

# **Detection of PVY strains in Potato Tubers at Different Developmental Phases by Melting Analysis of an Oligonucleotide Virus Probe.**

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In the Middle East there are two main cropping seasons: the spring season (**imported seeds**) and the following autumn-winter season (**locally grown seeds**)

### Spring season

**Planting (imported seeds)**

**Seed Harvest**



January February

March

April

May

June

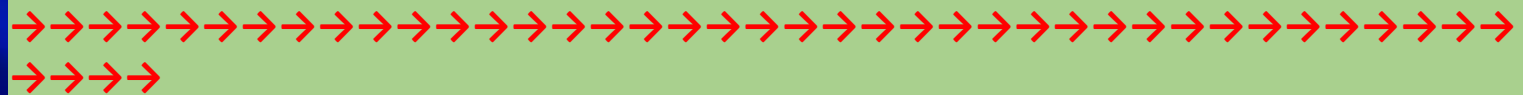
July

August

### Winter season

**Planting local seeds**

**Ware harvest**



September

October

November December

January

February

March



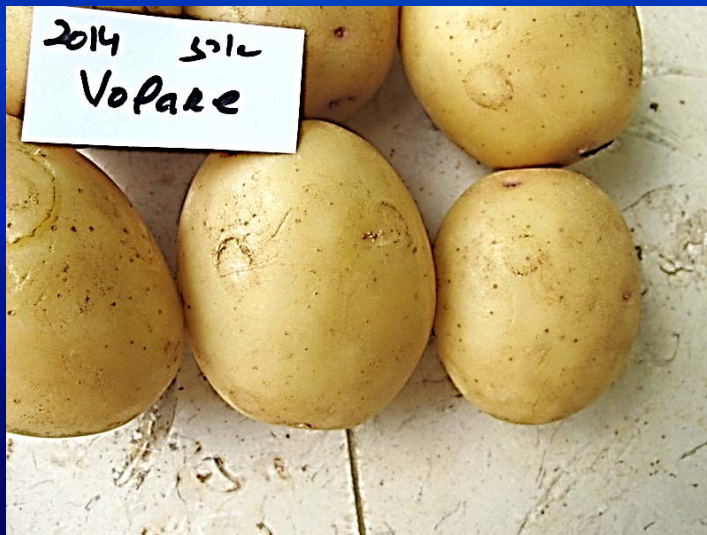
**Winter crop in the south-west Israeli Negev  
where 80% of potatoes are produced**

