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Prunus necrotic ringspot virus (PNRV) in sour cherry Symptoms, incidence in orchards and influence on fruit yield

Prunus nekrotisk ringplet virus (PNRV) i surkirsebær
Symptomer, udbredelse i plantagen og indflydelse på høstudbyttet

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Summary

Prunus necrotic ringspot virus (PNRV) is the cause of extensive bud- and tree decline in a number of older sour cherry orchards. In three 'Stevnsbär' orchards the symptoms and spread of PNRV in the field were monitored during a five year period.

Symptoms were observed every year on trees attacked by PNRV, the first year starting with a chock-reaktion.

The following years the symptoms in the three orchards were distinct from the end of bloom as chlorotic, curly leaves. In July and August these symptoms might be difficult to recognize.

As the virus attack starts from a single tree and is spread by pollen to neighbouring trees, the virus attack appears widespread in a few years.

Nearly 40% of 60 young 'Stevnsbär' trees planted near older trees with PNRV were infected within three years. The first symptoms were observed five years after planting.

In the two older orchards the PNRV spread from 24 to 59% of the trees within the registered area during three years, and 29 to 68% during a five year period respectively.

The effect of the virus disease on fruit yield was estimated in a 16 year old orchard.

After PNRV infection fruit yield was reduced to between 1/3 to 1/4 of the normal yield from symptom-free trees. During the year of shock reaction the yield was usually extremely low.

Key words: *Prunus* necrotic ringspot virus, PNRV, sour cherry, *Prunus cerasus* L., symptoms, field spread, ELISA, yield.

Resumé

Prunus nekrotisk ringplet virus (PNRV) er årsag til omfattende knop- og trædød i en række ældre surkirsebærplantager. I tre udvalgte 'Stevsbær' plantager blev symptomudviklingen og udbredelsen af PNRV fulgt i en femårig periode.

Der blev hvert år observeret symptomer på træer, der var angrebet af PNRV. Første år viste træet chok-reaktion. De følgende år viste symptomerne sig i de tre plantager tydeligst efter blomstring i form af klorotiske, buklede blade. Midt på

sommeren kan bladsymptomerne være svære at erkende.

Virusangrebet begynder på et enkelt træ og spredes derefter med pollen til nabotræerne, således at angrebet efter få år optræder i store pletter. Af 60 unge 'Stevnsbær', som var plantet tæt ved ældre PNRV angrebne træer, blev knap 40 pct. af træerne inficeret i løbet af en treårig periode. De første angreb blev konstateret fem år efter plantning.

Angrebene i observationsområderne i de to ældre plantager udvikledes fra 24 til 59 pct. i løbet af tre år, henholdsvis 29 til 68 pct. i en femårig periode.

Virusangrebets indflydelse på høstudbyttet blev vurderet i en 16 år gammel plantage.

Høstudbyttet blev efter PNRV angreb reduceret til mellem 1/3 og 1/4 af udbyttet hos sunde træer. Det år, hvor træet viste chok-reaktion, var udbyttet særlig lavt.

Nøgleord: *Prunus* nekrotisk ringplet virus, PNRV, surkirsebær *Prunus cerasus* L., symptomer, udbredelse, ELISA, udbytte.

Introduction

Bud- and tree decline causes problems in a number of Danish orchards with relatively old sour cherry trees *(Prunus cerasus* L.). A sudden wilting of single trees is observed shortly after bloom in 'Stevnsbär' which is the main cultivar in Denmark. According to growers observations, the diseased trees apparently »recovered« after a couple of years and consequently these trees are not grubbed. The aim of this investigation was partly to diagnose the cause of tree decline, partly to follow symptom development on trees, and incidence in selected orchard areas; thus being able to explain the effect of the attacks on tree growth and fruit yield.

Tree decline was due to *Prunus* necrotic ringspot virus (PNRV). In previous work techniques, and the most favourable time of the year for detection of PNRV were investigated (3, 13). The disease was recognized for the first time in Denmark in 1950 on mazzard, *Prunus avium.* In sour cherry, *P. cerasus,* the virus was noticed in 1955 (26).

