



## **Midterm Status Report 2002 and Application for Continuation in 2003**

For research projects financed by grants from  
The Directorate for Food, Fisheries and Agro Business  
under the Danish Ministry of Food, Agriculture and Fisheries

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### **1. Research program**

Research in organic farming 2000-2005 (DARCOF II)

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### **2. Project title and number**

1.14 Control of scab in organic apple growing

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### **3. Head of project**

Ass. Prof., John Hockenhull, Ph.D, (JH)  
Plant Pathology Section  
Department of Plant Biology  
The Royal Veterinary and Agricultural University  
Thorvaldsensvej 40  
1871 Frederiksberg C

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### **4. Participating institutes**

Plant Pathology Section  
Department of Plant Biology  
The Royal Veterinary and Agricultural University (KVL)  
Thorvaldsensvej 40  
1871 Frederiksberg C

Department of Horticulture  
The Danish Institute of Agricultural Sciences (DJF)  
Research Centre Aarslev  
Kirstinebjergvej 10  
5792 Aarslev

Department of Crop Protection  
The Danish Institute of Agricultural Sciences (DJF)  
Research centre Flakkebjerg  
4200 Slagelse

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## 5. Other project staff

KVL: Marianne V. Bengtsson (MVB), David B. Collinge (DBC), Hans J.L. Jørgensen (HJLJ)  
DJF: Marianne Bertelsen (MB), Hanne Lindhard Pedersen (HLP), Klaus Paaske (KP)

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## 6. Project period (month, year)

Start of project:	04.2002
End of project:	10.2004

## 7. Midterm description of the project, its results and progress, and application for continuation in 2003

### A. Project summary

Apple scab, caused by *Venturia inaequalis*, causes serious losses in quality and yield of organically grown apples in Denmark and elsewhere. Materials available to organic growers in Denmark for the control of scab are largely ineffective and scab is also a growing problem for conventional growers. No eradicated or curative fungicides are available for use by organic growers, and while protective, copper-based fungicides are permitted in most European countries, only sulphur is allowed in Denmark and sulphur is not very effective against scab. With respect for the principles of organic growing, the overriding aim of this proposal is to identify and begin to develop some new approaches and materials for the control of apple scab.

Following artificial inoculation of detached leaves and potted apple trees grown in the greenhouse, a range of potential control materials are to be collected and screened for efficacy against the scab pathogen. Candidate scab control materials include plant extracts, essential oils, compost tea, biocontrol agents, resistance inducers etc. Reference materials will include sulphur and Cu-fungicides. Where control of scab is found in the greenhouse screen, the materials are to be further investigated histopathologically to establish the mechanism of control whether it appears to be direct (fungicidal or fungistatic), biological (antibiosis, competition and /or hyperparasitism) or whether it appears to involve an activation of host plant resistance mechanisms (induced resistance). All materials showing activity against the scab pathogen are to be tested in organically managed orchards, firstly on single trees and next, for the most promising materials, on blocks of trees. Application will be made before apple scab infection periods occur, predicted by the PC-warning program RIMpro. More detailed studies will be made to characterise the effect of selected treatments on the secondary metabolites in apples, potentially possessing human disease-preventive properties.

**Table A.1: Work package list (from application)**

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s):
1	Screening of potential material for scab control	<u>JH</u> , MVB	1 330. 1	1	30	D1.1, D1.2, D1.3, D1.4, D1.5, D1.6, D1.7, D1.8, D1.9, D1.10
2	Histopathological investigations of host-pathogen interactions	<u>HJLJ</u> , MVB	336.9	5	24	D2.1, D2.2, D2.3.
3	Orchard testing of selected control compounds and secondary metabolite characterisation	<u>MB</u> , HLP, KP	446	13	30	D3.1, D3.2, D3.3, D3.4
4	□Molecular analysis of defence responses in apple	<u>DBC</u> , NN	60	17	30	D4.1, D4.2, D4.3

\* Responsible participants are underlined

□ Funding of WP4 is not covered by DARCOF II. The intention is to apply for funds elsewhere.

## B. Objectives and expected achievements

The overall aim is to identify and begin to develop new approaches and materials for the control of apple scab. The research has the following objectives:

- Following artificial inoculation of detached leaves and potted apple plants in the greenhouse, screen candidate materials for activity against the apple scab pathogen (WP1)
- Where control of scab is found in the greenhouse screen, determine by means of observations under the microscope, whether the controlling effect appears to be direct (fungicidal or fungistatic), biological (antibiosis, competition and/or hyperparasitism) or whether it appears to involve an activation of host plant resistance mechanisms (WP2). All materials showing activity will be considered for inclusion in further tests.
- Test the most promising anti-fungal materials under orchard conditions firstly on single trees and next, for the most promising materials, on blocks of trees (WP3).
- For a candidate material acting by induced resistance, characterise the gene expression induced by the treatment in relation to known defence responses (WP4) \*.
- Investigate whether treatment with antifungal materials results in differences in secondary metabolite profiles (phenols and volatiles) in apple material harvested from treated orchard trees (WP3).

