Menten constant for CO2 influence on O_2 uptake (kPa), Vmf_{CO2} = maximum fermentative CO_2 production rate (nmol/kg.s), Kmf_{O2} = Michaelis-Menten constant for the O2 influence on fermentative CO_2 production (kPa), RQox = RQ for oxidative processes, Resist = resistance for diffusion of neon (s/mm).

Delay in CA

The treatment of delaying the application of CA conditions for 5 weeks, and to store pears only in cooled conditions, was again compared with pears directly stored under CA conditions. Gas exchange characteristics were measured before and after the treatment. In contrast with 1997, the delay in CA did not cause great differences in gas exchange characteristics (table 2). Where in 1997 both respiration and fermentation increased considerably during only cooling, this was not the case in 1998. Therefore no differences in calculated internal gas composition was found. A different development stage at the start of the treatment as compared to 1997 could explain this (riper pears in 1997). We conclude that measurements on pears during both the harvest and the treatment is relevant only with a proper comparison of development stage.

Table 2: Results of the 'delay CA' experiment of 1998.

	Harvest	Direct CA	Delay CA
R^2	83.6	87.0	80.8
Vm_{O2}	17.1	20.3	21.4
Km_{O2}	0.329	2.99	1.44
Km_{CO2}	19.3	11.7	22.4
R^2	57.8	78.3	66.3
Vmf_{CO2}	19.5	7.8	14.0
Kmf_{O2}	0.068	0.70	0.18
RQx	0.832	0.83	0.84
Resist	637	523	483

The influence of CO2 on respiration and ethylene metabolism

Elevated CO_2 can both inhibit respiration and ethylene production. The precise mode of action on both processes still not fully understood. Uncoupling of oxidative phosphorylation by CO_2 may lead to mitochondrial damage and consequently inhibit respiration. The effect of inhibition on respiration may also be due to reduced activity or synthesis of various enzymes possibly through an effect on pH. There are some indications that the effect of CO_2 is mediated through an effect on ethylene synthesis and/or action. The current hypothesis about inhibition of autocatalytic ethylene production by CO_2 is that this inhibition is mediated through an effect on the ethylene receptor site.

In an experiment pears cv. Conference were stored at 2 °C under various O_2 partial pressures in combination with and 5 kPa CO_2. Respiration and ethylene production were measured. Ethylene production in relation to O_2 partial pressure could be described by a Michaelis-Menten equation. This has not been found before for whole fruit. The observed Michaelis-Menten curve probably reflects the conversion from ACC to ethylene by ACC oxidase which requires O_2. For describing this relation, the O_2 uptake model (Peppelenbos and van 't Leven, 1996) was modified for ethylene production. The ethylene production was best described with the model with noncompetitve type of inhibition by CO_2:

$$V_{C2H4} = \frac{Vm_{C2H4} * O_2}{(Km^e_{O2} + O_2) * (1 + CO_2 / Km^e_{CO2})}$$

Maximum ethylene production was 84 pmol.kg^{-1}.s^{-1}. Elevated CO_2 inhibited ethylene production with 34%. Also O_2 uptake rate was best described with the model with noncompetitive type of inhibition by CO_2. Elevated CO_2 inhibited O_2 uptake rate with 30%.

A second experiment was set up to study the site of inhibition of ethylene production by CO_2. For this purpose 1-methylcyclopropene (1-MCP) was used. 1-MCP is an effective inhibitor of ethylene responses because it blocks the receptor. The effect of elevated CO_2 on ethylene production and respiration was compared to the effect of 1-MCP Ethylene production was inhibited by CO_2 and by 1-MCP. The reduction in ethylene production by CO_2 was similar for 1-MCP treated and untreated pears. Elevated CO_2 therefore must have had an influence on ethylene production other than through ethylene perception.

Inhibition of ethylene production rate by CO_2 occurred simultaneously with an inhibition of O_2 uptake rate. A similar inhibition of ethylene production rate by 1-MCP was in most cases not accompanied by a reduction in O_2 uptake. These results indicate that there was no direct effect of ethylene on O_2 uptake rate. Maybe there is an inverse causal relationship between ethylene production and O_2 uptake rate. It is possible that a reduced ATP level by inhibition of respiration has affected ethylene production. Another possibility is that CO_2 inhibited ethylene production by depletion of ACC synthase protein or inactivation of ACC synthase activity. Also reducing the efficiency of the conversion of ACC to ethylene can lead to inhibition of ethylene production when ACC content is low.

Elevated CO_2 clearly affects respiration and ethylene production in pear. Knowledge about the site of inhibition by CO_2 will help to find an explanation for the occurrence of physiological disorders.

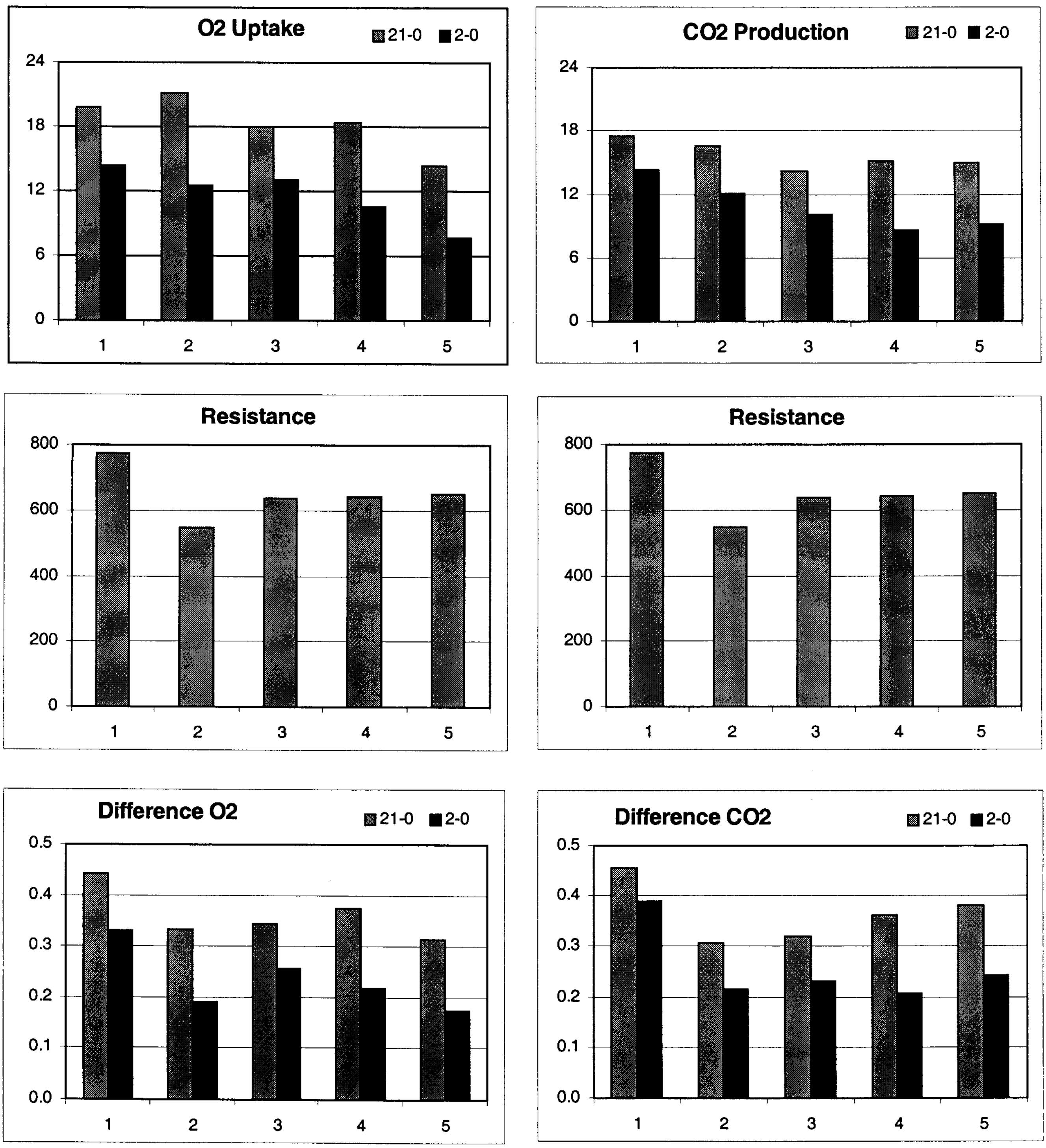

Figure 1: Comparison in gas exchange characteristics of harvest 1 to 5 (x axis). From top to bottom gas exchange, diffusion resistance and calcualted in ternal gas concentrations are shown. Left for oxygen, right for carbon dioxide.

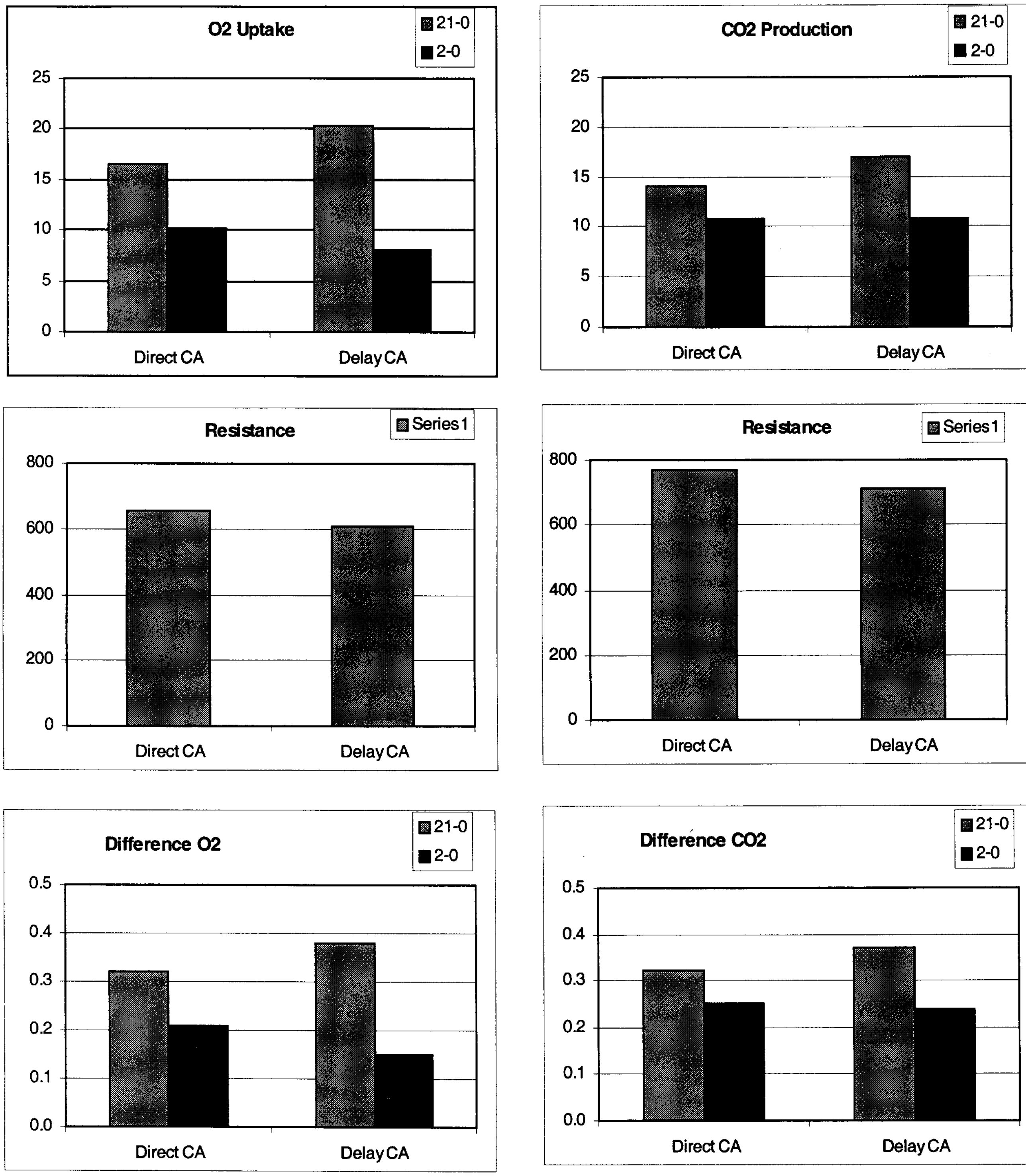

Figure 2: Comparison in gas exchange characteristics between pears directly subjected to CA conditions and pea subjected to CA after a colling period of 5 weeks (x axis). From top to bottom gas exchange, diffusion resistance an calcualted in ternal gas concentrations are shown. Left for oxygen, right for carbon dioxide.

Task 7

Ascorbic acid and browning

There are no clear differences in ascorbic acid levels of pears from either different orchards or picking dates. Also r relation can be established between ascorbic acid levels in these pears and browning later during storage (results n shown). However, ascorbic acid levels are relatively low in the core of the fruit, which fits well with the start of brow core development in this part of the fruit. Generally, peel tissue and cortex tissue just beneath the peel of the fruit not affected by browning. Except for extreme cases of the disorder, the outer appearance of affected fruits is norma

Ascorbic acid concentrations in the peel of the fruit are significantly higher than values in the fruit flesh (results not shown). Specific atmospheric conditions lead to continuously decreasing levels of ascorbic acid; e.g. storage at lowered oxygen and increased carbon dioxide concentrations (Fig. 3). Browning of the fruit flesh occurs when ascorbic acid levels drop below circa 1.3 mg/100g fresh weight (Fig. 4), while browning can be avoided to a great extent, and ascorbic acid levels are enhanced, when carbon dioxide concentrations are lowered at the moment this level is reached (Fig. 5 and table 3). Ascorbic acid levels show a continued drop when conditions are not changed, and severe browning becomes inevitable. At this moment it is not clear whether the drop in ascorbic acid is the main step yielding brown core. The function of other antioxidants, like α-tocopherol and glutathione, for instance, was not studied. However, ascorbic acid may well serve as a marker for browning. As our results show, brown core can be avoided by monitoring ascorbic acid levels (Veltman et al., 1998, submitted to Physiologia Plantarum).

