DEVELOPMENT OF A METHOD TO SCREEN SOUR CHERRY (*PRUNUS CERASUS* L.) FOR RESISTANCE TO PRUNUS NECROTIC RINGSPOT VIRUS

BY

Randal L. Hamilton

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture
Plant Breeding and Genetics

1987

ABSTRACT

DEVELOPMENT OF A METHOD TO SCREEN SOUR CHERRY (*PRUNUS CERASUS* L.) FOR RESISTANCE TO PRUNUS NECROTIC RINGSPOT VIRUS

BY

Randal L. Hamilton

Ten isolates of Prunus Necrotic Ringspot Virus (PNRSV) were collected from 'Montmorency' sour cherry orchards in Michigan. Five isolates were submitted to herbaceous host range studies, and based on the results one Michigan isolate (1A1) was equivalent in symptom severity to a known severe PNRSV strain from Prosser, WA (strain 107-57). One Michigan PNRSV isolate (Jurczack 2) received a severity ranking intermediate between the known severe strain and Fulton's type-virus strain (NRSV-G). Three other Michigan isolates received severity rankings equal to or slightly greater than strain NRSV-G. Aseptic shoot culture and conventional propagation from heel-cuttings of *Prunus cerasus* L. cv. 'Meteor' and *Prunus cerasus* L. x *Prunus fruticosa* L. (clone 173/9) is described. Inoculation of 'Meteor' and clone 173/9 with single PNRSV-infected buds was most effective in transmitting the virus (76-96%). Leaf-rub, root-rub, and bark-flap inoculation methods were tested individually and in combination. None of these methods when used individually or in combination produced consistent acceptable infection rates. Additionally, there were no consistent significant differences in infection rate when PNRSV strains were compared. Enzyme-linked immunosorbent assay (ELISA) was used to detect virus infection. The critical absorbance value of twice the healthy mean A_{405nm} value was appropriate for comparing treatments in the inoculation experiments. ELISA tests eight to 12 weeks post-inoculation appeared to detect the maximum number of virus-infected plants.

TABLE OF CONTENTS

This thesis is dedicated to Jean and Paul Hamilton. Their continuous support made it all possible.

I thank Dr. Amy Iezzoni and Dr. Donald Ramsdell for their support and guidance through the this thesis project. Additionally, I would like to thank Dr. James Hancock for his suggestions on experimental design and for reading the thesis. Finally, thanks to Dr. Wayne Adams for reading the thesis on short notice.

Table

1. Her
ide
PNR

2. Pru
(PD

3. Rea
ima

4. Des
aft
rir
Jur
str
Ful

5. Sev
Pro
str

6. Ing
aic
'Me
(c)

7. Ing
pro
'Me
(c)

8. Per
cv.
ste

9. Per
ELI
Pla

List of Tables

Tab

10.

11.

12.

13.

14.

15.

16.

17.

18.

Table Page

Table

19.

20.

21.

22.

23.

24.

25.

Table Page

Table

26.

B1.

B2.

B3.

B4.

B5.

B6.

B7.

Table Page

Figu

1.

2.

3.

4.

5.

A1.

A2.

A3.

A4.

LIST OF FIGURES

Figure Page

affe

erou

infe

tion

weat

Beca

for

grow

facto

sole

is t

resis

resis

lop a

and t

purpo

isolat

and pr

were

and e

GENERAL INTRODUCTION

Sour cherry (*Prunus cerasus* L.) production is adversely affected by a virus disease known as Sour Cherry Yellows (SCY). Numerous reports have shown that SCY gradually reduces the yield of infected trees to only 10 to 50% that of healthy trees. Yield reductions can also fluctuate dramatically from year to year depending on weather conditions immediately following flowering of infected trees. Because of the erratic nature of the yield reductions, yearly production for regions of Michigan are unpredictable. As a result sour cherry growers sell in a market that is unstable from year to year. Other factors contribute to unpredictable yields, but the affect of SCY is of sole interest in this thesis.

One possible solution for eliminating the variability in yields is to identify sour cherry genotypes that possess heritable resistance to SCY.