\* Funding of WP4 is not covered by DARCOF II. The intention is to apply for funds elsewhere

## C. Midterm results and progress

### C.1 Description (summary) of main results and conclusions

WP1 (Screening of materials) is progressing. Systems for production of inoculum of *V. inaequalis* have been established and screening systems in the growth chamber and green house are now functioning. A collection of candidate materials for screening is being made and the first screenings are about to be made. WP2 is about to begin.

### C.2 Fulfilment of deliverables and milestones

<b>WP 1: Screening of candidate materials for scab control</b>	Time schedule according to application	Deviations, if any*
Deliverables		
1.1 Collection of materials for batch 1	Month 2	Month 5
1.2 Growing of strains of <i>V. inaequalis</i> and establishment of inoculation procedures	Month 2	Month 6
1.3 First screening of batch 1 of scab control materials in growth chambers. Material supplied to WP2 for microscopy	Month 4	Month 7
1.4 Second screening of promising materials for batch 1	Month 7	Month 10
1.5 Collection of materials for batch 2	Month 10	
1.6 Supply of material to WP3 for orchard experiments (chosen mainly from batch 1)	Month 13	
1.7 First screening of batch 2 of scab control materials in greenhouse	Month 16	
1.8 Second screening of promising materials from batch 2	Month 19	
1.9 In co-operation with WP2, supply of leaf material to WP4 (molecular study of induced resistance)	Month 17-28	

1.10 Supply of material to WP3 for orchard experiments (chosen from batch 1 and 2)	Month 24-28	
Milestones		
1.1 Materials for batch 1 collected	Month 2	Month 5
1.2 Growing and inoculation procedures established	Month 2	Month 6
1.3 First screening of batch 1 completed. Material for microscopy supplied to WP2	Month 4	Month 7
1.4 Second screening of batch 1 completed	Month 7	Month 10
1.5 Materials for the second batch of screening collected	Month 10	
1.6 Supply of materials to WP3 for orchard testing completed	Month 16	
1.7 First screening of second batch completed	Month 16	
1.8 Second screening of batch 2 completed. Material supplied to WP2 for microscopy	Month 19	
1.9 Supply of materials to WP3 for orchard trials completed	Month 28	
1.10 Materials for WP3 for orchard trials supplied	Month 28	
1.11 Supply of leaf material to WP4 completed	Month 28	

\* *Deviations are to be further discussed in D*

<b>WP 2: Histopathological investigations of host-pathogen interactions</b>	Time schedule according to application	Deviations, if any*
Deliverables		
2.1 A protocol or a set of criteria to be used to test whether induced resistance is involved in protection offered by a control agent	Month 6	Month 8
2.2 Results of test of selected batch 1 material	Month 11	
2.3 Results of test of selected batch 2 material	Month 24	
Milestones		
2.1 A protocol for examining whether induced resistance is involved in the protection against disease is established	Month 6	Month 8
2.2 Test of control material in batch 1 for ability to induce resistance completed	Month 11	
2.3 Test of material in batch 2 for ability to induce resistance is completed	Month 24	

• *Deviations are to be further discussed in D*

<b>WP 3: Orchard testing of selected control compounds and secondary metabolite characterisation</b>	Time schedule according to application	Deviations, if any*
Deliverables		
3.1 Results of field screening of selected compounds 2003	Month 19	
3.2 Results of field testing of selected compounds in combination with tree growth in 2004	Month 29	
3.3 Analysis of volatiles and phenolic compounds of apples	Month 29-30	
3.4 An international publication in 2004/2005 (together with WP1,2 & 4)	Month 30	
Milestones		
3.1 Selections of promising compounds in 2003	Month 22	
3.2 Testing of promising compounds in 2004	Month 25	
3.3 The analyses of secondary metabolites are completed	Month 30	
3.4 National publication 2004	Month 30	

3.5 International publication	Month 30	
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- *Deviations are to be further discussed in D*

WP 4: Molecular analysis of defence responses in apple	Time schedule according to application	Deviations, if any*
Deliverables		
4.1 Macroarray of apple genes	Month 27	
4.2 Macroarray data for induced resistance in apple	Month 27	
4.3 One scientific paper in collaboration with other participants and potentially Japanese and Korean collaborators	Month 30	
Milestones		
4.1 The analyses are completed	Month 30	
4.2 The analyses are completed	Month 30	
4.3 Publication completed	Month 30	

\* *Deviations are to be further discussed in D*

□ WP4: Funding of WP4 is not covered by DARCOF II. The intention is to apply for funds elsewhere.