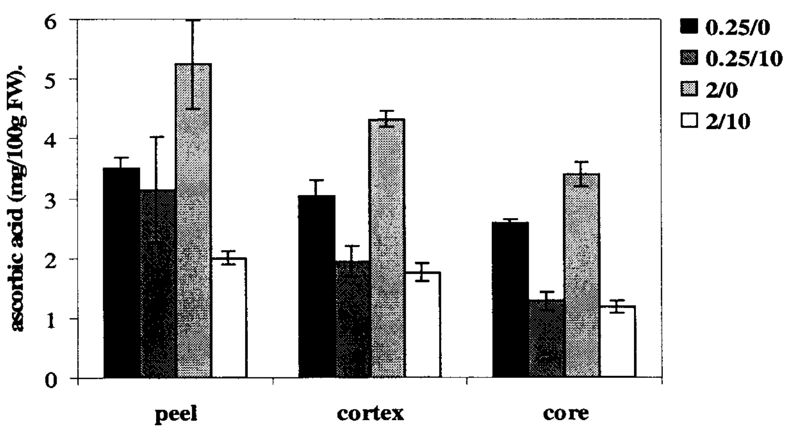

Fig. 3. Ascorbic acid content in conference pears after 60 days storage in a flow-through system (5 °C) at 0.25% oxygen, no carbon dioxide (0.25/0), 0.25% oxygen, 10% carbon dioxide (0.25/10), 2.0% oxygen, no carbon dioxide (2/0, control) or 2.0% oxygen, 10% carbon dioxide (2/10). Fruits were from orchard 1 (pick 2). Bars are the mean of mixed samples of 5 pears ± SE (n=4).

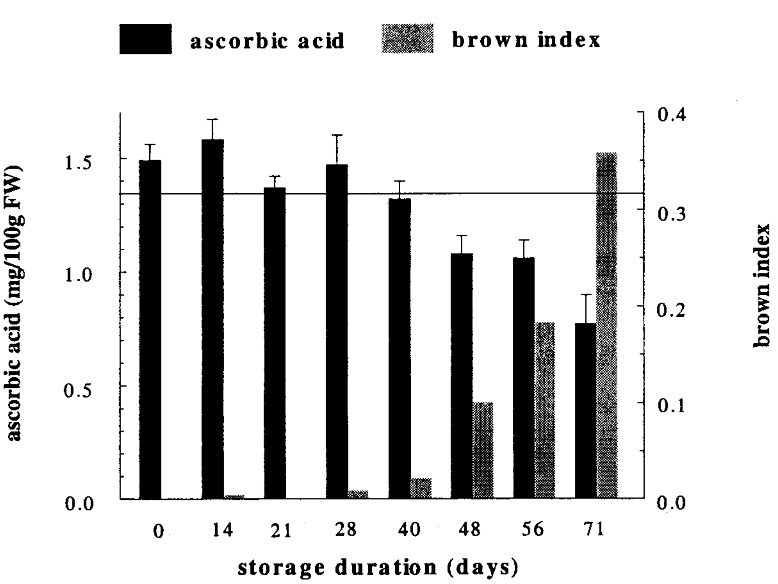

Fig. 4. Ascorbic acid and browning in pears during storage at 2% oxygen and 10% carbon dioxide in the flow-through system (5 °C). The ascorbic acid content and the brown-index of pears were monitored for 71 days. For the

ascorbic acid determinations two mixed samples of 10 pears each were taken from 4 containers (n=4). Before ascorbic acid was determined, pears were judged on browning. When ascorbic acid levels drop below ca. 1.3 mg/100g FW (horizontal line in graph) browning seems to be initiated.

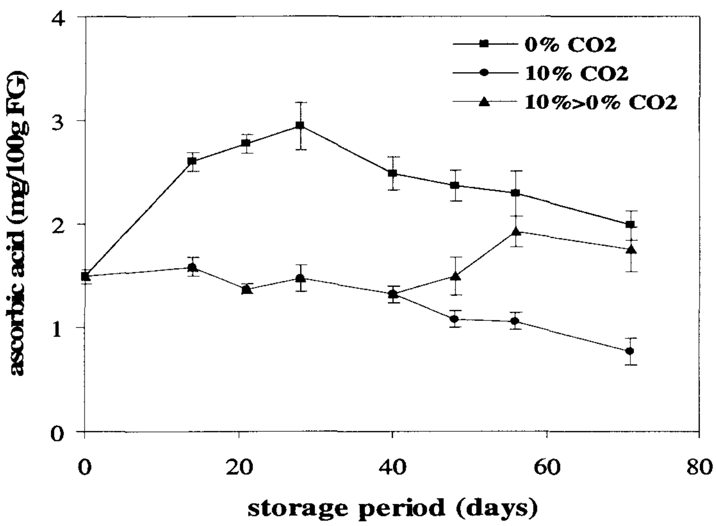

Fig. 5. Ascorbic acid in pears as an indicator for browning before and after switching gas conditions. Fruits from orchard 3 were stored at 2% oxygen without carbon dioxide (control, n=4) or under 2 % oxygen and 10% carbon dioxide (n=8). After 40 days of storage four of the enhanced carbon dioxide containers were switched to the contrc condition (10%>0% CO_2). During storage ascorbic acid was determined in two mixed samples of 10 pears from each container (n=4 or 8). Values are expressed as mg /100g FW ± SE.

percentage of pears with brown core

storage condition	2/0	2/10	2/10>0
stage of browning			
healthy (0)	41.3	23.9	74.7
slight (I)	36.7	14.7	6.1
moderate (II)	16.6	13.0	11.6
severe (III)	5.4	48.5	7.7
total brown-index	0.29	0.62	0.17

Table 3. Brown core in pears after 83 days of storage. Pears from orchard 3 were stored at 2 % oxygen, with and without 10 % carbon dioxide (2/0 and 2/10, n=4). One series of containers was switched from high carbon dioxide (10%) to no carbon dioxide (2/10>0) after 40 days (n=4). Pears were classified as healthy (class 0), and slightly (class I), moderately (class II) or severely (class III) brown. For every condition (2/0, 2/10 and 2/10>0) circa 120 fruits were examined. Values are expressed as percentages. From the percentages the brown-index is calculated.

Task 8

Non destructive measuring of brown pears was tested using an Peleg device. Fifteen amplitudes were tested on a ranş of frequencies. In total 99 pears were measured, and afterwards a score for brown was given. One amplitude w:

selected where big differences were found between signals for brown and healthy pears. The method to be validated is the signal at 1100 Hz – the signal at 700 Hz (see graphs). After a first validation with a different group of pears, the number of wrong predictions was low: 3 pears were predicted to be brown, but were healthy, and 3 pears were predicted to be healthy, but were brown. In total that gives (3+3)/99 = 6% wrong predictions.

Planning

Various experiments will be carried out in the second half of this storage season. These experiments will be described shortly:

1. Changes in gas exchange during the storage period (task 6)
2. Mode of CO2 action on respiration and fermentation (ethylene pathway) (task 6)
3. Comparison diffusion resistance method with actual internal concentrations (task 6, with partner 6).
4. Ethane emissions (task 7)
5. Energy metabolism and fermentation (task 7)

6 Validation non-destructive measurement (task 8)

7 Modelling (task 10, with partner 5).

Ad 4: Ethane

Preliminary experiments showed that brown pears emit ethane. Measurements were done with the aid of photoacoustic laser techniques. Future research should elucidate if ethane as a trace gas is suitable as a marker for brown core. The main question here is if pears that are not brown produce ethane under stress conditions.

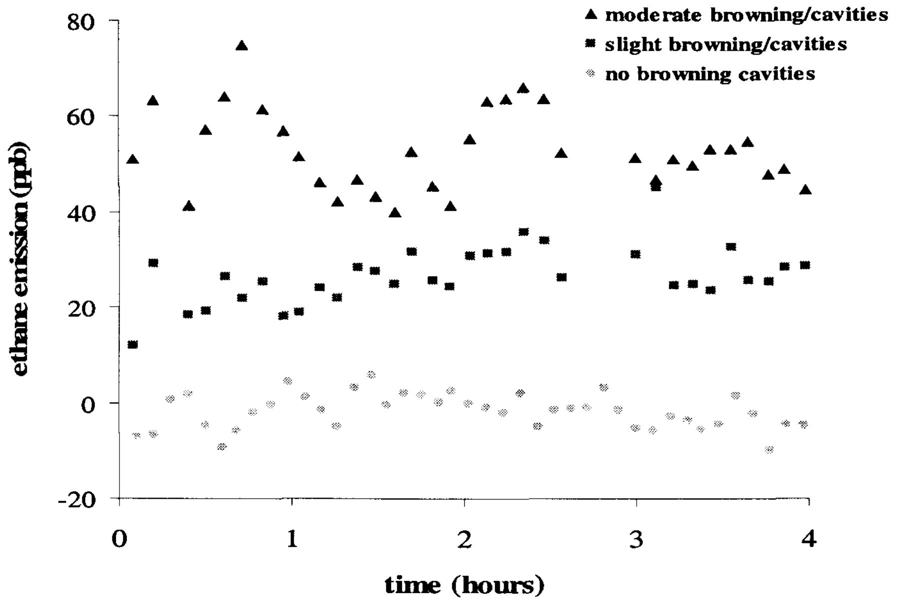

Fig. 6. Photoacoustic laser measurements on the ethane emission of brown pears. Before the experiment fruits were stored at 0.5% oxygen and 3.0% carbon dioxide for 5 months, to initiate aberrations. Three cuvettes were connected to the flow-through system of a photoacoustic CO laser, both with three pears, at 2.5 % oxygen and 8% carbon dioxide. The total flow through the cuvette was 5 liter/h. Ethane emission is expressed as ppb. Pears were judged afterwards. (▲): Moderate browning/cavities; (∝): slight browning/cavities; ( ) no browning or cavities.

Ad5: Energy metabolism: fermentation

Controlled atmosphere (CA) storage of fruit benefits storage duration, by inhibition of respiration and ethylene production. But, CA conditions, especially carbon dioxide, can also induce storage disorders, when these conditions are too stressful. Ethanolic fermentation –described by Pasteur as 'la vie sans l'air'- is a response to low oxygen concentrations. Ke et al. (1994) also found formation of ethanol at extreme high carbon dioxide concentrations. Ethanol is usually the major product formed during low oxygen stress in fruits. To form ethanol, pyruvate is reduced in a two step reaction, with acetaldehyde as an intermediate. The first reduction step is catalyzed by pyruvate decarboxylase (PDC), the second step by alcohol dehydrogenase (ADH). PDC is a key factor during short-term anoxia, while ADH activity does not seem to be limiting in the fermentation process. Peppelenbos et al. (1998) suggested that fermentation

is not necessarily coupled to anoxia or hypoxia during storage, but is always active, even at normoxia. An explanatic for this phenomenon is an oxygen gradient in bulky fruits, like in apples and pears, at higher oxygen concentration Unless high oxygen concentrations, conditions in the fruit tissue can be hypoxic, or even anoxic.
References on the effect of carbon dioxide on fermentation are scarce. Carbon dioxide is known to suppress respiratio It can inhibit succinic dehydrogenase strongly, which accounts for an accumulation of succinic acid in the mitochondri According to Ke et al. (1994) and many other authors, in contrast with its inhibitory effect on oxidative respiratio enhanced carbon dioxide concentrations induce the fermentation process. This is in contradiction with our results.] preliminary results we found that fermentation is also inhibited by carbon dioxide (fig. 7). One of our hypotheses is th respiration is stronger inhibited by carbon dioxide than the fermentation route. This could imply that fermentation relatively stimulated at ULO conditions when carbon dioxide is added to the storage container. This could explain tl 'stimulation' of fermentation seen by Ke.
In literature fermentation and oxidative respiration are often seen as two separated processes. Our goal is to present model, in which both pathways and the effect of carbon dioxide on these two pathways are more integrated. Both shoı term and long-term experiments should be done. By means of laser driven techniques short term, reversible effects ‹ carbon dioxide on the energy metabolism can be monitored. In the future we hope to correlate a model for energ metabolism to the appearance of disorders in pears.