**Seed tubers for the autumn-winter seasons are produced locally in the preceding spring season.**

**The subtropical climate promotes high incidences of aphid populations with the consequent hazards of PVY infection.**



# The presence of NTN can cause sever damage to the growers.



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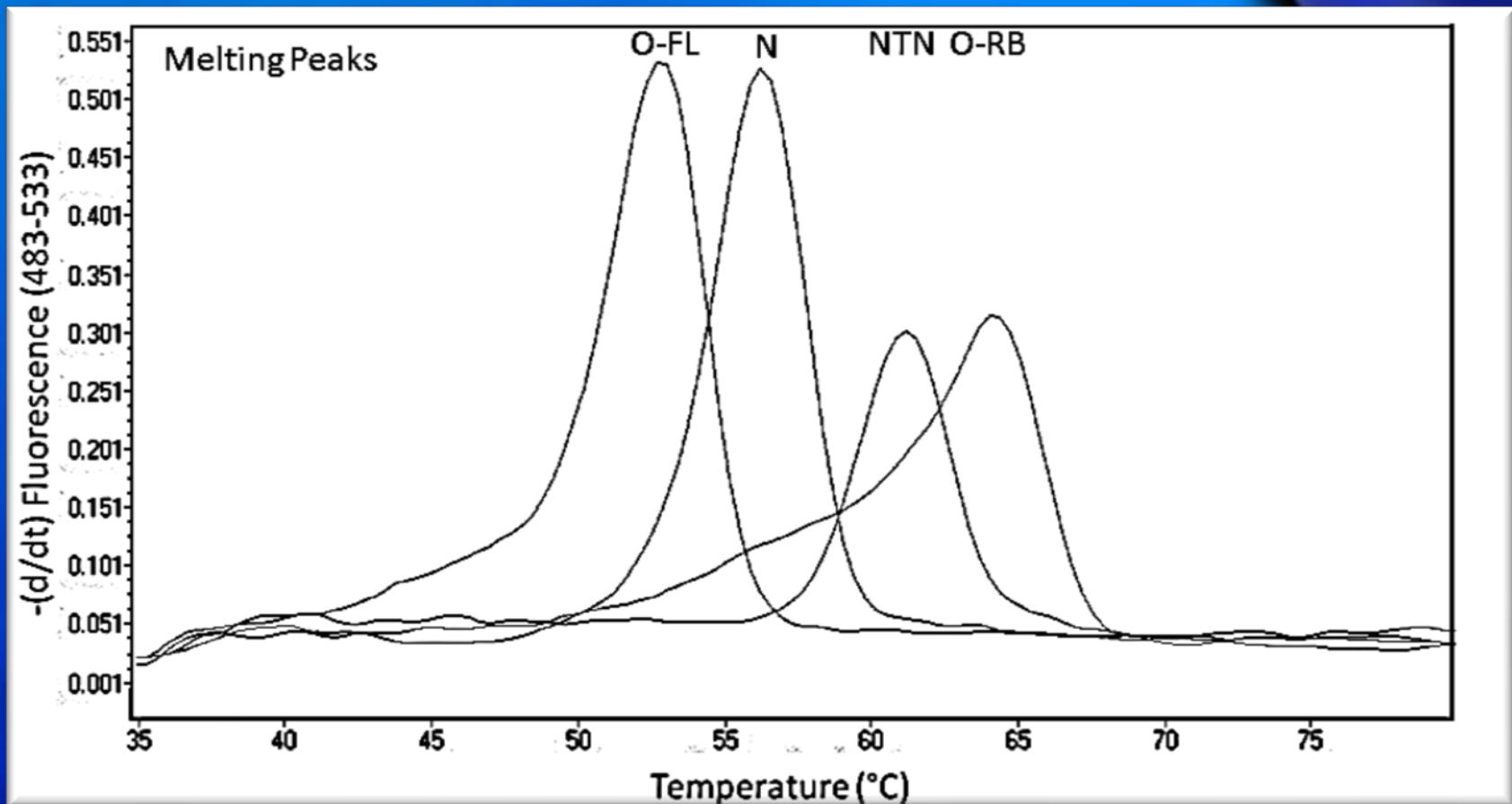
SHORT COMMUNICATION