PNRV might cause heavy losses in the following stone fruit plantations: Apricot, peach, sour cherry and sweet cherry. In addition, the virus occurs in almonds, cherry plum, plum and *P. mahaleb,* but is also common in hop, and causes a mosaic disease of rose (8). *P.* × *cistena* is a natural host of PNRV (25), however, infected shrubs show no foilage symptoms or any other signs of disease (11, 25). Immunity to PNRV is not found in any of 122 tested *Prunus* species (11).

The distribution of PNRV is worldwide in temperate regions (8) and the virus is widespread in sour cherry all over Europe (10,14).

PNRV is transmitted by pollen to seed and to pollinated plants (4,7,8,9,16,20,21,23,26,31) and also by budding (24,31). The virus exists as different strains causing varying degrees of disease and loss (20). PNRV particles from infected pollen pass from the flower or fruit into the mother tree within two weeks from flowering (9). The virus particles are translocated in the phloem and are thereby distributed throughout the whole tree (30). In greenhouse grown rootstocks the spread was 8 cm/day (30).

Methods

Identification of PNRV

Cucumber seedlings were inoculated with sap from diseased sour cherry leaves (13). After one week virus symptoms were visible on the seedlings and further identification was made by ISEM, immunosorbent electron microscopy (3). Mass testings were made by ELISA, enzyme-linked immunosorbent assay (5).

Registration of symptoms

Areas including trees with typical symptoms of »cherry tree decline« were selected in three orchards. All the trees in these areas, diseased as well as symptom-free were monitored by visual registration two times in each growing season: shortly after bloom, that is at the end of May or beginning of June, and at harvest in August.

Fig. 1. Orchard A
Plantage A

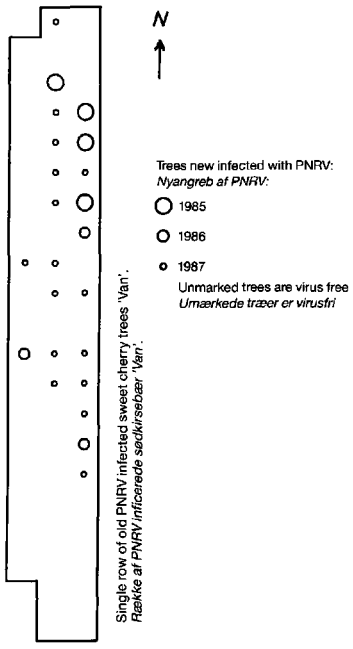
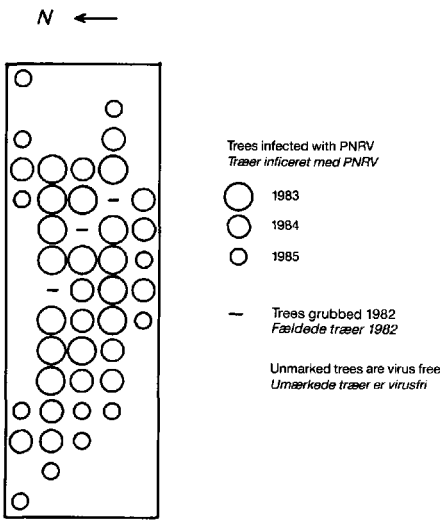


Fig. 2. Orchard B. Surveyed area
Område af plantage B



Incidence of PNRV

The surveyed trees in the three orchards were tested by ELISA (5) every year in the period 1983-1987 to index field spread of PNRV.

In the winter period the samples for serological testings consisted of buds (1), and in May very young leaves were used. The samples were taken from the tip of 10 annual shoots situated along the whole crown circumference (29). One-half gram of frozen cherry buds or leaves was homogenized in 5 ml grinding buffer PBS-Tween-PVP, pH 7.5. The ELISA plates contained controls known to produce positive as well as negative reactions. The positive controls consisted of lyophilized cucumber leaves heavily infected with PNRV. The negative controls were from healthy sour cherry trees.

All solutions used in ELISA and tests were conducted as described by *Clark* and *Adams* (5). Antiserum PVAS 22 from American Type Culture Collection was used in dilution 1:1000 or 1:2000. Enzyme reactions were measured at 405 nm by spectrophometer, Titertek Multiskan, after 30 minutes and one hour.