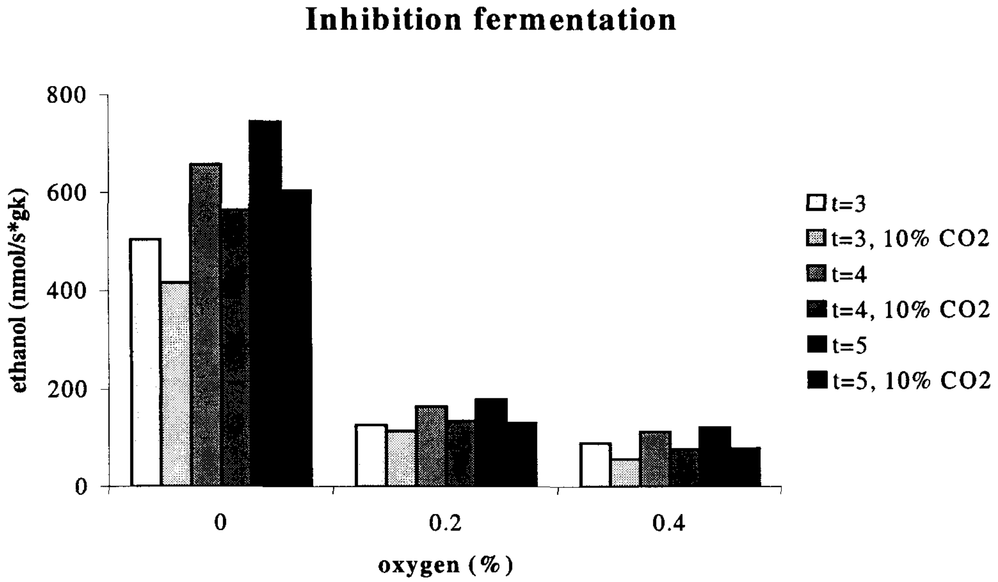

Fig. 7. Fermentation in Conference pears. Fruits were stored in 70 litre containers (ca. 25 kg per container) at 0%, 0.2% and 0.4% oxygen combined with 0% or 10% carbon dioxide for 5 days. At day 3, 4 and 5 ethanol, a product of fermentation expressed as nmol/kg*s, was determined in the containers. Although preliminary, the results indicate that in the low oxygen range fermentation is inhibited by carbon dioxide.

Output related to the project

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

3 Veltman R.H., Peppelenbos H.W., 1998. Healthy Conference pears in CA storage thanks to vitamin C (in Dutc Fruitteelt, 22: 14-15.
2 Peppelenbos H.W., Jeksrud W.K., 1998. A method for the simultaneous measurement of gas exchange aı diffusion resistance of fruits and vegetables under various gas conditions. Acta Horticulturae, 464: 333-338.
2 Peppelenbos H.W., Oosterhaven J., 1998. A theorethical approach on the role of fermentation in fruits aı vegetables. Acta Horticulturae, 464: 381-386.
2 Peppelenbos, H.W., 1998. Gas exchange models and the prediction of disorders in fruits. Proc. COST915 Copernicus CIPA-CT94-0120 workshop on Food Quality Modelling. Leuven, 3-6 June 1997, pp 69-74.
2 Veltman R.H., van Schaik A., Peppelenbos H.W. and Oosterhaven J., 1999. Core browning in Conference pe relation vitamin C and storage conditions. Proc. Int. workshop on Antioxidants in higher plants. April 13-15, 19 Ravensburg, Germany (in press).
1 De Wild H.P.J., Woltering E.J. and Peppelenbos H.W., Carbon dioxide and 1-MCP inhibit ethylene producti and respiration of pear fruit by different mechanisms. J. Exp. Botany (accepted)

1 De Wild H.P.J., Peppelenbos H.W., Use of pressure readings in gas exchange measurements. HortScience (submitted)

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

1 R.H. Veltman, M.G. Sanders, S.T. Persijn, H.W. Peppelenbos, and J. Oosterhaven. 1998. Decreased ascorbic acid levels and brown core development in pears (Pyrus communis L. cv. Conference). (Submitted to Physiologia Plantarum).

1 R.H.Veltman, A.C.R. van Schaik, M.G. Sanders and J. Oosterhaven. 1998. On the relation between ascorbic acid and tissue browning in pears (Pyrus communis L., cv. Rocha and Conference) subjected to low O_2 concentrations. (Submitted to Postharvest Biology and Technology).

<u>**LIMITED REPORT – WAGENINGEN, JANUARY 1999**</u>

<u>**Partner 2: IRTA**</u>

<u>Objectives</u>:

At the view of the results of the next year, we planned the following activities:
1 - define the effects of spraying the fruits with different Ca^{++} treatments
2 - estimate the effect both on quality and B.H incidence of high CO_2 treatments before storage.
3 - repeat and complement the study on the determination of the biochemical underlying mechanisms leading to the increase in susceptibility when the fruits are picked more mature.
4 - study the biochemical changes due to high CO_2 levels on a short-term basis.

a) <u>Preharvest Ca $^{++}$ treatment</u>:

a) <u>Material and methods</u>:
- 3 treatments:
Seniphos: 310 g/l P_2O_5 + 56 g/l CaO
Stopit: 224 g/l CaO
Metalosate: P_2O_5 (12 %) + CaO (6.7 %)
- dates of treatment: each 3 weeks from 15 abril to 15 july
- parameters of the study: mineral content, changes in quality and effect on B.H incidence.

b) <u>Preliminary results</u>:
- No significant differences in the mineral composition of the fruits
- Treated fruits showed a slight increase in maturity but no significant changes in firmness at harvest.

c) <u>Work planned for the next 6 months</u>:
- determine the effect of the Ca^{++} treatment on quality after storage
  the effect on B.H disorders
  and the relationship between these two parameters

b) <u>High CO_2 treatments before storage</u>

a) <u>Material and Methods</u>:
- 4 treatments:
control
3 d at 10 % CO_2 before storage
5 d at 10 % CO_2 before storage
3 d at 15 % CO_2 before storage
- parameters of the study : changes in quality (storage), effect on the fermentative and oxidative metabolism (after treatment), effect on B.H incidence (storage).

b) <u>Preliminary results</u>:
- high CO_2 treatment don't affect significantly the fermentative metabolism (no toxic effect) but cause a significant increase in the antioxidant-scavenging enzyme SOD ➪ positive effect for a further protection of oxidative damage

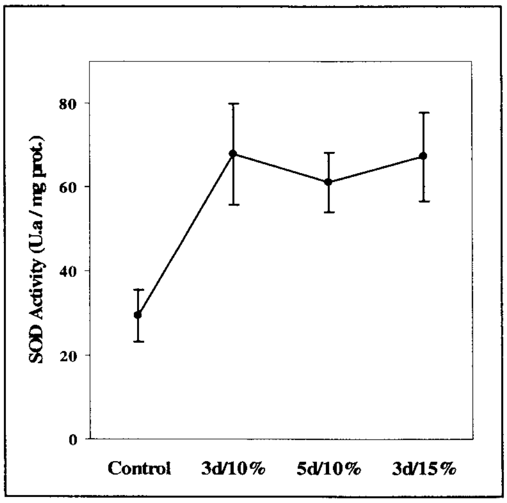

Figure 1: Changes in SOD activity in fruits treated with high CO_2. Analysis was carried out immediately after treatment.

- slight (and positive) effects on LOX activity
- significant increase of firmness values in CO_2 treated fruits after storage ➪ may be beneficial for a control of B.H disorder.

	Color (a value)	Firmness (lb)	SSC (%)	Acidity (g/l)
Control	-17.6	12.8	12.9	0.85
3d/10%	-11.1	14	14.2	0.64
5d/10%	-12.8	15.2	14.1	0.62
3d/10%	-13.6	14.1	13.8	0.62

Table 1: Change in quality parameters in fruits treated with high CO_2. Analysis was carried out after 3 months storage at 2 % O_2 and 5 % CO_2.

c) Work planned for the next 6 months:

At the view of these results we noted a beneficial effect of high CO_2 treatment on quality and oxidative scavenging potential. These results will be confirmed on a long-term basis and correlated with the changes in quality and with B.H incidence after storage.

3- Biochemical changes in mature (delay in harvest) fruits:

During year 1997/98, we showed (cf progress report), that the more mature fruits showed a significant decrease in their enzymatic capability of defense against oxidative damage and peroxidation. Significant decrease in Superoxide Dismutase (SOD), Catalase (CAT) and increase in Ascorbate Peroxidase activity were found.

This new year we repeat this experiment and complement it determining the levels of antioxidants such as Ascorbate and Glutathione.

a) Material and Methods:

Fruits were picked at one week interval, the 17, 24/08 and 01/09. Immediately after harvest, we determined the following parameters: content in ascorbate and glutathione, activity of SOD, CAT and APX.

b) Complementary results:

As regards antioxidants, we showed that:

- delayed harvest leads to a significant decrease in ascorbate and glutathione content

we noted a change in the reduction state of ascorbate

Maturity stage	**ASA + DHA ($mg\ kg^{-1}$)**	**ASA ($mg\ kg^{-1}$)**	**DHA ($mg\ kg^{-1}$)**	**DHA / ASA ratio**	**np - SH ($\mu moles\ kg^{-1}$)**
-7 d	**53.3 (9.9)**	**44.9 (5.7)**	**8.4**	**0.18**	**237.1 (18.4)**
	NS	**(*)**			**(**)**
0	**45.2 (9.6)**	**37.4 (8.8)**	**7.8**	**0.21**	**128.0 (12.8)**
	NS	**(*)**			**(**)**
+ 7 d	**37.9 (8.9)**	**26.1 (7.7)**	**11.8**	**0.45**	**104.7 (9.2)**
	NS	**(*)**			**(**)**

Table 2: Effect of harvest date on levels of ASA, DHA and non-protein thiols in Conference pears. The fruit was picked at optimal maturity (0) or 7 days before (-7d) or after (+7d). Each value represents the mean of at least six independent samples. SD values are shown in parentheses. Asterisks indicate significant differences between harvest dates by Duncan's multiple range test, NS: non significant, (*): significant at P=0.05, (**): significant at P=0.01.

These changes and the variations in enzyme activities presented before (in 1997) and confirmed in 1998, showed that delayed harvest leads to reduction in the antioxidative defense system in Conference pears and likely explain the harvest-maturity related susceptibility of this fruit to physiological disorders such as B.H.

c) Publication:

A new paper entitled " delayed harvest leads to reduction of the antioxidative defense system in Conference pears" has been sent to J. Amer. Soc. Hort. Science.

4- Biochemical changes, short-term basis:

a) Material and Methods:

- Immediately after harvest, fruits were put in microchambers at 2 % O_2 and 0.7 or 5 % CO_2. Determinations were carried out after 2, 4, 8 and 21 days of storage.
- Parameters of the study : effect of short-term storage on the parameters of the oxidative metabolism (antioxidants, oxidant scavenging enzymes) and relationships with B.H incidence.

b) Results:

Metabolic changes on a short-term basis are important and likely related to the occurrence of storage disorders.

Short-term changes in oxidative defense systems are a key element and detrimental effects particularly affects:

- the level in antioxidant (Vit.C, glutahione)

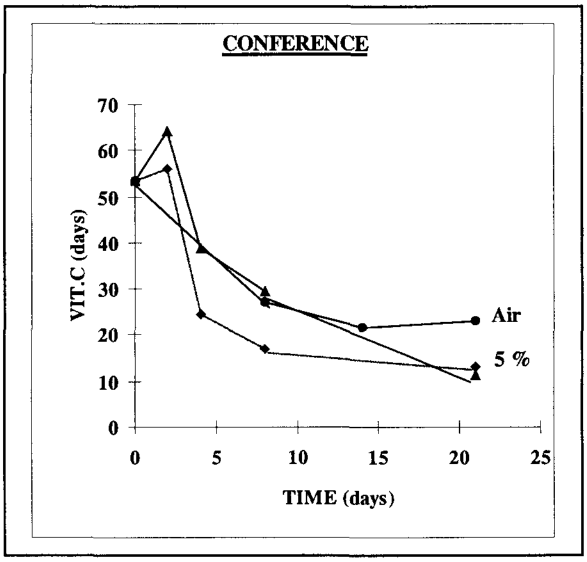

Figure 2: Short-term related changes in the content of ascorbate

- the activity of the SOD

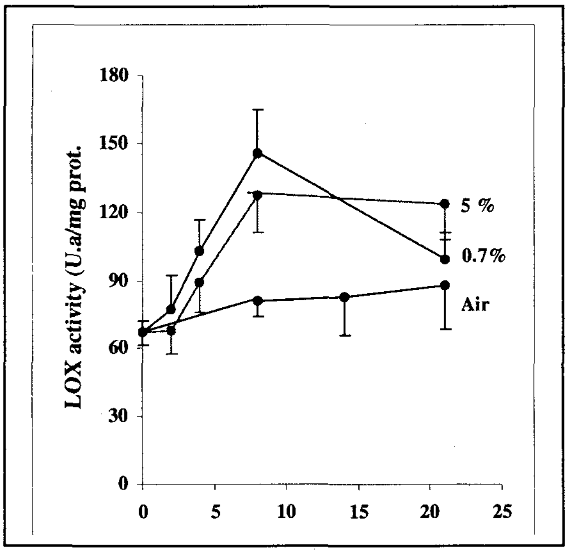

Figure 3: Short-term related changes in the activity of LOX

- the activity of LOX

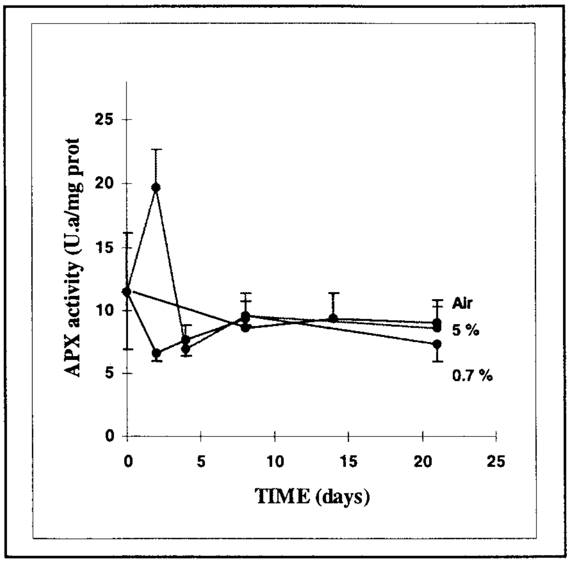

Figure 4: Short-term related changes in the activity of LOX

- the activity of APX and as a consequence the level in ascorbate

5- Work planned for the next 6 months:

Different experiments are in progress and will be completed:
- E_1 : *N_2 fertilization*, relationship with quality and B.H incidence will be determined.
- E2 : *Ca^{++} treatments*, relationship with quality and B.H incidence will be determined.
- E3 : *high CO_2 treatment*, relationship with quality and B.H incidence will be determined.
- E4 : *Orchards*, the changes in quality parameters and B.H incidence after storage will be determined for the 3 orchards
- E5 : *Biochemical studies* : metabolic changes in the antioxidant pathways (ascorbate, glutathione, enzymes) will be followed on a long-term basis and correlated to the changes in quality and B.H incidence. Complementary results on polyamines will be performed. Specific biochemical changes in damaged fruits will be also established.