# Detection and Differentiation of Potato Virus Y Strains by Melting Analysis of an Oligonucleotide Virus Probe

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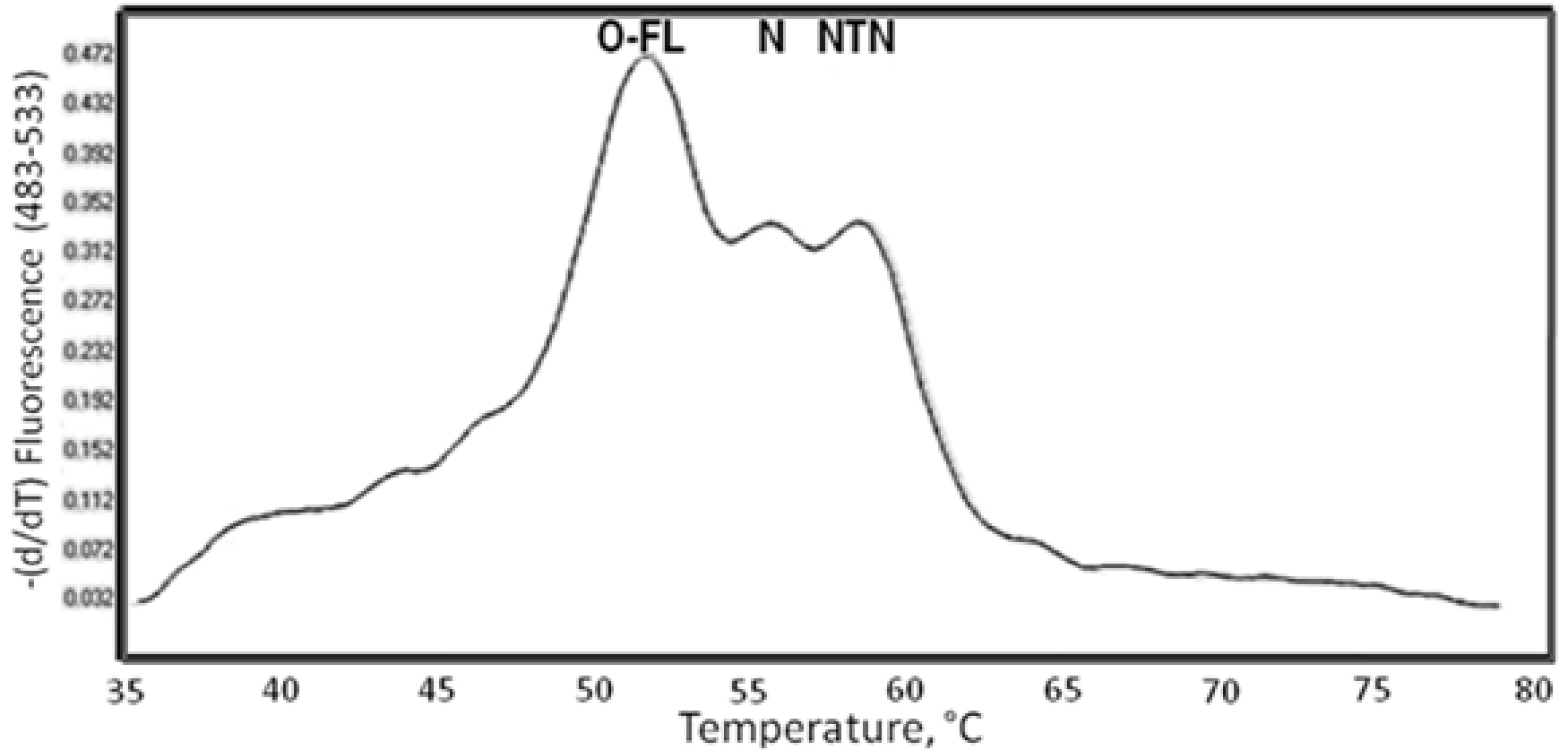
Physiological phase	Tuber source	Variety	No. of tubers	PVY positive
● Pre-dormant	Spring 2016-prior to foliage desiccation	VR808	34	10 ●
● Pre-dormant	Winter-prior to foliage desiccation	VR808	28	27
● Pre-dormant	Spring 2017-prior to foliage desiccation	VR808	10	10 ●
Dormant	Winter harvest	VR808	36	18
Dormant	Spring harvest	Caruso	12	10 ●
Post dormant-not sprouting	Spring harvest	VR808	24	17 ●
Post dormant-not sprouting	Imported seed tubers	VR808	12	1
Initial sprouting	Spring harvest	Gabriel	24	4 ●
Initial sprouting	Spring harvest	Joshua	25	6 ●
Sprouts (5mm)	Imported seed tubers	VR808	12	1
Sprouts (10 mm)	Imported seed tubers	VR808	12	4

- **PVY was detected in tubers at various developmental phases**
- **Locally grown tubers (spring season) are prone to high PVY infection**