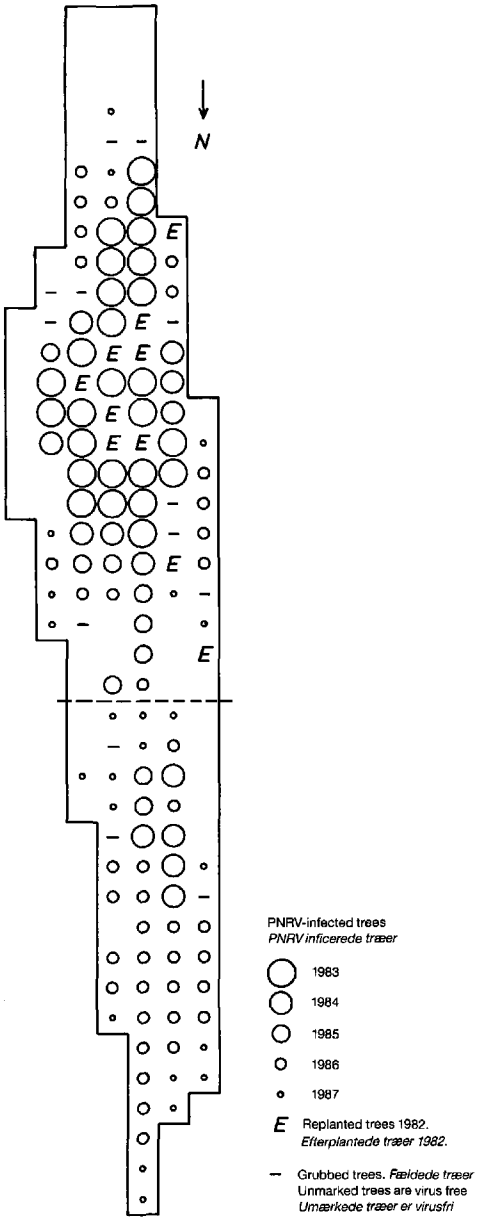
Optical density readings were considered positive when $OD_{405}>1.2$ after 30 minutes. In negative samples OD_{405} did not exceed 0.06.

Orchard A: Research area consisting of 60 sour cherry trees cv. 'Stevnsbär' planted immediately west of a row of PNRV infected sweet cherry trees cv. 'Van' planted in 1972 (Fig. 1).

The two rows of 'Stevnsbär' next to the sweet cherries were planted in autumn 1980. The third row of 'Stevnsbär' was planted in autumn 1981. Samples from the trees were taken in May 1985, 1986, and 1987 and incidence of PNRV was tested by ELISA or ISEM.

Orchard B: 'Stevnsbär' planted 1971. Incidence of PNRV was surveyed in an area consisting of 75 trees planted in five rows (Fig. 2). Samples from all the trees were taken in November 1983 and assayed for PNRV by ELISA. Leaf samples were taken and tested in May 1984 and May 1985. The plantation was grubbed autumn 1985 since the plot contained another large area with PNRV infected trees.

Fig. 3. Orchard C. Surveyed area
Område af plantage C



Orchard C: Incidence of PNRV was indexed in an area of 'Stevnsbär' trees planted 1968. The area consisted of 115 trees planted in seven rows (Fig. 3). Samples from all the trees were taken for PNRV testing in November 1983 and in May 1986. In May 1984, 1985 and 1987 samples were taken from trees in the outskirts of the PNRV area; so that the trees in which symptoms appeared for the first time and the following two symptom-free trees in the row were tested (Fig. 3).

PNRV attack was observed in a number of trees close to the surveyed area, and therefore an additional 59 trees – under the dotted line in Fig. 3 – were sampled and tested by ELISA in May 1986.

In 1982, when this investigation began, trees were grubbed or young trees were replanted in the middle of the surveyed areas of orchard B and C (Figs 2 and 3). Typical attacks of PNRV were the cause of the grubbed trees (3 trees in orchard B; orchard C: 7 replanted trees). In Table 1 the grubbed/replanted trees are therefore calculated to be PNRV-infected in 1983.