- Results will be send for publication in international reviews.

Participant no.3:
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(Research Institute for Agricultural Products Technologies)
via Venezian 26
I-20133 Milan, Italy

Task 2: Harvest of the pears

Results and Discussion

Harvest date. With delaying harvest time significant differences were found in fruit weight (about +20% from first to third harvest), in hue of fruit colour (increase of yellow hue) and in soluble solids (Table 1). As regards firmness, starch hydrolysis and Streif and deJager indexes of maturity, only the fruits of the first harvest were significantly different from the fruits harvested later, and no difference was significant between fruits from the 2nd and 3rd harvests. The values of the parameters found in the second year are very similar to those found in the first year of experiment, indicating that the pears were picked at a comparable stage of maturity.

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

Task 6: Measuring gas exchange and diffusion rates

Diffusion resistance.
Diffusion resistance increased significantly after 4 months' storage in 2% oxygen, and was significantly higher in 3rd harvest fruits (Table 3).
No difference was found in 3rd harvest fruits cooled for 5 weeks as compared to those measured at harvest.
If these results are confirmed and, according to Graham's law, diffusion resistance of O_2 and CO_2 is proportional to that found for neon, it means that in late harvested, mature fruits after a certain storage time, diffusion resistances to O_2 and CO_2 can reach very high values so that the entry of oxygen in the fruit from external atmosphere and the diffusion of CO_2 out of the fruit could be hindered. These results are consistent with the fact that 3rd harvest fruits are more susceptible to brown heart, and that brown heart develops after some time in storage.

Gas exchange measurements.
Gas exchange rates are reported in Tables 4 - 8.
Harvest date. At harvest only O_2 consumption, but not CO_2 production, was significantly affected by harvest time: respiration rate decreased with delaying harvest time, and it was the lowest in third harvest fruits (Table 4).
For fruits from all harvests the oxygen consumption rate at harvest was lower in 21% than in 2%O_2, with no difference between low and high CO_2.

Carbon dioxide production at harvest was significantly lowered by the presence of high CO_2 in the atmosphere, with little or no difference between 2 and 21% O_2 (Table 5).

Delayed CA experiment. In fruits of the 3rd harvest after 5 weeks' cooling in air storage at –1/0 C, O_2 consumption rate increased to a level higher than that of 1st harvest fruits at harvest (Table 4) while pears stored for the same time in 2 % oxygen and 0.7 % CO_2 had respiration rates as low as at harvest. Fruits cooled for 5 weeks had high respiration rates also in 21% O_2. Cooling did not significantly affect CO_2 production rate (Table 5). The latter was lower in 3rd harvest fruits stored for 6 weeks in 2% O_2 and low CO_2 (Table 6).
Peppelenbos found increased respiration and fermentation rates in pears stored in cool air for 5 weeks (First Europear report, 1998). We found similar results, although only the increase in respiration rate was significant.

Storage period.
Gas exchange rates were similar after 2 or 4 months' storage (Tables 5 and 7). On the average after 2 months' storage at 2% O_2 both O_2 and CO_2 exchange rates in air were higher in fruits which had been stored in high CO_2 than in those stored in low CO_2.
CO_2 production was significantly lowered by the presence of 5% CO_2, indicating that high CO_2 inhibits CO_2 production.
In fruit stored in low CO_2 some differences due to harvest were significant after 2 months, 3rd harvest fruits showing higher gas exchange rates than 1st and 2nd harvest fruits (Table 8).
In conclusion the effect of harvest on gas exchange rates tends to disappear with increasing length of storage, but it is very important in the first weeks after harvest.

Internal gas concentration. The average sampled volume of internal gas was 0.91 ml, with a range from 0.2 to 3.5 ml. Often the volume was insufficient to flush the GC. Almost all the analysis of internal atmosphere resulted in relatively high O_2 concentration, indicating that air had entered the GC or the syringe so diluting the sample atmosphere. However several samples, although with a higher level of oxygen than expected and therefore apparently diluted by air, showed higher CO_2 concentration than that computed on the hypothesis of a steady state condition and on the base of gas exchange rates and diffusion resistance. The means of these samples are reported in Table 9. These results are not exhaustive, but suggest that the actual internal CO_2 concentration could be still higher than that found, indicating perhaps a higher diffusion resistance to CO_2 than that considered here (based on diffusion resistance to neon modified according to Graham's law). Further research is needed on this point, and a better sampling method for internal gas analysis should be found.

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

Results and Discussion

Brown heart and storage disorders. The only fruits examined until now are those used for gas exchange measurements in October and in December. Only 1 fruit examined in December showed the symptoms of brown heart. Total number of fruits was not high: 48 fruits were examined in October and 60 fruits in December. According to the results of last year, the first symptoms of brown heart were shown already in December.

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

Tasks 1, 2 , 4 and 6. The tasks were completed.

Task 3 - In January and March fruits will be taken out of storage at IVTPA for analysis of quality parameters and for examination of storage disorders. The same analyses will be carried out on fruits stored in commercial stores.

Task 7 -
The analysis of quality parameters and the evaluation of incidence of brown heart and other storage disorders will be carried out on fruits of the harvest experiment (one sample /harvest/ storage atmosphere /storage time), of the position experiment (one sample /position /storage atmosphere only in March) and of the delayed CA experiment at IVTPA, and on fruits of the harvest and delayed CA experiments at the commercial stores.

Quality analysis of fruit.
The following measurements will be made the day after the end of storage on samples of 20 fruits: background colour of the skin (Minolta CR-200) on the largest and greenest part of the fruit; largest diameter; height; weight; firmness with 8 mm plunger (Instron UTM); soluble solids.

Brown heart and storage disorders.
Fruits will be cut and examined for brown heart and other storage disorders. The experimental unit will be made of one box per subset and treatment.

Biochemical studies.
Samples of the inner and the outer part of the fruits have been stored for further analysis of ascorbic acid content at harvest, in October and in December. The analysis is now in progress.

E. DISSEMINATION

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

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

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

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

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

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

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

Table 3. Means of diffusion resistance for Ne in pears from different harvests and at different storage times.

storage time	harvest	R_{Ne} s/mm	
at harvest		481,2257	a
october		567,8397	a
december		736,8706	b
	1	543,7181	a
	2	566,5397	a
	3	728,2956	b

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

Table 4. O_2 consumption rate (nmol/kg/s) of Conference pears from different harvests.

gas conditions ($\%O_2+\%CO_2$)	at harvest			after 5 wks cooling	after 6 wks in low CO_2
	1st harv	2nd harv	3rd harv	3rd harv	3rd harv
0+0	0	0	0	0	0
2+0	15.7	14.9	11.9	16.2	10.0
2+5	15.0	13.6	12.8	14.6	11.3
21+0	10.5	9.9	8.8	17.6	7.2
21+5	11.1	10.1	5.2	15.9	8.6
mean	*13.1*	*12.1*	*9.7*	*16.1*	*9.3*
	a	*ab*	*b*		
			b	*a*	*b*

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

Table 5. CO_2 production rate (nmol/kg/s) of Conference pears.

gas conditions ($\%O_2+\%CO_2$)	at harvest	after 5 wks cooling (3rd harvest)	after 2 months in		after 4 months in	
			low CO_2	high CO_2	low CO_2	high CO_2
0+0	16.0	18.4	11.8	11.8	12.5	10.8
0.5+0	-	-	-	-	8.8	-
0.5+5	-	-	-	-	-	8.9
2+0	13.3	15.0	8.1	-	9.1	-
2+5	9.7	10.8	-	6.1	-	9.3
21+0	16.9	14.3	8.5	10.7	8.4	10.1
21+5	7.9	8.3	3.5	4.4	3.9	4.2

Table 6. Mean CO_2 production rates (nmol/kg/s) in Conference pears of the 3rd harvest, at harvest, after 5 weeks cooling and after 6 weeks in storage with 2% O_2 and low CO_2.

	at harvest	after 5 wks cooling	after 6 wks in low CO_2
mean	*11.6* *a*	*13.4* *a*	*8.8* *b*

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

Table 7. O_2 consumption rate (nmol/kg/s) of Conference pears.

gas conditions ($\%O_2+\%CO_2$)	after 2 months in		after 4 months in	
	low CO_2	high CO_2	low CO_2	high CO_2
0+0	0	0	0	0
0.5+0	-	-	8.9	-
0.5+5	-	-	-	7.5
2+0	9.2	-	8.6	-
2+5	-	9.7	-	6.0
21+0	6.4	13.0	5.2	6.7
21+5	7.1	9.0	5.5	6.0

Table 8. Mean O_2 consumption and CO_2 production rates of fruits of different harvests after 2 months storage in low CO_2.

	harvest	O_2 (nmol/kg/s)	CO_2 (nmol/kg/s)
	1	7.3 b	7.5 b
	2	6.8 b	7.1 b
	3	8.6 a	9.3 a

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

Table 9. Internal CO_2 concentration (%) of Conference pears after 4 days in different gas conditions computed according to diffusion and gas exchange results, and actually found by GC analysis. Only data of fruits where CO_2 found was higher than that computed are reported. Differences of CO_2 and O_2 concentrations are also reported, showing that, even if diluted by air, internal CO_2 concentration is still higher than expected.

storage time	gas conditions		N	% internal CO_2 computed	% internal CO_2 found	ΔCO_2 (% found - % computed)	ΔO_2 (% found - % computed)
	$\%O_2$	$\%CO_2$		mean (min; max)	mean (min; max)	mean (min; max)	mean (min; max)
	0	0	4	0.45 (0.43; 0.48)	0.62 (0.51; 0.67)	0.17 (0.09; 0.19)	5.89 (3.80; 10.12)
at harvest	2	5	1	5.18 (5.18; 5.18)	5.52 (5.52; 5.52)	0.34 (0.34; 0.34)	1.76 (1.76; 1.76)
	21	0	4	0.53 (0.40; 0.60)	0.82 (0.67; 0.99)	0.29 (0.27; 0.39)	-0.35 (-0.53; -0.08)
	21	5	2	5.31 (5.29; 5.32)	5.79 (5.55; 6.03)	0.48 (0.26; 0.71)	-0.37 (-0.42; -0.33)
	0	0	13	0.34 (0.23; 0.50)	0.52 (0.33; 0.97)	0.18 (0.10; 0.48)	4.75 (1.00; 11.58)
	2	0	7	0.93 (0.87; 1.07)	1.11 (0.94; 1.45)	0.18 (0.07; 0.38)	1.46 (0.36; 2.91)
october	2	5	3	5.21 (5.15; 5.32)	5.80 (5.62; 6.00)	0.59 (0.46; 0.67)	1.75 (0.07; 3.07)
	21	0	11	0.32 (0.20; 0.61)	0.68 (0.26; 1.73)	0.36 (0.05; 1.12)	-0.14 (-2.68; 0.72)
	21	5	6	5.12 (4.88; 5.20)	5.35 (5.00; 5.73)	0.23 (0.12; 0.53)	0.06 (-0.12; 0.27)
	0	0	9	0.32 (0.18; 0.46)	0.50 (0.24; 0.90)	0.18 (0.06; 0.44)	4.72 (1.43; 12.80)
	2	0	2	0.97 (0.94; 1.01)	1.02 (0.95; 1.09)	0.05 (0.02; 0.08)	2.58 (2.12; 3.04)
december	2	5	3	5.27 (5.26; 5.29)	5.64 (5.54; 5.71)	0.37 (0.28; 0.42)	2.15 (1.26; 3.74)
	21	0	7	0.26 (0.21; 0.35)	0.40 (0.24; 0.56)	0.14 (0.03; 0.21)	-0.02 (-0.47; 0.30)
	21	5	1	5.14 (5.14; 5.14)	5.36 (5.36; 5.36)	0.22 (0.22; 0.22)	0.02 (0.02; 0.02)

4th Europear meeting 18/19 Januari 1999 Wageningen

Presentation of progress and planning at FPO
Anton de Jager
Petra Willeboer

Content of the presentation

progress 01-07-1998 to 31-12-1998

1. EXPERIMENTS

Task 3 (storage of pears)

a. 5 picking dates in 7 orchards
b. measurement at pick of quality (weight, firmness, ground colour, starchpattern, sugar- and acid concentration
measurement of nutrient concentration at pick 3
measurement of vitamin C concentration at pick 1, 3 and 5
c. two CO_2 concentrations (0.5 and 5.0%) at standard scenario
7 days cooling (-0.5°C) followed by CA storage at -0.5°C, 2%O_2 at two CO_2 concentrations.
d. three O_2 concentrations: 2, 3 and 4% after 21 days of cool storage
e. 1 box containing 17 kg (ca. 75 pears) for each of the 140 objects

Task 4 (Nutrition/crop load/position)

* *Nutrition*
a. 2 orchards, 5 picking dates
b. normal and high levels of Ca, N and K (3 trees per treatment)

* *Crop load*
- hand thinning (end of June/beginning of July)
a. 2 orchards, 5 picking dates
b. 3 levels of bearing by hand thinning (3 trees per treatment)
- level of flowering
a. 1 orchard
b. 3 levels of flowering

* *Position of fruit in the tree*
a. 2 orchards, 5 picking dates
b. 5 positions (top, high, inner, 2*outer; 3 trees per treatment)

* *General for all three experiments*
c. quality measurement at pick of quality (weight, firmness, ground colour, starchpattern, sugar- and acid concentration measurement of nutrient concentration at pick 3
measurement of vitamin C concentration at pick 5
d. storage at standard scenario: 7 days cooling (-0.5°C) followed by CA storage at -0.5°C, 2%O_2 and 0.5% CO_2;
e. total number of objects: 60 + 30 + 50 = 140 (1 box per object)

Task 5 (storage scenario, stress test)

** storage scenario*
a. 7 orchards, pick 3, 4 and 5
b. quality measurement at pick of quality (weight, firmness, ground color, starch pattern, sugar- and acid concentration; measurement of nutrient concentration at pick 3; measurement of vit C at pick 3 and 5
c. period of cooling before CA: 7, 21, 33 and 80 days
d. total number of objects 126 (1 box per object)

** stress test*
a. 1 or 2 days at 18°C, followed by 18 or 17 days at 4°C, 2%O_2 and 5% CO_2.
b. 4 or 5 days at 18°C, followed by 15 or 14 days at 4°C, 2%O_2 and 0.5% CO_2.