## D. Description of deviations and subsequent adjustments of plans

Initiation of the project was delayed because of the uncertainty earlier in the year about funding from DARCOF II. WP1 (screening of materials for scab control), including the purchase of plant material, inoculum production of *V. inaequalis*, collection of candidate materials for screening etc., and delivery of material to WP2 for microscopy therefore were delayed. We have therefore applied for the technician salary to be moved from year 1 to year 2 so that the screening backlog in WP1 & WP2 can be overcome in the early months of 2003. By so doing we will be able to deliver the candidate control materials to WP3 on time.

## E. Project publications and other products

### 1. Articles in international, scientific journals with review procedures

#### 2. Papers presented at congresses, symposiums, etc.

Bengtsson, M. & Hockenhull, J. 2002. Control of apple scab (*Venturia inaequalis*) in organic growing. StopScab: A Danish research programme for screening substitutes to copper fungicides. Presented as paper, poster and oral presentation at "The 6<sup>th</sup> IOBC/WPRS Workshop on Pome Fruit Diseases", 31.8-5.9.2002, Lindau, Germany.

#### 3. Reports, articles in agricultural journals, etc.

Bengtsson, M. & Hockenhull, J. 2002. Kampen mod frugttrækraft. *Frugt & Grønt* 4/2002: 158-159.

#### 4. Oral presentations, public meetings, field days, etc.

Hockenhull, J. & Bengtsson, M. "Temamøde for Frugt og bæravlerer", 30<sup>th</sup> January 2002, DEG Odense

Bengtsson, M. & Hockenhull, J. Orchard open day arrangement for apple growers, Vente-

godtgård, Li Skensved, 28<sup>th</sup> June 2002.

Bengtsson, M. Oral presentation of “Control of apple scab (*Venturia inaequalis*) in organic growing. StopScab: A Danish research programme for screening substitutes to copper fungicides”, presented at ”The 6<sup>th</sup> IOBC/WPRS Workshop on Pome Fruit Diseases”, 31.8-5.9.2002, Lindau, Germany.

## F. Scientific education

Post doc. Marianne Bengtsson (MVB) is to make a study visit to Institute of Plant Sciences Pathology, Swiss Federal Institute of Technology, ETH Zürich, Switzerland, October 2002, to get experience in microscopical techniques for studying the infection biology of *V. inaequalis* (will be used in WP2: Histopathological investigations of host-pathogen interactions).

## G. National and international cooperation

### National:

Internally the research consortium involved in this project is a strong team as it links researchers with different specialisations, coming from different departments and groups at two different institutions (KVL and DJF). Thus, at DJF, three groups are involved, The Research Group for Food Quality and Natural Products Chemistry, The Pomology Research Group and The Research Group for Effects of Pesticides. Participants from KVL belong to different research groups, each with its focus on plant-pathogen interactions: Biological Control Group, Induced Resistance and Histopathology Group and Molecular Plant Pathology Group.

Our research activities is linked to the DARCOF project no. I.2. Development of sustainable production for apples.

We have long-standing and excellent relations with the fruit consultant Svend Ramborg and organic fruit consultant Maren Korsgaard.

We have initiated and formalised co-operation with the Danish company Nor-Natur ApS, Kannaholmen 2, DK-2650 Hvidovre, regarding supply of plant extracts the first batch of which was obtained August 2002.

### International:

M. Bengtsson (MVB) and J. Hockenhull (JH) have attended the 6<sup>th</sup> Workshop of Pome Fruit Diseases, 31. August – 5. September 2002, Lindau, Germany. Contacts were strengthened with key researchers working with apple scab including:

- Odile Carisse, Agriculture and Agri-Food Canada, Québec, Canada.
- Cesare Gessler & Eve Silberberg-Dilworth, Institute of Plant Sciences Pathology, Swiss Federal Institute of Technology, ETH Zürich, Switzerland.
- William MacHardy, University of New Hampshire, USA.
- Luciana Parisi, INRA, Beaucouzé, France.
- Arne Stensvand, Norwegian Crop Research Institute, Aas, Norway.
- Peter Triloff, Marktgemeinschaft Bodenseeobst, Friedrichshafen, Germany.

JH is invited to attend the 10<sup>th</sup> Meeting on Apple Scab, January 2003, Italy, for researchers and consultant working with apple scab in Europe and North America.

JH and MVB will attend the 11<sup>th</sup> International Conference on Cultivation Technique and Phyto-

pathological Problems in Organic Fruit-Growing and Viticulture, February 2003, Weinsberg, Germany.