In orchard C a further 13 trees were grubbed or replanted in the surveyed area during the period of observation. This was due to overturning of the original trees during autumn storms. These 13 trees are not included in Table 1.

Yield

Fruit yield in the surveyed area in orchard C was assessed in the period 1984-1988. The estimation was made in August, when the cherries were red, on the basis of the fruit growers experience in visual yield assessment. To compare actual and estimated yield, the cherries from every tree in one of the surveyed rows were weighed separately at harvest.

Results

Identification of PNRV

All leaves with visual symptoms caused chlorosis or systemic tip killing in cucumber seedlings used as indicators. In the following serological tests it was established, that the virus causing symptoms on sour cherry was *Prunus* necrotic ringspot virus, PNRV. This is in agreement with the symptoms observed on the cucumber plants.

Registration of symptoms

Symptoms were observed every year in the period 1982-1988 on PNRV infected 'Stevnsbär' trees. The initial effect of PNRV infection was a pronounced shock-reaction. The following year leaf chlorosis were prevalent in the three surveyed orchards.

Shock-reaction can easily be seen from a distance: The infected trees are brownish compared to the white/green blossom on symptom-free neighbouring trees (Fig. 4). This discoloration is due to shrivelled, reddish, shortstalked, unopened flowers, and small leaves where approx. half of the leaf plates are necrotic. New infections often emerge on that part of the crown facing a PNRV infected neighbouring tree.

A single branch, part of the crown or the whole tree might show shock-symptoms. Leaves in the vegetative buds at shoot tips turn brown and wither shortly after foliation. Due to tip-killing the PNRV infected branches have fewer and smaller leaves and can easily be distinguished from healthy trees. The year of shock-reaction results in nil or very low yield as the main part of the flowers on affected branches died.

The following years symptoms are distinct during spring and early summer. Foliation of PNRV infected trees is about four days delayed compared to healthy trees. Newly foliated leaves of infected trees in the three orchards are mainly chlorotic and curly. Chloroses are distinctly seen, when leaves are held in backlight. In midsummer the leafsymptoms might be difficult to distinguish because later formed leaves are nearly without symptoms. Symptoms will remain on the oldest leaves at the base of the annual shoot.

The PNRV attack causes poor bud development and the infected trees are therefore 'thin' leaved and have reduced growth of annual shoots compared to healthy trees. Many of the virus infected trees died during the period of observation.

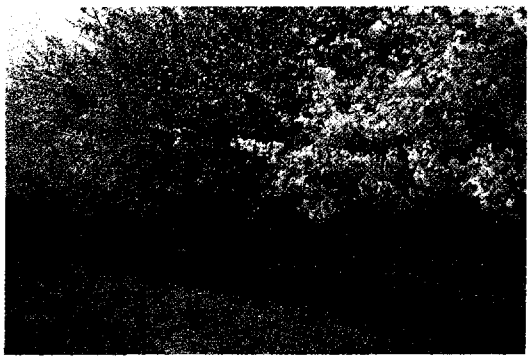


Fig. 4. Shock-reaction due to PNRV-infection is seen on the two sour cherry trees in the middle.
Chok-reaktion forårsaget af PNRV-angreb ses på de to surkirsebærtræer midt i billedet.

Identical results were obtained every year in the observation period from the serological tests and symptom registration made one or two weeks after bloom.

Incidence of PNRV

New PNRV infected trees in per cent of tested trees are given in Table 1.

Orchard A: The first virus infection was observed five years after planting in the trees nearest to the old PNRV infected sweet cherry trees (Fig. 1). Every spring the sweet cherries had weak leaf symptoms, mainly chlorotic ringspots. New infection was observed on 27% of the trees in 1987, which means that nearly 40% of the trees were infected with PNRV seven years after planting.

Table 1. Field spread of *Prunus* necrotic ringspot virus (PNRV) in areas in three sour cherry orchards cv., 'Stevnsbär'.
Udbredelse af Prunus nekrotisk ringplet virus (PNRV) i områder i tre surkirsebær-plantager cv., 'Stevnsbær'.