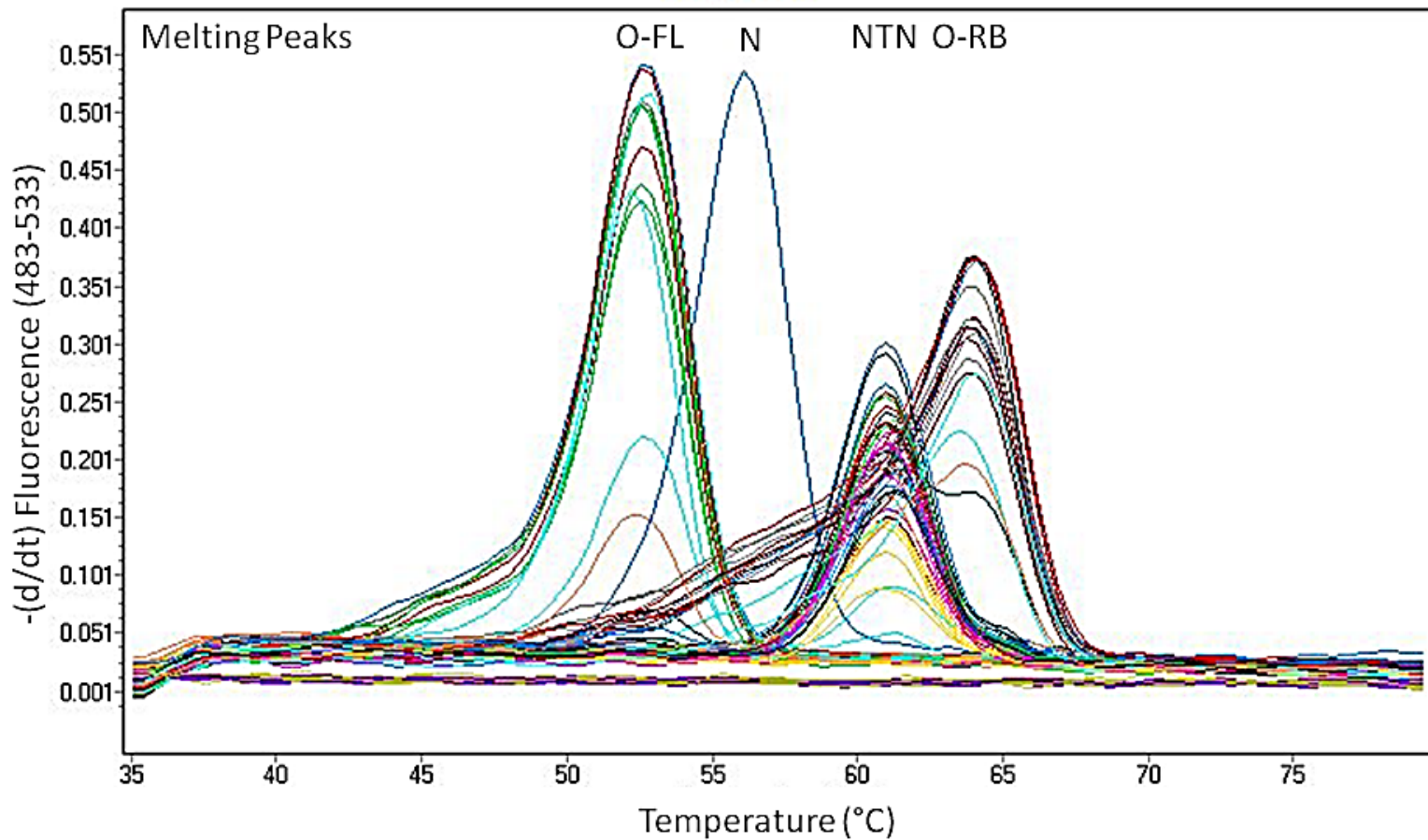


**Melting curves of four classified reference PVY strains: O-FL, O-RB, N and NTN, analyzed with the RT-PCR-Light-Cycler 480.**





**Naturally occurring mixture of the Potato Virus Y (PVY) strains O-FL, N and NTN identified in a field grown tuber. Tuber extracts were analyzed by the RT-PCR Light-Cycler 480.**



**Melting curves of individual extracts from 55 tubers randomly picked from commercial fields and of four reference strains. Tuber-extracts analyzed using the RT-PCR-Light-Cycler 480.**

## **The “pre-dormant’ tubers experiment – spring 2017**

**Prior to the chemical top kill in the spring, 10 plants of the cv. VR808 were sampled in the field.**

**From each plant, senescent leaves and pre-dormant tubers of 40-50 mm in diameter were sampled and assayed instantly (‘rose end’ and ‘heel end’).**

**The assayed tubers and their intact sister tubers were stored in 4 °C for 5 months followed by a month at room temperature.**

**A grow-out test was conducted.**



16



17



18



19



100



15



14



9.5.17 perse 1/8

13

VR 808 (SE)