** summary of vit C determinations at harvest:*
- Task 3: in 7 orchards at pick 1, 3 and 5
- Task 4: at pick 3

** proposal for vit C determinations at take out:*
Task 3:
a. 7 orchards, pick 1, 3 and 5, standard scenario: 21 samples
b. also at 5% CO_2 and at 4%O_2 : 42 samples
Task 4:
a. 2 orchards, 6 objects, pick 3: 12 samples (nutrients)
b. 2 orchards, 3 objects, pick 3: 6 samples (bearing)
c. 2 orchards, 5 objects, pick 3: 10 samples
Task 5:
a. 7 orchards, 6 scenario's: 42 samples

Total of 133 samples = Dfl 13.300

2. RESULTS

At this moment we have no results available since all pears are still in storage. The planned period of observation ('take out') is 22 March to 1 April.
We do have, however, information, that might be interesting, concerning a possible application of the automatic measurement of the degree of starch degradation using the french apparatus 'amidomètre'. This apparatus is developed a .o. at the Ctifl institute at St. Remy de Provence and produced by COPA-technologie S.A., 13103 St. Etienne du Gres, France (tel ** 33 4 90490521, fax ** 33 4 90490521).
Since the degree of starch degradation is rated visually up till now, the determination is rather depending on the person actually performing the rating, even though a map with standard pictures ranging from 1 (black) to 10 (white) is available. The french apparatus might solve

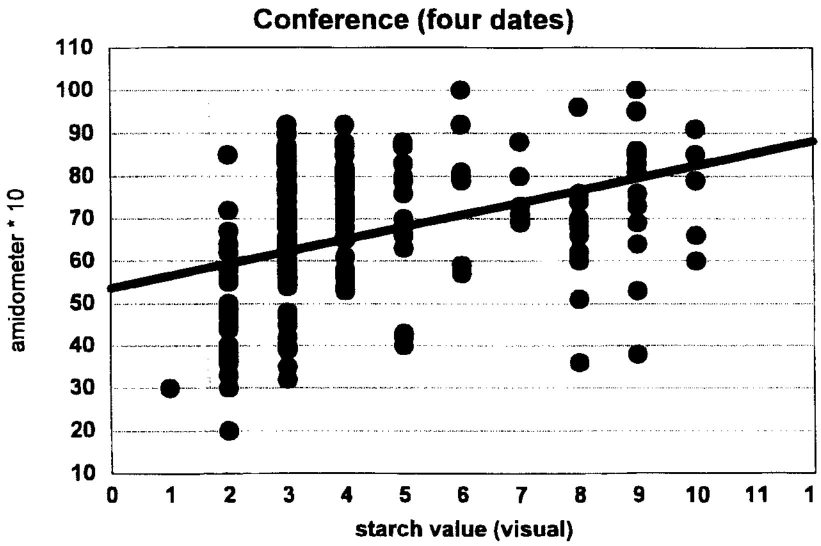


Fig. 1. Automatic determination of the proportion of white surface area in the KI/I_2 starch test, against visual rating of the 'decoloration stage' (according to standard maps) using 4 samples of 25 fruits each during the ripening period.

the problem of reproducibility (same person) and of agreement between persons.
The figure shows, however, that the agreement between person and apparatus is very poor for pear, much worse than for apple. This may be because of the rather faint type of black that is typical for starch coloration by the KI/I_2 mixture in Conference.

PLANNING SEASON 1999-2000

1. Depending on requirement for data of the modeling people.
2. Depending on the results of nutrition, bearing and position experiment.
3. More emphasis on stress test.
4. Tentative practical application: guiding a number of growers with 'high risk orchards' using a provisional scheme to be worked out later but including at least

a. normal and late pick
b. keeping pears from low bearing trees and from top apart
c. stress test
d. choosing between standard scenario (21 days cooling before CA) and regular storage for each of the four lots.
e. parallel screening of these pears in research facilities (inspection in two week intervals)

"Quality improvement of pears
by predictive and adaptive technology"

Limited Progress Report for the EUROPEAR-meeting at 18/19 January 1999

Period: 01-06-98 to 31-12-98

University Hohenheim, Institut für Obstbau, Bavendorf

Scientific team

Team leader: Dr. Josef Streif

Other scientific staff: Ing. Haibo Xuan, Ing. Claudia Rabus,

Technician Eva Gorgus 2 months

RESEARCH ACTIVITIES DURING THE PERIOD 01-06 to 31-12-1998

Task 1: Cultivation of pears

The same orchards and experimental design as in 1997

Hand thinning was applied for regulation the crop load,

no growth regulators were used

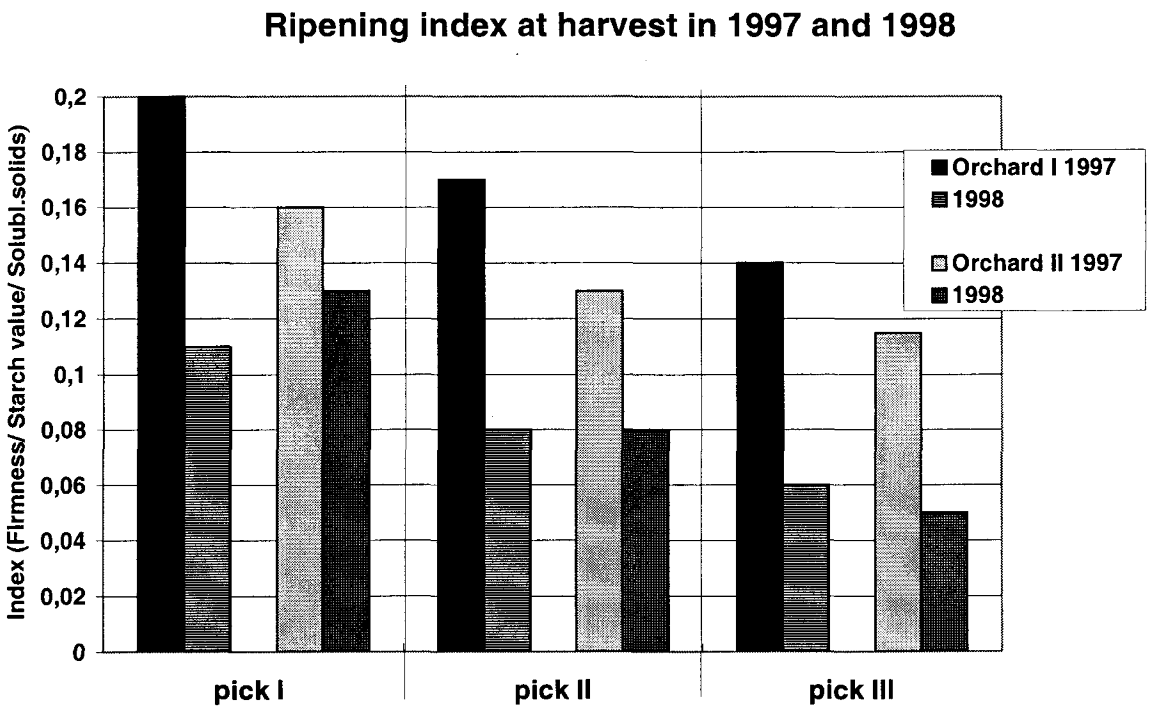
Task 2 Harvest of pears

The same picking procedure as in 1997. Because of advanced ripening in 1998 the begin of harvest was one week earlier. The Streif index was lower than 1997

Tab. 1 Fruit quality and ripening parameters of Conference pears from two orchards at harvest 1998

Orchard I									
sampling date	harvest date	storage cond. %CO2+%O2	size mm	firmness kg/0.5cm2	solubl sol. %	acidity mval	starch 1-10	colour CIE a	ripening Index
1	I (03-Sept.)	begin	60,70	6,02	12,35	1,85	4,50	-17,64	0,11
	II (10-Sept.)	begin	65,50	5,52	13,15	1,82	5,00	-16,95	0,08
	III (17-Sept.)	begin	69,67	5,50	13,77	1,59	7,00	-15,60	0,06
Orchard II									
sampling date	harvest date	stor.cond. %CO2+%O2	size mm	firmness kg/0.5cm2	solubl sol. %	acidity mval	starch 1-10	colour CIE a	ripening Index
1	I (03-Sept.)	begin	63,83	6,43	12,87	2,01	4,17	-16,14	0,13
	II (10-Sept.)	begin	60,17	5,45	12,37	1,81	6,17	-16,08	0,08
	III (17-Sept.)	begin	63,67	5,38	12,63	1,84	8,83	-15,21	0,05

Fig 1



Task 3 **<u>Storage of pears</u>**

Storage devices are the same as 1997

The following 2 CA-storage conditions were used at temperatures of -0.5 to -1 °C:

2.0 % O_2 plus 0.7 % CO_2 (conditions avoiding core browning)

2.0 % O_2 plus 5.0 % CO_2 (conditions provoking core browning)

The observations of browning disorders were intensified in the first three months of storage period in order to detect the very first beginning of damages. The risk for brown heart seems very low in 1998/99, however.

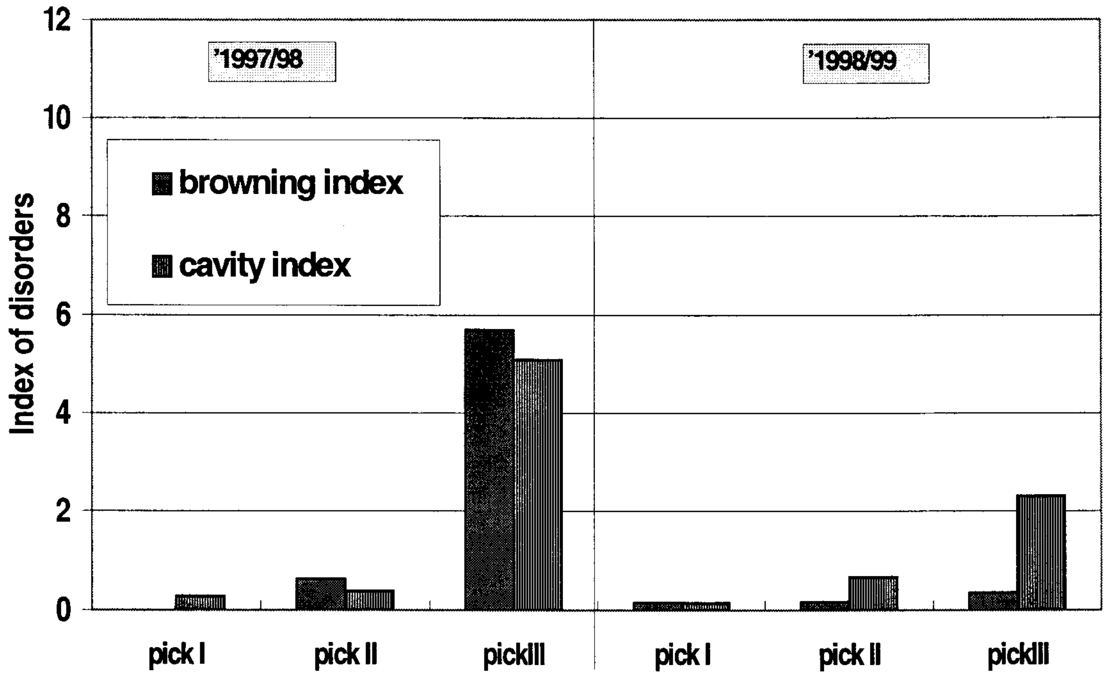
Nevertheless the strong relationship between the incidence of browning disorders on the one hand and the picking date and the amount of CO_2 concentration in the storage atmosphere on the other hand could be observed.