Orchard *Plantage*	Year of planting *Planteår*	No. of trees tested by ELISA *Antal træer undersøgt ved ELISA*	New PNRV infected trees, % *Træer med nyangreb af PNRV, pct.*					Total trees with PNRV, % *Træer med PNRV i alt, pct.*
			1983[1)]	1984	1985	1986	1987	
A	1980/81	60	0	0	6.7	5.0	26.7	38.4
B[2)]	1971	75	24.0	18.7	16.0	–	–	58.7
C	1968	115	28.7	7.0	6.1	13.0	7.0	61.8
C[3)]	1968	59				54.2[1)]	25.4	79.6

[1)] Orchard B and C. PNRV infection was established when this investigation started. *Plantage B og C. PNRV angrebet var etableret ved undersøgelsens start.*

[2)] The trees were grubbed autumn 1985. *Træerne blev fældet efteråret 1985.*

[3)] Extended area of investigation in orchard C. *Udvidelse af undersøgelsesområdet i plantage C.*

Table 2. Estimated fruit yield in orchard C 'Stevnsbär'.
Skønnet høstudbytte i plantage C 'Stevnsbær'.

Year *År*	No. of trees estimated *Antal træer vurderet*	Symptom-free trees[1] *Symptomløse træer[1]*		PNRV infected trees *PNRV inficerede træer*	
		Number *Antal*	Average kg/tree[2] *Gns. kg/træ[2]*	Number *Antal*	Average kg/tree *Gns. kg/træ*
1984	65	36	24.5	29	6.1
1985	60	29	27.4	31	6.2
1986	81	38	27.3	43	7.8
1986[3]	127	53	25.2	74	9.4
1987	75	27	25.9	48	5.9
1987[3]	126	34	25.2	92	7.8
1988	59	14	24.2	45	6.2

[1] All the trees were tested by ELISA in 1983 and 1986 and found free from PNRV. In 1984, 1985, 1987 and 1988 symptomfree neighbouring trees to PNRV infected trees were tested by ELISA.
Alle træerne blev i 1983 og 1986 undersøgt ved ELISA og fundet fri for PNRV. De øvrige år blev symptomløse nabotræer til PNRV angrebne træer undersøgt ved ELISA.

[2] Actual fruit yield was higher than this estimation.
Kontrolvejning viste, at høstudbyttet var højere end anført i vurderingen.

[3] Include extended area in orchard C.
Omfatter udvidet område i plantage C.

Orchard B: Serological testings of samples from all the surveyed trees taken in November 1983 revealed PNRV infection in all the neighbouring trees to the three grubbed trees (Fig. 2.). Nearly 60% of the surveyed trees were PNRV infected 14 years after planting, and infection had spread to the row north of the surveyed area.

Orchard C: ELISA test of samples taken from all the 115 indexed trees in November 1983 showed that many trees around the seven replanted trees in the middle of the surveyed area (Fig. 3) were attacked by PNRV. The anewed trees were not infected with PNRV during the period of observation. Many PNRV infected trees were observed in the area north of the surveyed area (Fig. 3.), and therefore in May 1986 the number of monitored trees was extended with 59 to a total of 174 trees. At the termination of this investigation 68% of these 174 trees were PNRV infected.

Yield

Table 2 gives an estimate of fruit yield in orchard C from PNRV infected trees and healthy-looking trees found uninfected in ELISA tests for PNRV.

PNRV infection caused yield reduction to on average one third to a quarter of estimated yield in uninfected trees in 1984-1988. Control weighing of actual yield from a single row of surveyed trees showed that yield was estimated 10-20% too low in various years.

Average fruit yield from trees already infected with PNRV in the first ELISA testing in 1983 is stated in Table 3. The yield from these trees stayed low during the five years of observation, and about half of the infected trees were grubbed because of debilitation or death.

Table 3. Estimated fruit yield in orchard C from trees infected with PNRV when tested by ELISA in 1983.
Skønnet høstudbytte på træer, der ved ELISA-testning i 1983 var angrebet af PNRV. Plantage C.