## H. Critical reflection on the project

So far we have not found the need to radically change any of the approaches described in the original proposal. However, thanks to advice from international contacts some of the methods are being adapted and improved. Systems for production of inoculum of *Venturia inaequalis* have been established and screening systems in the growth chamber and green house are now functioning. A collection of candidate materials for screening is being made and the first screenings are about to be made. Good contacts have been established with major apple scab researchers in Europe and North America (see mention the recent IOBC meeting in Lindau under G) and with respect to organic apple production, JH attended the Organic Fruit and Viticulture conference in Weinsberg in 2001 and he and MVB plan to attend the next meeting in 2003.

Despite the uncertainty earlier in the year about funding from DARCOF II and the subsequent delayed starting date, reasonably good progress has been made with WP1 (Screening of candidate materials for scab control). However, particularly because of the delay in starting the project, WP1 is behind schedule with regards to delivery to WP2 (histopathological screening) of promising control materials. We have applied for technician salary to be moved from year 1 to year 2 so that the screening backlog in WP1 & WP2 can be overcome in the early months of 2003. By so doing we will be able to deliver the candidate control materials to WP3 on time.

## 8. Budget

### A. Account for any change in budgets

Concerning the work at the Plant Pathology Section, KVL, we apply for the technician salary to be moved from year 1 (according to application) to year 2 so that the screening backlog in WP1 & WP2 can be overcome in the early months of 2003. By so doing we will be able to deliver the candidate control materials to WP3 on time.

### B. Budget for the whole project (1.000 DKK)

Total consumption of funds from DARCOF and expected consumption this year and coming years

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	-	9	22	20		51
Scientific personnel	-	9	13	13		35
Technical personnel	-	-	9	7		16

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	-	286	662,4	663	-	1.611,4
Scientific personnel	-	286	438	475	-	1.199
Technical personnel	-	-	224,4	188	-	412,4
Other operational costs	-	33	80	84	-	197
Equipment	-	0	0	0	-	0
Others (please specify)	-	0	0	0	-	0
Direct costs	-	319	742,4	747	-	1.808,4
Indirect costs (20% of direct costs)	-	63,8	148,5	149,4	-	361,7
Total	-	382,8	890,9	896,4	-	2.170,1

**Comments:**

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## 9. Signatures and stamps

Name	Institute	Date	Signature
Head of project John Hockenull			

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## Appendix I. Detailed budget

### A. Budget for each participating institute (1.000 DKr)

Name of Institute and department:

**Plant Pathology Section**

**Department of Plant Biology**

**KVL**

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	-	9	20	12	-	41
Scientific personnel	-	9	12	9	-	30
Technical personnel	-	-	8	3	-	11

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	-	286	599	394	0	1.279
Scientific personnel	-	286	400	314	0	1.000
Technical personnel	-	-	199	80	0	279
Other operational costs	-	33	70	54	0	157
Equipment	-	0	0	0	0	0
Others (please specify)	-	0	0	0	0	0
Direct costs	-	319	669	448	0	1436
Indirect costs (20% of direct costs)	-	63,8	133,8	89,6	0	287,2
Total	-	382,8	802,8	537,6	0	1.723,2

#### Comments:

Scientific personnel: Months based on 32 hours/week

Technical personnel salary is moved from year 1 to year 2, according to application.

## B. Budget for each participating department (1.000 DKK)

Name of Institute:

**Department of Horticulture / Department of Crop Protection  
Research Centre Årslev / Research Centre Flakkebjerg  
DJF**

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	-	-	2	8	-	10
Scientific personnel	-	-	1	4	-	5
Technical personnel	-	-	1	4	-	5

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries	-	-	63,4	269	-	332,4
Scientific personnel	-	-	38	161	-	199
Technical personnel	-	-	25,4	108	-	133,4
Other operational costs	-	-	10	30	-	40
Equipment	-	-	-	-	-	-
Others (please specify)	-	-	-	-	-	-
Direct costs	-	-	73,4	299	-	372,4
Indirect costs (20% of direct costs)	-	-	14,7	59,8	-	74,5
Total	-	-	88,1	358,8	-	446,9

**Comments:**

**C. Budget for co-financing from each participating institute (1.000 DKK)**

Name of Institute:

**Department of Plant Biology****KVL**

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months	-	1	3	3	-	7
Scientific personnel	-	1	2	2	-	5
Technical personnel	-	-	1	1	-	2

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel						
Technical personnel						
Other operational costs						
Equipment						
Others (please specify)						
Direct costs						
Indirect costs (20% of direct costs)						
Total						

**Comments:**

The Department of Plant Biology, KVL, will cover one month's salary for one month for a scientist in 2002, 2003 and 2004 for WP2 and one month's salary for one technician and one scientist in 2003 and 2004 for WP4.