Tab. 2 Incidence of core browning and cavities in Conference pears in the first 3 months of CA storage

After 1 Month							
sampling date	torage cond. %CO2+%O2		total nr. of fruits	healthy fruits %	fungus infected %	browning index	cavity index
1 month storage	CO2 0,7+2 O2	pick I	74,00	100,00	0,00	0,00	0,00
		pick II	64,67	99,47	0,00	0,17	0,00
		pickIII	61,67	100,00	0,00	0,00	0,00
	CO2 5+2 O2	pick I	66,67	99,49	0,00	0,00	0,00
		pick II	66,33	98,97	0,51	0,17	0,00
		pickIII	65,00	98,96	0,52	0,17	0,00
After 2 Months							
sampling date	torage cond. %CO2+%O2		total nr. of fruits	healthy fruits %	fungus infected %	browning index	cavity index
2 months storage	CO2 0,7+2 O2	pick I	69,33	99,53	0,47	0,00	0,00
		pick II	66,33	99,50	0,50	0,00	0,00
		pickIII	60,33	100,00	0,00	0,00	0,00
	CO2 5+2 O2	pick I	73,33	99,55	0,00	0,15	0,15
		pick II	67,00	97,73	0,00	0,17	0,66
		pickIII	62,67	91,99	0,00	0,35	2,30
sampling date	torage cond. %CO2+%O2		total nr. of fruits	healthy fruits %	fungus infected %	browning index	cavity index
3 months storage	CO2 0,7+2 O2	pick I	40,33	99,22	0,78	0,00	0,00
		pick II	34,00	95,21	4,79	0,00	0,00
		pickIII	37,67	99,15	0,85	0,00	0,00
	CO2 5+2 O2	pick I	69,00	96,18	1,37	0,81	0,32
		pick II	67,00	91,64	0,98	1,49	1,33
		pickIII	63,00	73,14	0,54	5,64	6,00

Abb. 2

Incidence of disorders in Conference pears after 2 months of CA storage (2%O2+5%CO2)



Task 4 <u>Climate and orchard factors</u>

The climate characteristics for the two orchard were monitored in the same way as in the year before. In 1998 the temperature was generally somewhat higher, mainly in June but lower in September comparing with 1997.

Orchard I	Temperature (in 2m)			Humidity (%)			Radiation	Precip.
Month	Average	Max.	Min.	Average	Max.	Min.	(W m^{-2})	(mm)
1997								
May	13,2	20,7	6,8	67	92	40	4970	54
June	15,7	21,8	11,0	74	93	49	4256	187
July	16,7	23,1	11,6	76	95	50	4270	107
August	19,0	26,8	12,9	73	94	44	4422	73
Septemb	14,5	22,4	8,7	77	95	47	3568	32
Average 1997	15,8	23,0	10,2	73	94	46	21485	453
1998								
May	14,4	21,2	8,0	65	89	42	4914	49
June	17,3	24,1	11,3	68	92	42	4857	92
July	18,0	24,0	13,0	71	92	46	4282	74
August	18,3	25,4	12,4	67	91	41	4429	50
Septemb	13,1	18,9	9,3	80	94	53	2642 !!	151
Average 1998	16,2	22,7	10,8	70	92	45	21123	417

Orchard II

1998	16,1	23,5	9,4	67,5				440,2
1997	16,2	24,9	9,0	78,4				335,0

Crop load was regulated by thinning according to last year.

The effects of various crop loads in respect to the occurence of disorders will be evaluated after 6 months of CA storage.

Task 5 <u>Postharvest treatments</u>

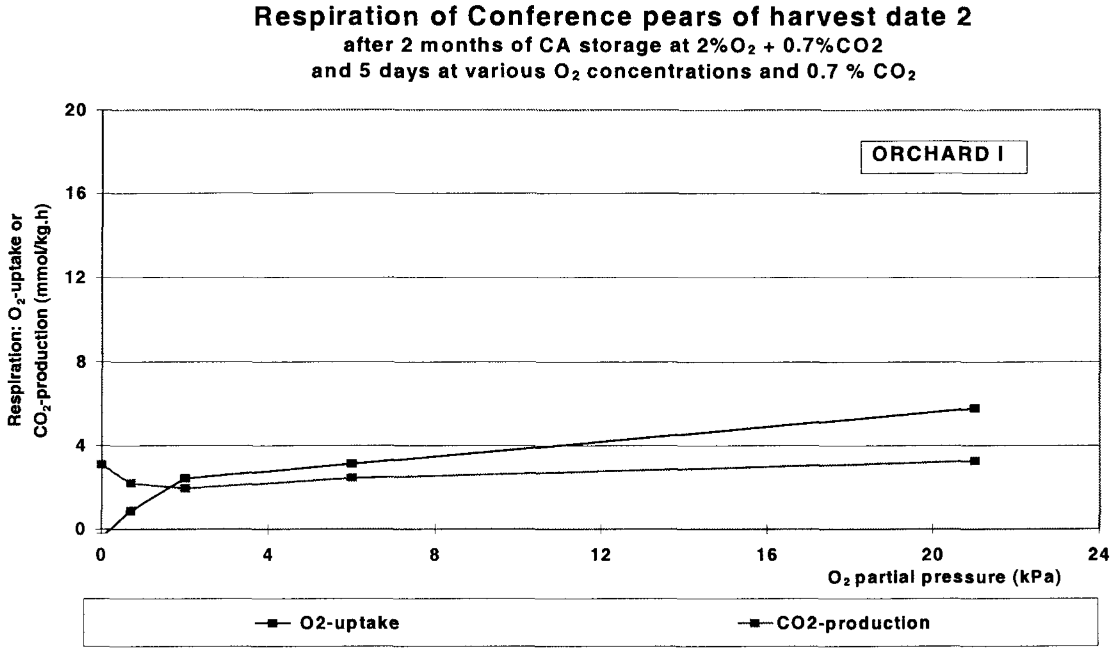
Our special aim was to examine which CA regime and which time of storage periode are most favorable for the induction of browning disorders.

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 periode. 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. The experiment is still in progress.

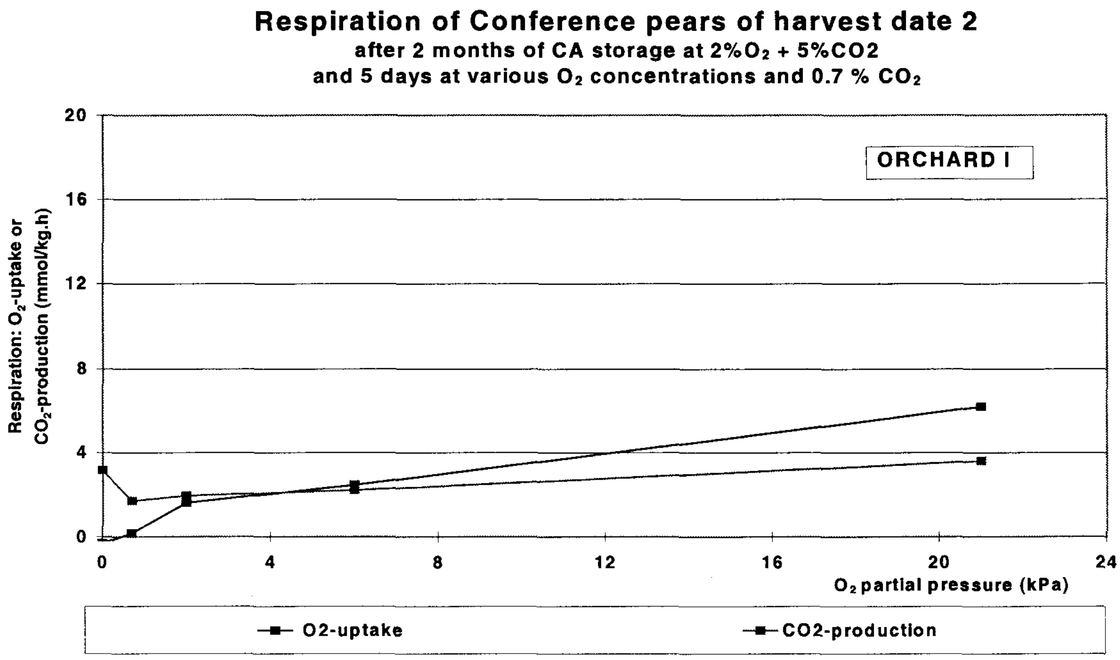
Task 6 <u>Gas exchange and diffusion measurement</u>

<u>Respiration measurements:</u>

Respiration measurements are done by headspace GC anlysis in the same way as last year. Respiration and RQ of fruits from pick 3 were higher than of fruits from the earlier picks

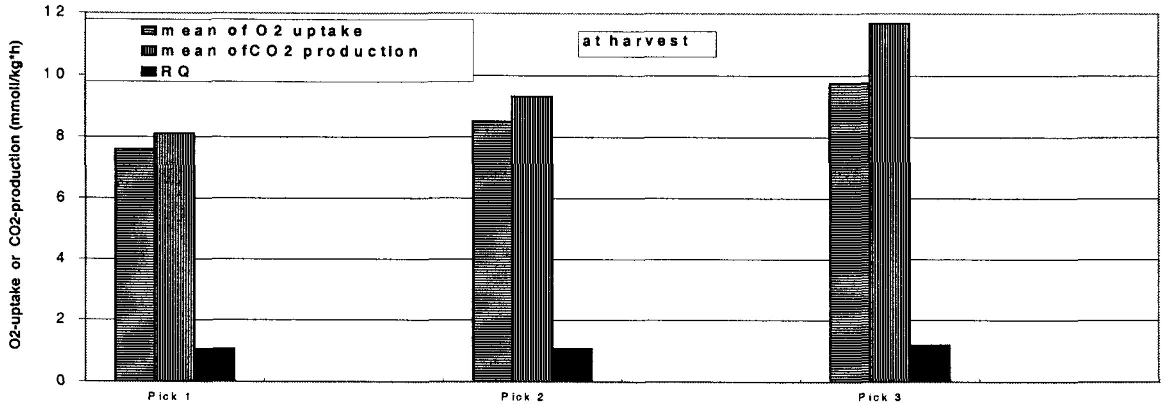


Respiration of Conference pears of harvest date 2
after 2 months of CA storage at 2%O2 + 0.7%CO2
and 5 days at various O2 concentrations and 0.7 % CO2
ORCHARD I
Respiration: O2-uptake or CO2-production (mmol/kg.h)
O2 partial pressure (kPa)
0
4
8
12
16
20
24
O2-uptake
CO2-production

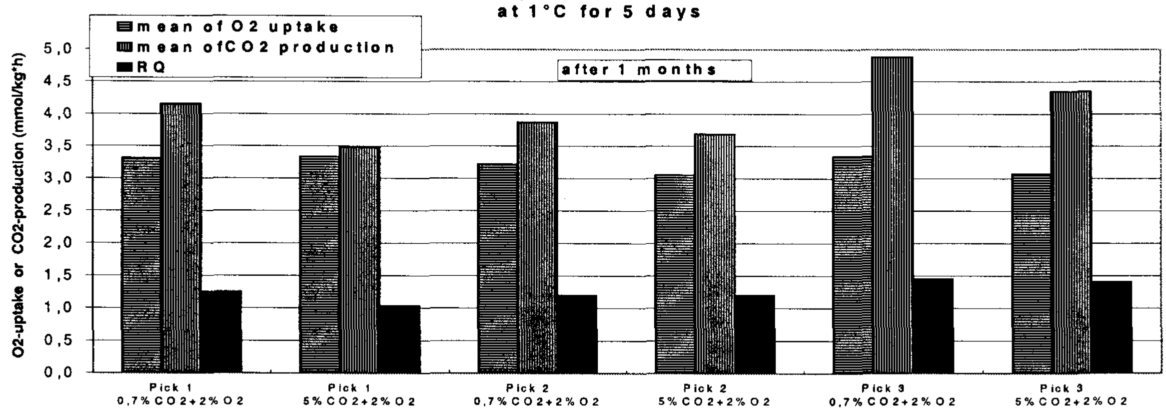


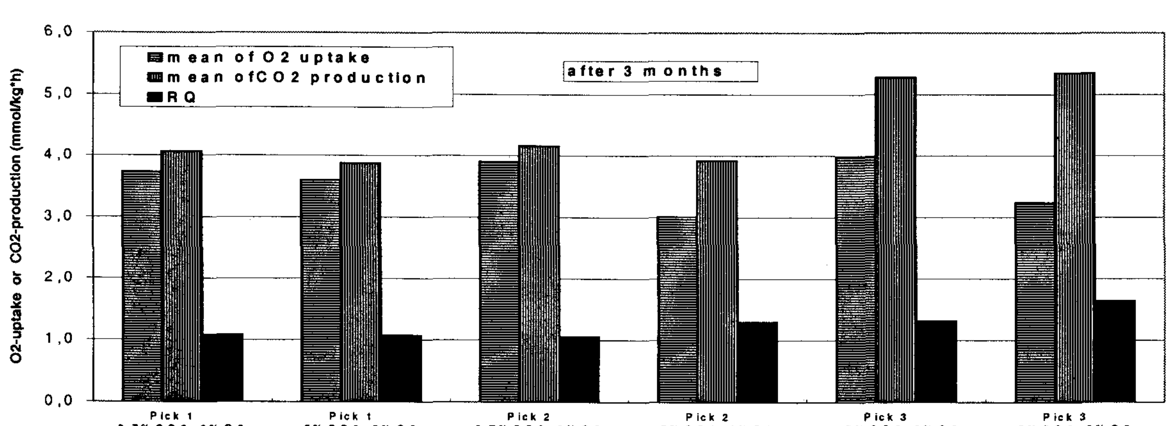
Respiration of Conference pears of harvest date 2
after 2 months of CA storage at 2%O2 + 5%CO2
and 5 days at various O2 concentrations and 0.7 % CO2
ORCHARD I
Respiration: O2-uptake or CO2-production (mmol/kg.h)
O2 partial pressure (kPa)
0
4
8
12
16
20
24
O2-uptake
CO2-production