Year *År*	No. of trees *Antal træer*	Average fruit yield kg/tree *Gennemsnitlig høst kg/træ*
1984	21	8.1
1985	17	5.9
1986	15	10.9
1987	14	7.8
1988	11	6.7

Table 4. Course of yield from 'Stevnsbär' being infected with PNRV in the period 1984-1988. Orchard C.
Udbytteforløb hos 'Stevnsbær', der i årene 1984-1988 blev angrebet af PNRV. Plantage C.

Year of shock-reaction *År for chok-reaktion*	No. of trees *Antal træer*	Average yield symptom-free trees kg/tree[1] *Symptomløse træer gns. kg/træ*[1] Year before infection *År før udbrud*			PNRV shock-reaction average yield kg/tree *PNRV chok-reaktion gns. kg/træ*	PNRV infected trees average kg/tree *PNRV angrebne træer gns. kg/træ* Year after PNRV infection *År efter PNRV angreb*			
		3.	2.	1.		1.	2.	3.	4.
1984	6				2.0	11.3	7.3	2.6[2]	2.3[4]
1985	5			26.0	3.0	4.8	7.0	5.5[3]	
1986	6		24.7	25.5	4.2	5.5	6.8[2]		
1987	4	26.8	27.8	34.3	7.5	4.8			
1988	1		35.0	21.0	1.0				

[1] The trees were tested by ELISA every year and found free from PNRV. *Årlige testninger ved ELISA viste, at disse træer var fri for PNRV.*
[2] Average 5 trees. *Gns. 5 træer.*
[3] Average 4 trees. *Gns. 4 træer.*
[4] Average 3 trees. *Gns. 3 træer.*

Table 4 gives course in yield from trees being infected with PNRV during the period 1984-1988. Before infection the trees had a constant high yield varying from 20-40 kg per tree. The year of shock-reaction yield was in most cases reduced to 2-3 kg per tree, while higher yield was observed in trees, where only a part of the crown had shock symptoms. After establishment of PNRV in the trees, the average yield stayed low, often 6-8 kg per tree.

Discussion

Registration of symptoms

Shock-reaction, appearing in the year following infection (7), is also described in other sour cherry varieties (6,7,14,15,17,18,20). Necrotic leafspots were often observed the following years. The necrosis consist of dead tissue, which often falls out of the leaf plate, leaving it in a tattered condition, as described by *Kegler* (14). These symptoms were not observed in the three surveyed orchards, but occur in other Danish sour cherry orchards, and are also observed in sweet cherry. The existence of different strains of PNRV-virus might be a possible explanation to the varying symptoms among orchards with the same sour cherry variety (8,15). PNRV infection causes growth reduction (14,15,24) which was also observed in the Danish orchards. Infected trees became debilitated and were often grubbed.

Several investigators have found, that infections became latent in the years after shock-reaction (1,6,8,10,20). In Bulgaria up to 30% of the trees were by testing found to be latently infected (10), while *Davidson* (7) registered only a few trees without symptoms in some years. Latent infections were not found in Denmark. However symptoms could be difficult to see and detect by testings in the middle of the summer (1,3,13,29) because old leaves contain substances with inhibitory effect (22). Furthermore the symptom expression depends on the susceptibility of the *Prunus* species (11) and variety (14,31) and the strain of the infecting virus (15,20,26,31).

Incidence of PNRV

PNRV attack starts from single trees in discrete groups (28), which also has been registered in young Danish orchards. From these trees PNRV is naturally spread by pollen to healthy adjacent trees (4,6,7,8,9,10,12,20) and gradually the infection is distributed like »rings in the water«. This »clumbed« distribution was also indexed by other authors (6,12,28). Shock reaction in the Danish orchards is often characterized by its emergence on branches facing already infected trees. This might suggest that transmission by root grafting also is possible. *Thresh* and *Edwards* (28) have found groups of infected plants around surviving debris of the former infected variety. However

Cameron et al. (4) assumes spread by root-grafting unlikely.

In orchard A the source of PNRV infected pollen was from 13 year old 'Van' sweet cherry trees. Bloom in this variety overlaps with 'Stevnsbär'. *Howell* and *Mink*'s (12) investigation shows that spread is not influenced by pollen compatibility, suggesting that fertilization is not required for transmission to occur.