12



11

Senecsent  
leaves

Pre-dormant tubers

Post storage assayed  
tubers

Post storage Sister tubers

Plant

Tuber

Young leaves

Young leaves

Rose end

Heel end

DAP 19

DAP 40

DAP 19

Dap 40

1	O-FL+NTN	O-FL+N+NTN	O-FL+N+NTN	None	O-FL+NTN	None	N
2	None	O-FL+N+NTN	O-FL	None	O-FL	O-FL	O-FL
3	None	O-FL+NTN	O-FL+NTN	O-FL	O-FL	None	NTN
4	None	O-FL+NTN	O-FL+NTN			O-FL	O-FL
5	O-FL+NTN	O-FL+NTN	N	O-FL	None	None	None
6	None	O-FL+N	O-FL+NTN	O-FL+NTN	O-FL+NTN	O-FL+NTN	O-FL+NTN
7	None	O-FL+NTN	O-FL+NTN			None	O-FL
8	O-FL+NTN	O-FL	O-FL			O-RB	O-RB
9	None	O-FL+NTN	N			O-FL+O-RB	O-FL+O-RB
10	None	O-FL+NTN	O-FL+N+NTN			N	NTN

- PVY detection in senescent leaves did not correlate with virus incidence in the tubers of the same plants.
- 75% of the samples in the tubers contained a mixture of strains.
- Occurrence of strain variability in the same tuber is evident.

### Senescent leaves

### Pre-dormant Tuber

#### Rose end

#### Heel end

O-FL+NTN

O-FL+N+NTN

O-FL+N+NTN

None

O-FL+N+NTN

O-FL

None

O-FL+NTN

O-FL+NTN

None

O-FL+NTN

O-FL+NTN

O-FL+NTN

O-FL+NTN

N

None

O-FL+N

O-FL+NTN

None

O-FL+NTN

O-FL+NTN

O-FL+NTN

O-FL

O-FL

None

O-FL+NTN

N

None

O-FL+NTN

O-FL+N+NTN

In the grow-out tests, virus detection tends to be improved in the later test (DAP 40)

Post storage Sister tubers	
Young leaves	
DAP 19	Dap 40
None	<b>N</b>
<b>O-FL</b>	<b>O-FL</b>
None	<b>NTN</b>
<b>O-FL</b>	<b>O-FL</b>
None	None
<b>O-FL+NTN</b>	<b>O-FL+NTN</b>
None	<b>O-FL</b>
<b>O-RB</b>	<b>O-RB</b>
<b>O-FL+O-RB</b>	<b>O-FL+O-RB</b>
<b>N</b>	<b>NTN</b>

**Barker et al. (1993) failed to detect the virus in PVY infected tubers after 20 weeks in storage at 10° C and suggested that a slowdown in virus replication led to a reduction in viral RNA to a level below the limits of assay detection in the stored tubers.**



Pre-dormant tubers		Post storage assayed tubers		Post storage Sister tubers	
Tuber		Young leaves		Young leaves	
Rose end	Heel end	DAP 19	DAP 40	DAP 19	Dap 40
O-FL+N+NTN	O-FL+N+NTN	None	O-FL+NTN	None	N
O-FL+N+NTN	O-FL	None	O-FL	O-FL	O-FL
O-FL+NTN	O-FL+NTN	O-FL	O-FL	None	NTN
O-FL+NTN	N	O-FL	None	None	None
O-FL+N	O-FL+NTN	O-FL+NTN	O-FL+NTN	O-FL+NTN	O-FL+NTN
O-FL+NTN	N			O-FL+O-RB	O-FL+O-RB
O-FL+NTN	O-FL+N+NTN			N	NTN

**PVY strains may vary in the tuber and in the young leaves in a grow-out test**

## Naturally occurring mixtures of PVY strains:

**O-FL**, **O-RB**, **N** and **NTN** were detected in the 'rose end' and the 'heel end' of the tubers and in the young leaves grown in a grow-out assay.