Mean of O2-uptake and CO2-production and RQ of Conference fruits at harvest stored under various O2-concentrations at 1°C for 5 days



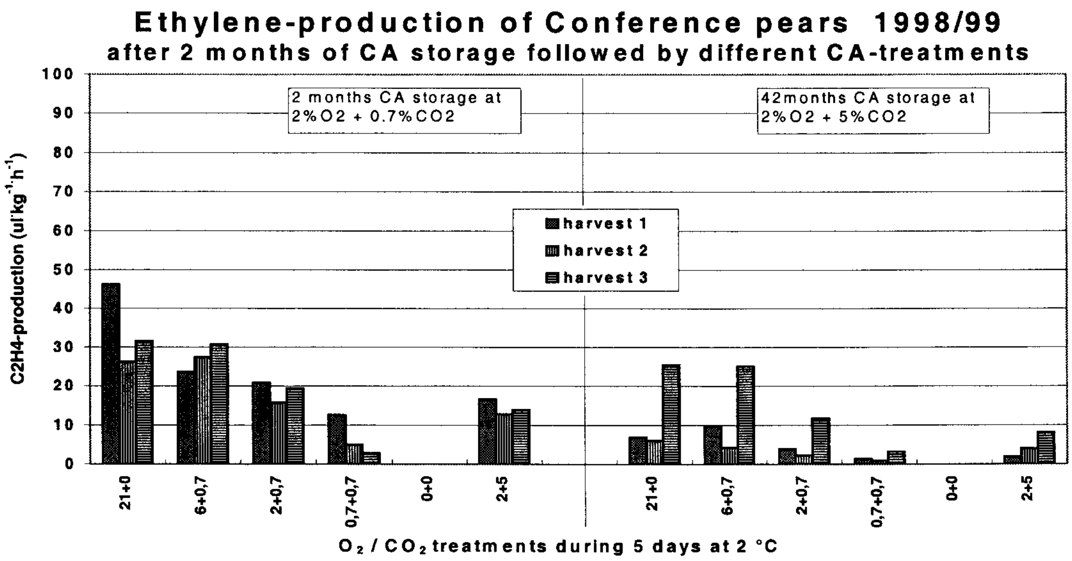
Mean of O2-uptake and CO2-production and RQ of Conference fruits after 1 months of CA-storage and various O2-concentrations at 1°C for 5 days

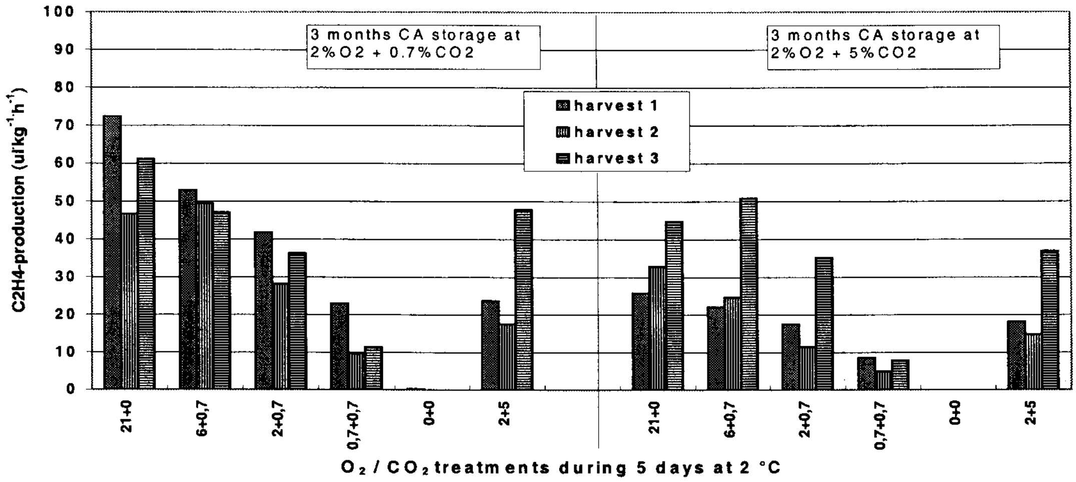




Ethylene production: At harvest and after 1 month of CA storage no ethylene could be detected. In the ongoing storage periode the amount of ethylene production is highly influenced by the O_2 concentration in the respiration jars.

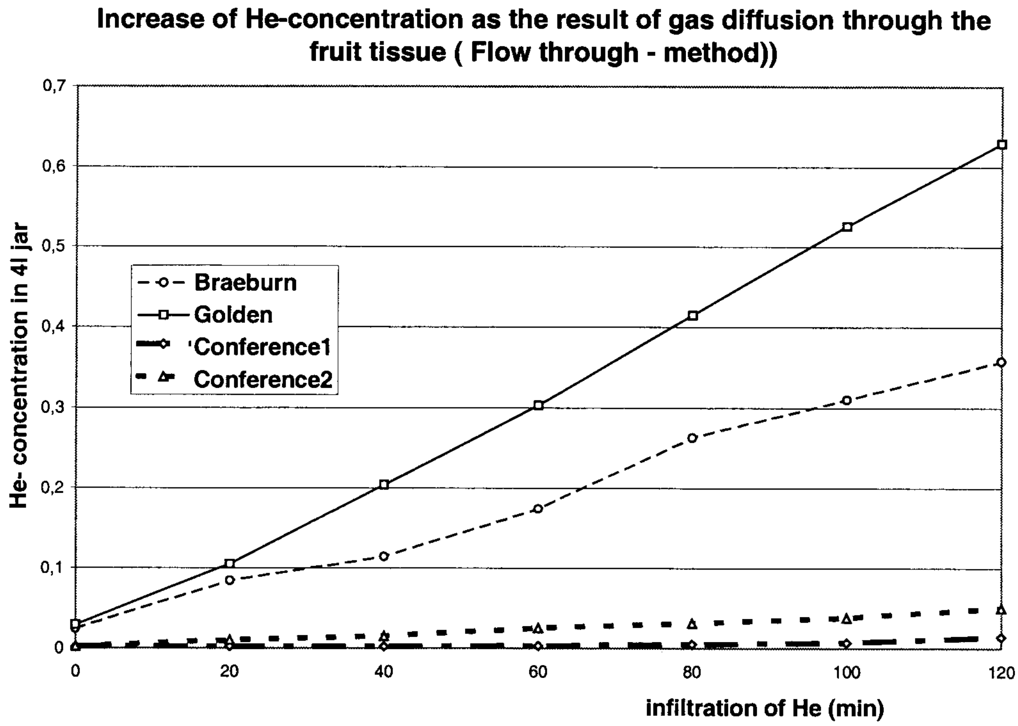
The influence of CO_2 concentration (0.7% or 5% CO_2) and picking was not very pronounced.





Gas diffusion: Different methods of gas diffusion measurements were tested

In a new developped methode the penetration of Helium from the core of the fruit through the skin was measured. The Helium was flushed through the core continously with needles injected in the core and connected with an medicinal infusion device. Big differences could be detected in tissue resistance between apple and pears and between different cultivars.



Task 7 <u>Destructive measurements</u>

a: Fruit quality evaluation

Methods and evaluation of fruit quality as changed under the different harvest time and storage conditions are the same as last year but the amount of fruits and the number of sampling dates are reduced. Investigations are still in progress.

b: Biochemical analysis

<u>Vitamin C and ascorbic acid content:</u>

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 observations of Vitamin C metabolism was concentrated in the first three months of storage period in order to detect relations between changes in Vitamin C and the first beginning of browning disorders.

Tab: Vitamin C and ascorbate content in the inner and outer part of the cortex of Conference pears from 2 orchards at harvest and after differrent time of CA storage

Orchard I mg/100g FS

I. Ernte **Ascorbate**

	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	1,50	1,46	1,28	1,41
0,7+2 outer	1,98	1,88	1,52	1,45
5+2 inner	1,50	0,94	0,90	0,98
5+2 outer	1,98	1,60	1,78	1,50

II. Ernte **Ascorbate**

	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	1,52	1,69	1,18	1,48
0,7+2 outer	1,96	1,27	1,65	1,24
5+2 inner	1,52	0,61	0,75	0,67
5+2 outer	1,96	1,38	1,02	1,20

III. Ernte **Ascorbate**

	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	2,57	1,27	1,11	0,82
0,7+2 outer	2,78	2,45	1,81	1,58
5+2 inner	2,57	0,67	0,66	0,61
5+2 outer	2,78	1,93	1,25	1,16

I. Ernte **DH-Ascorbate**

	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	6,28	2,68	1,84	1,24
0,7+2 outer	5,50	2,78	1,59	0,99
5+2 inner	6,28	1,93	0,67	0,30
5+2 outer	5,50	2,35	0,18	0,60

II. Ernte **DH-Ascorbate**

	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	4,07	2,40	1,09	0,68
0,7+2 outer	3,19	3,08	1,06	1,20
5+2 inner	4,07	2,31	0,78	0,67
5+2 outer	3,19	1,98	1,25	0,34

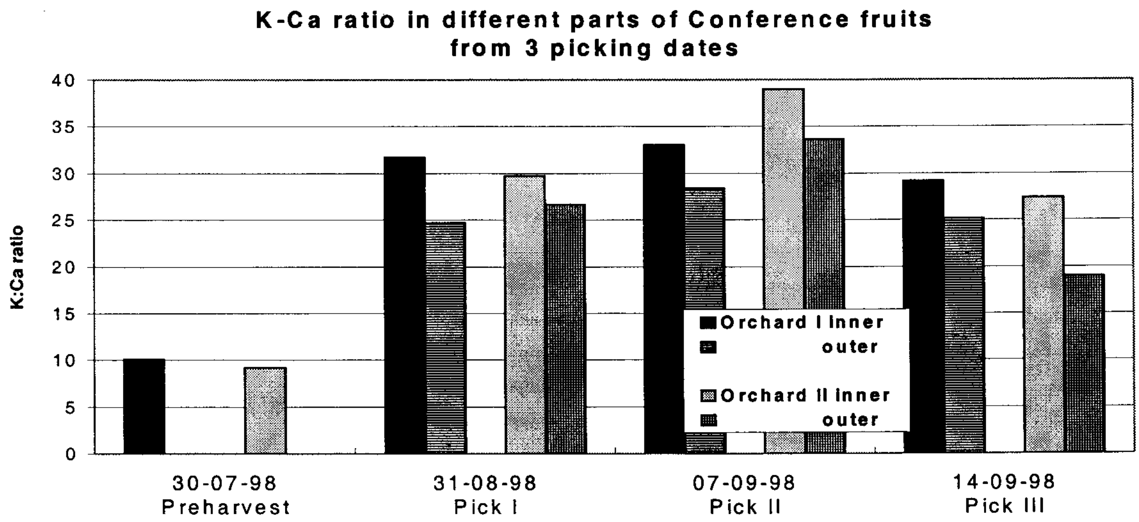
III. Ernte **DH-Ascorbate**

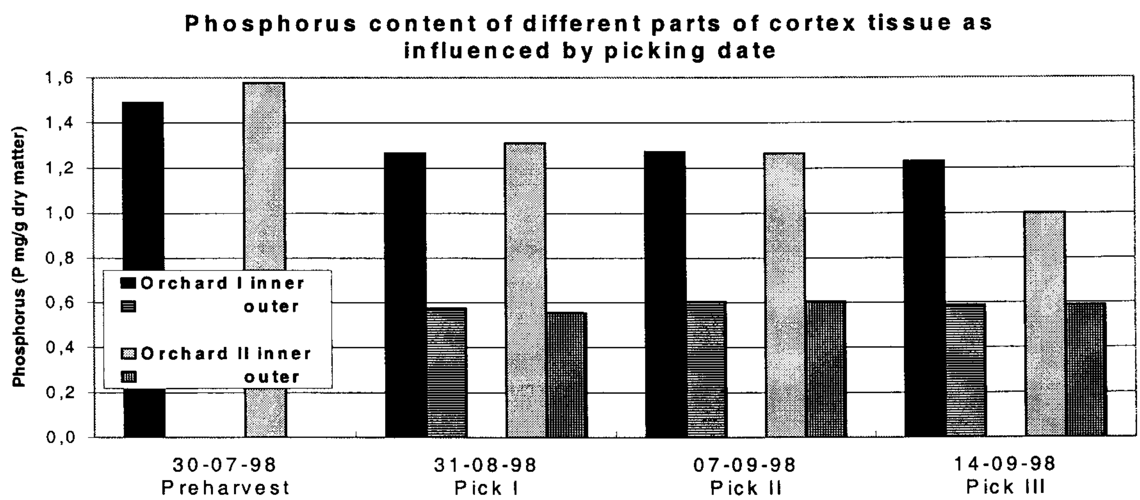
	beginn	after 1 month	after 2 months	after 3 months
0,7+2 inner	4,80	2,82	1,46	0,87
0,7+2 outer	5,30	3,06	1,33	1,77
5+2 inner	4,80	3,14	0,70	0,67
5+2 outer	5,30	2,73	1,11	0,42

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.

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. The cortex tissue of the pears was also separated for the mineral analysis in inner and outer parts. For K, Ca and K:Ca-ratio no clear correlation is found between in respect to incidence of browning disorders.