The number of infected trees rises rapidly among trees of fruit bearing age if large numbers of blossoms are pollinated with diseased pollen (4,6,7). In orchard A the rate of spread was rapid seven years after planting. This is in coincidence with the findings of *Davidson* (6,7).

In the two older 'Stevnsbär' orchards B and C per cent newly infected trees varied from 6 to 19% during a four year period. Similar infection rates were recorded in sour cherry varieties by *Gerginova* (10) and *Davidson* and *George* (6). Ten years after planting 68% infection in 'Schattenmorelle' was measured by *Kunze* (17). The main factors affecting the rate of spread are volume of inoculum (diseased pollen e.g. from mature adjacent trees) and tree age (6,7).

Yield

Yield assessment in orchard C revealed that no recovery occurred in the PNRV infected trees. Shock-reaction reduced yield by approx. 90%. Control weighing showed that yield estimates were too low. However during the five year period yield was always significantly lower from PNRV infected trees; approx. one third to one fourth of normal yield from symptom-free trees. The actual yield from trees without symptoms, with this spacing, is considered to be above average in commercial orchards *(J. Vittrup*, pers. com.). Several sour cherry varieties have been found to be infected with PNRV and yield reductions were measured (15,17,18,28).

Kunze (17) and *Meyer* (20) found a yield reduction in 'Schattenmorelle' from 74-90% the year of shock-reaction, followed by an average reduction of 43 and 35% respectively. Again, yield reduction is dependent on the PNRV strain involved: *Kegler et al.* (15) measured a reduction of 76-93%. in the heaviest attacks. In 'Montmorency' sour cherry, where virus attack became latent 1-2 years after infection, *Parker et al.* (24) found 20% yield reduction. *Lewis* (18) measured 42% reduction the year of shock-reaction in the same variety.

According to *Basak* (2) and *Nyéki* and *Vértesy* (23) the yield reduction is due to impaired pollen quality and -vitality in PNRV infected trees. A smaller percentage of the infected pollen grains will germinate, and the following pollen tube formation is slow compared to virus-free pollen resulting in poor fertilization and little fruit set. During the year of shock-reaction the main part of the flowers will shrivel resulting in a very low yield.

To reduce incidence of this virus in the future, growers should purchase nursery trees, propagated from virus-free seed and buds. In Denmark disease-free budwood can be bought from The Danish Growers' Elite Plant Station. Propagation of disease-free 'Stevnsbär' on their own root has been started.

Megahed and *Moore* (19) indexed large variation in per cent infected seeds from different PNRV infected *Prunus* species. *Mink* and *Aichele* (21) recommend use of certified seed which is less often infected by seed-borne virus diseases than uncertified seed lots. However the demand for certified seed often exceeds the supply available (21, 27). A source of infection is reseeded mazzard cherry in hedges near cherry orchards (20, 27). On the average 22 % of the seedlings from wild growing Danish PNRV infected *P. avium* trees were infected (27).

Conclusion

- Symptoms were observed every year in the period 1982-1988 on PNRV infected 'Stevnsbär' trees. ELISA-testing of the trees did not reveal any latent infections.
- Most distinct symptoms are seen from the end of bloom and the following weeks. Identical results were obtained from the serological test during winter, and visual registration of symptoms during the following spring.
- Shock-reaction is registered in the first year of symptoms: Flowers shrivel and turn reddish. Leaves are small and partly necrotic and shoot tips die. During this year fruit yield is nearly nil, if the whole crown is affected.
- The following years after infection leaves are mainly chlorotic and 'curly'. In other orchards necrotic leaf spots have been observed.
- PNRV infection causes yield reduction to on average one third to a quarter of estimated yield in uninfected trees.

- PNRV infection spreads from single infected trees to neighbouring trees and gradually the affected area increases like »rings in the water«. In the young orchard the field spread of PNRV is high in the seventh growth season.
- It is recommended to grub PNRV infected trees as soon as possible to prevent natural spread by infected pollen. The following spring trees neighbouring the grubbed ones should be inspected for symptoms.
- New orchards are established with healthy trees and the greatest possible distance to older sour cherry orchards, which might be infected with PNRV.

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