**O-FL** was the dominant strain detected in **60%** of the samples followed by **NTN** in **40%** of the samples.

The dominant mixture was **O-FL+NTN** (**30%**)

## Akcnowledgments:

We are grateful to Dr. Xianzohou Nie,  
Potato Research Center, Fredericton, Canada,  
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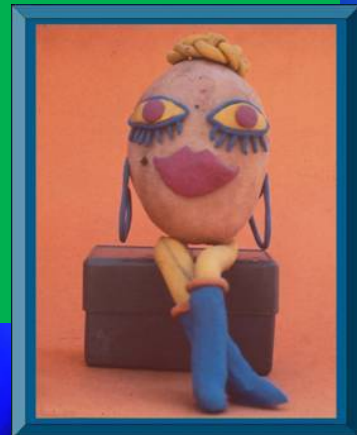


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## **Development and Improvement of Local Seed Potato Production in the Middle East.**

Participants: Morocco, Egypt, Lebanon, Jordan, Israel

Funded by: the U.S. Agency for International Development (USAID- MERC).

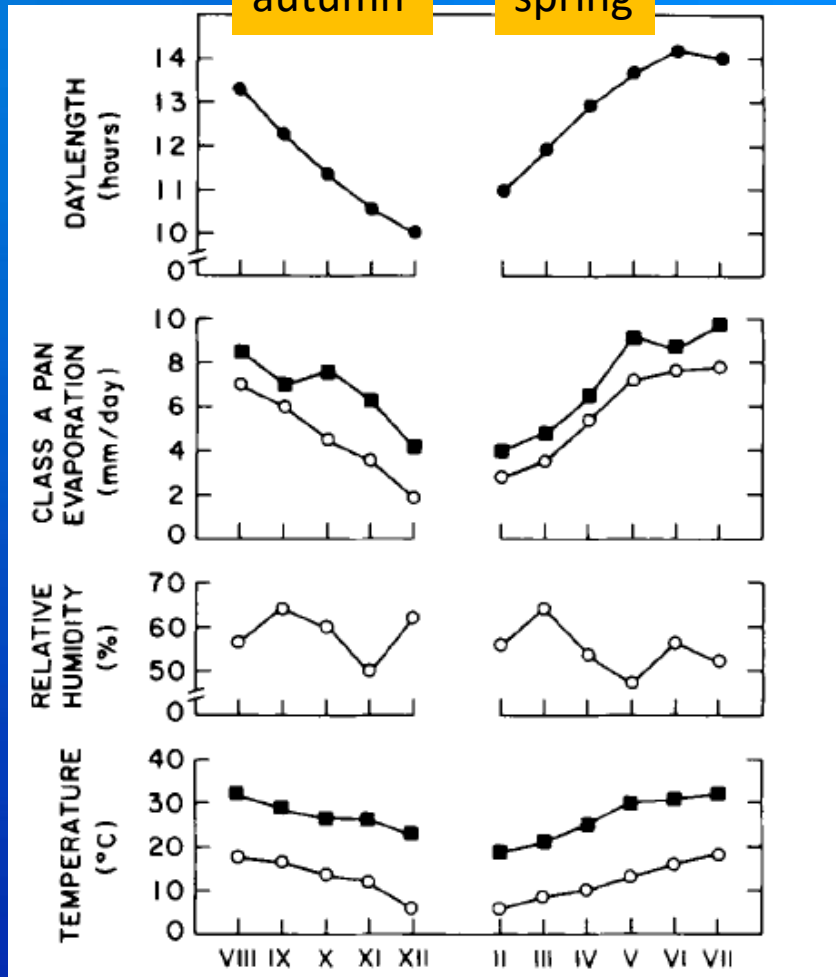


**The ability to detect PVY decreased substantially after tubers had been stored for 20 weeks at 10~ ATA-ELISA detected virus in only half the tubers from infected plants. However, PCR detected PVY very inefficiently in infected tubers after 20 weeks storage.**

**Barker et. al. 1993**

autumn

spring



Regional averages

The mean monthly maximum/minimum temperatures are: 18.3/5.6, 22.9/7.6, 26.8/10.3, 31.9/14.5 and 33.4/14.5 respectively for February, March, April, May and June (averages of 17 years' data).

The probe provider:

(RND-DYN-3001-POTATO, Genotyping  
DNA Markers DYN R&D, Caesarea, Israel)

**Oligonucleotide** – a relatively short fragments of nucleic acids with defined chemical structure (sequence).