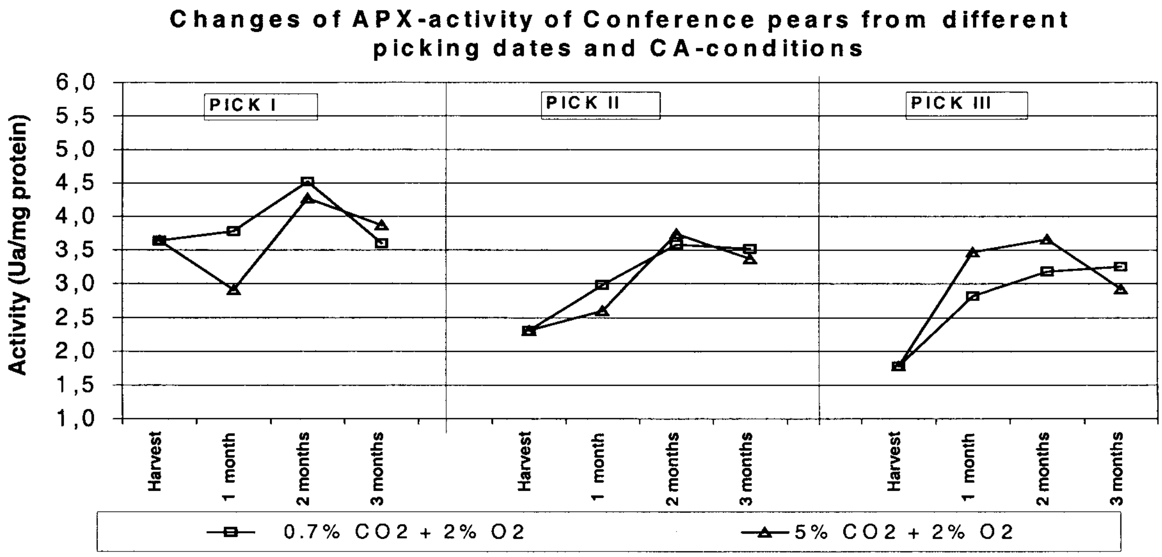


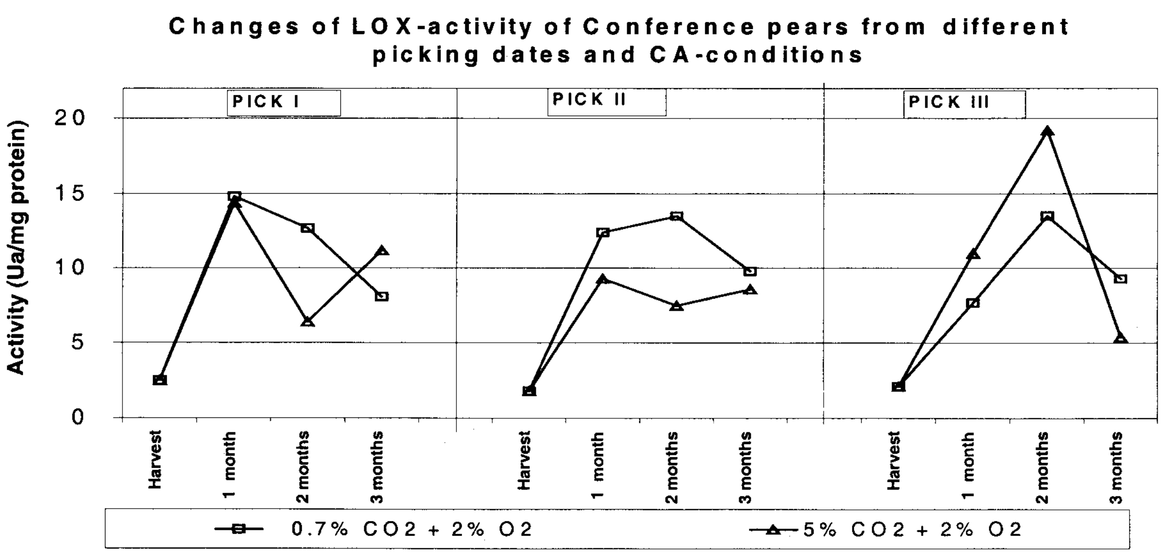
But it seems to exist a correlation between the occurence of disorders and the phosphorus content of pears from the last harvest date.

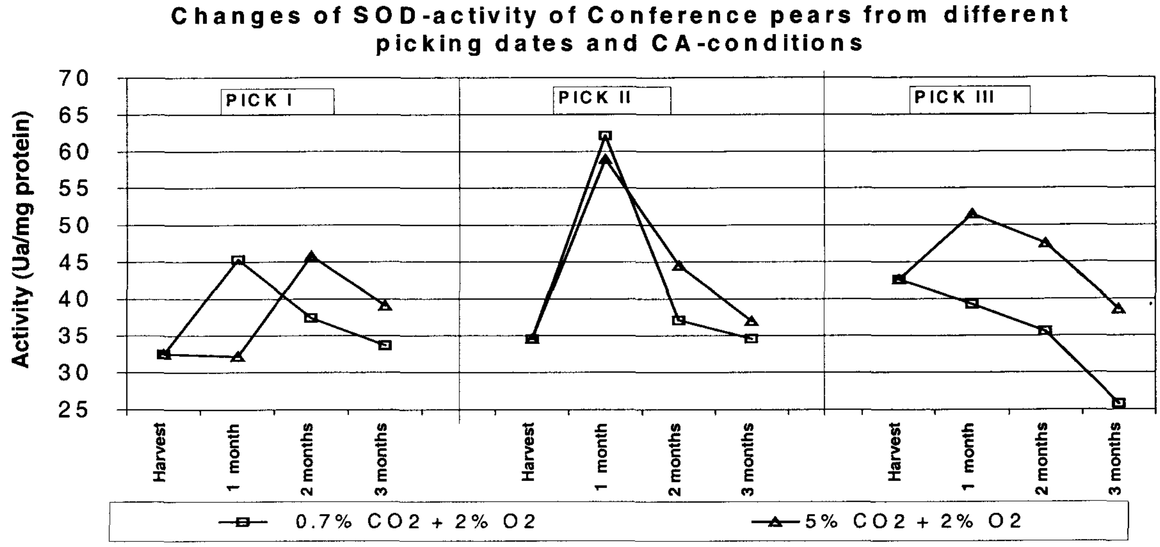
Enzyme analysis:

In the ongoing investigation periode we focused our biochemical work in determination of enzymes which are involved in the maintenance or destruction of membrane integrity. A Ph.D. studend of our group has visited IRTA in Lleida (partner 2) to learn more about the methods for enzymatic analysis.

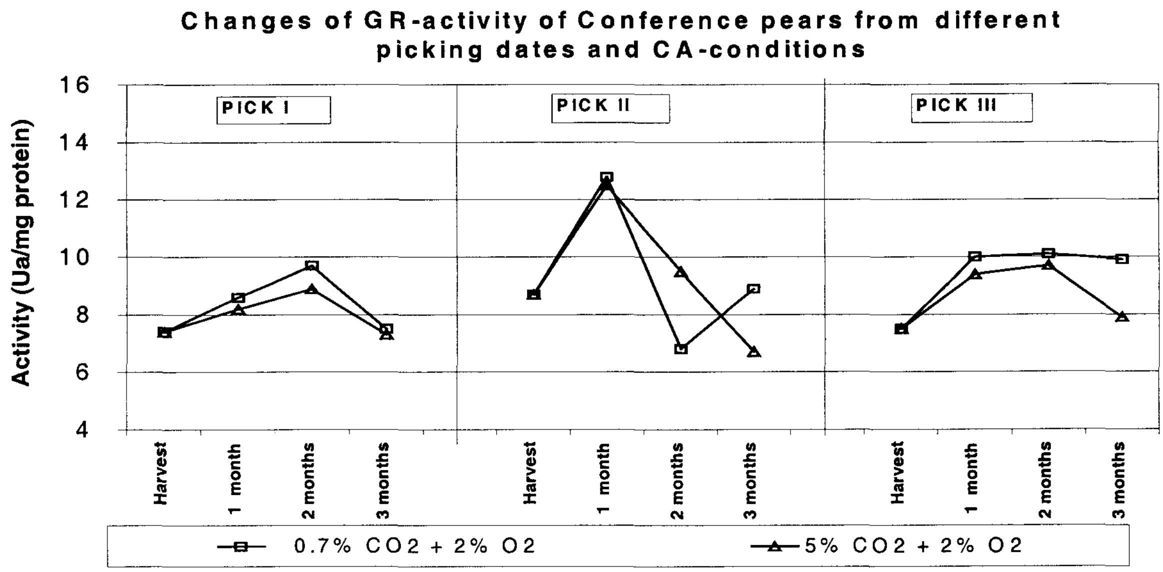
We started now with determination of ascorbate peroxidase (APX), lipoxygenase (LOX), superoxide dismutase (SOD), catalase (CAT) and glutathion reductase (GR). Methods for determination of monodehydro-ascorbat reductase and dehydro-ascorbate reductase as well as the antioxidative potential are still in development.



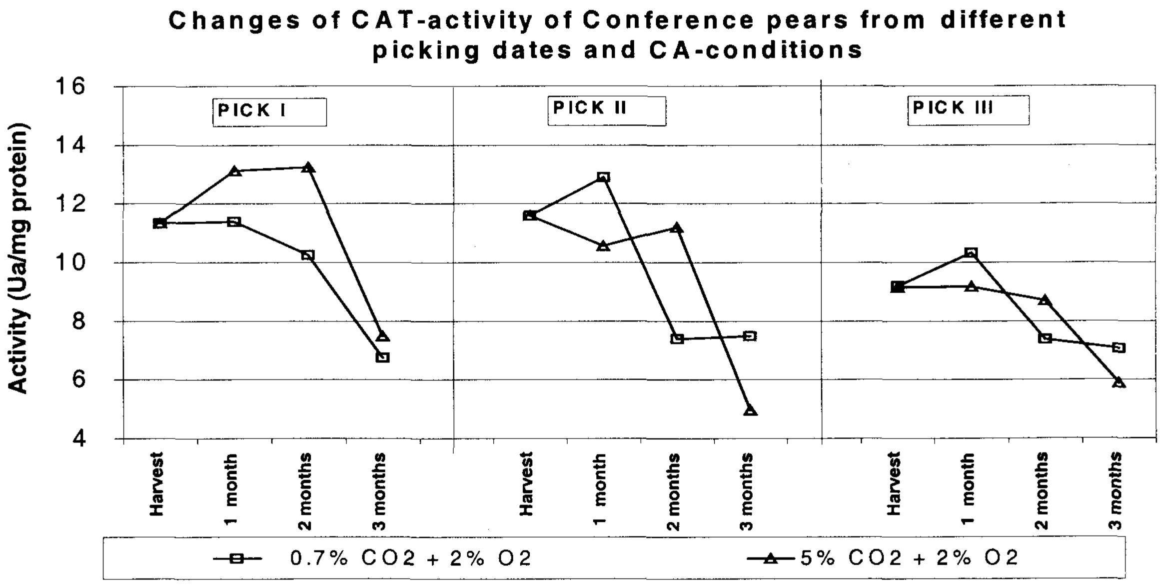




Changes of SOD-activity of Conference pears from different picking dates and CA-conditions
Activity (Ua/mg protein)
70
65
60
55
50
45
40
35
30
25
PICK I
PICK II
PICK III
Harvest
1 month
2 months
3 months
0.7% CO2 + 2% O2
5% CO2 + 2% O2



Changes of GR-activity of Conference pears from different picking dates and CA-conditions
Activity (Ua/mg protein)
16
14
12
10
8
6
4
PICK I
PICK II
PICK III
Harvest
1 month
2 months
3 months
0.7% CO2 + 2% O2
5% CO2 + 2% O2



Changes of CAT-activity of Conference pears from different picking dates and CA-conditions
Activity (Ua/mg protein)
16
14
12
10
8
6
4
PICK I
PICK II
PICK III
Harvest
1 month
2 months
3 months
0.7% CO2 + 2% O2
5% CO2 + 2% O2

Energy metabolism

Moreover, we started with investigations about the energy metabolism of oxidative and fermentative pathways. This work will be done in Conference pears and Jonagold apples stored under conventional and extreme CA-conditions.

Untill now the protocols for determination of different nucleotides were tested and first samples of fruits from the ongoing storage experiments were analysed.

Planned research activities for the next 6 months

In the next time periode the storage experiments will be continued and the last sampling date after 6 months of storage with the different tasks must be carried out in March.

Special interest will be put in task 7 (Biochemical analysis) on the investigations of enzymes (analyse of collected fruit samples of other experiments) and energy metabolism in relationship with fermentative metabolism (ethanol, ethylacetate, acetaldehyde, ADH,PDC)

Diffusion resistance measurement of pears with different methods will be continued with more fruits.

Samples for mineral analysis will be taken at the end of the storage periode in March in order to confirm the relation between low phosphorus content and higher occurence of disorders of fruits from the latest picking date.

Publications planed

Streif, J. Storage of apple and pears with special respect to storage disorders in Braeburn and Conference fruits. Oral presentation. Neustadt, 8.01.99 and Bitzfeld-Öhringen, 8.03.99

Rabus, C.: Gas exchange and diffusion measurement in fruits, Oral presentation planed for a seminar at Fruit Science Instiute in SS 1999, Hohenheim University.

Xuan H.: Enzymatic defence mechanisms against oxidative stress in fruits, Oral presentation planed for a seminar at Fruit Science Instiute in SS 1999, Hohenheim University.

Oral presentation and publications on browning disorders in pears as influenced by pre and postharvest conditions are planed for the OHD-meeting in St.Remy-de-Provence at 17/18 June 1999 and also for the meeting of the German Horticultural Society (DGG).