This thesis was begun as a first step toward identifying such resistant sour cherry genotypes. The purpose of this work was to develop a method by which diverse sour cherry genotypes could be propagated and tested for their reaction to the causal viruses of SCY. For this purpose three goals were set: (1) to collect and positively identify isolates of the causal viruses prunus necrotic ringspot virus (PNRSV) and prune dwarf vrus (PDV), (2) to determine if strains of PNRSV and PDV were present among the virus isolates by using herbaceous host ranges and enzyme linked immunosorbent assay (ELISA), (3) to propagate suffi-

cient *Prunus* plant material by micro-propagation or by conventional propagation methods, and (4) to identify the most effective inoculation method(s) for infecting known susceptible sour cherry plants.

The Dis

diseas

and C

(Barne

1943;

Thomas

obser

fulne

SCY s

year

yield

(194

init

obse

the

tree

Yor

Sym

Yie

Sym

bea

LITERATURE REVIEW

The Disease

Sour Cherry Yellows (SCY) is recognized as one of the major virus diseases affecting sour cherry (*Prunus cerasus* L.) in the United States and Canada. Reduced yields due to SCY have been widely reported (Barnett and Fulton, 1969; Hildebrand *et al.*, 1942, Keitt and Clayton, 1943; Posnette *et al.*, 1969; Lewis, 1951; Swenson and Milbrath, 1944; Thomas and Hildebrand, 1936; Way and Gilmer, 1962). Moore (1946) observed in Wisconsin orchards little or moderate reduction in fruitfulness during the first and second year following initial appearance of SCY symptoms. Yield reductions increased during the next two to three year period. Trees which had been infected five or more years suffered yield reductions of approximately 50 to 62 percent. Keitt and Clayton (1943) also observed slight yield reductions in the first years after initial symptom expression in Wisconsin. More serious reductions were observed after four years of symptom expression. For example, in 1941, the average yield of healthy (nonsymptomatic) trees was 139.1 pounds per tree, as compared to 81.3 pounds per tree in symptomatic trees. In New York, Klos and Parker (1960) found that during the first year of symptom expression, sour cherry yields were equal or slighty less than yields in healthy trees. In succeeding years, yields decreased in symptomatic trees. Trees with significantly reduced yields tended to bear larger individual fruit with no reduction in soluble solid content.

On th

repor

cherr

Sympt

and H

is ca

labil

necro

to 23

of ap

is a

total

parti

Alone

top

icles

5.2 t

virus

few m

to 18

vatio

the i

mately

sucros

On the Niagara peninsula in Ontario, Canada, Davidson and George (1964) reported that SCY caused a 36 to 56 percent reduction in yield of sour cherries.

Symptoms and Causes

Symptoms of Sour Cherry Yellows were first described by Thomas and Hildebrand (1936), and named by Moore and Keitt (1944). The disease is caused by a complex of two serologically unrelated ILAR (icosohedral labile ringspotting) viruses known as prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) (Fulton, 1968).

PNRSV virions are quasi-spherical and range in diameter from 22 to 23 nm. The protein coat consists of subunits with a molecular weight of approximately 2.5 x 10 ^{6} daltons (Barnett and Fulton, 1968). PNRSV is an RNA virus with the nucleic acid accounting for about 16% of the total particle weight. Loesch and Fulton (1975) found three species of particles that have sedimentation coefficents of 72S, 90S and 95S. Alone, the bottom particles are slightly infectious while the middle and top particles are noninfective. By mixing the bottom and middle particles infectivity was markedly increased. Particle weights range from 5.2 to 7.3 x 10^{6} daltons. The A260/A280nm ratio for PNRSV is 1.56. The virus is unstable in crude, undiluted sap; infectivity is lost within a few minutes at room temperature. In diluted sap maximum longevity is 9 to 18 hours. When the virus is in a stabilizing buffer, thermal inactivation point (ten minute exposure) ranges from 55 to 62°C, depending on the isolate of the virus (Waterworth and Fulton, 1964).

PDV virions are also quasi-spherical, with a diameter of approximately 19 to 22 nm. Halk and Fulton (1978) found that PDV sedimented in sucrose density gradients as five closely spaced zones of about 75, 81,

85, 98,

been de

sediment

virus i

in und

tions

divalen

minutes

the is

and ha

Mink

polle

requi

from

80%

the

tes

(Fr

Mil

wh

tr

te

tr

re

c

85, 98, and 113S. The composition of the virus protein coat has not been determined. The virus contains 14% RNA. Only the faster sedimenting particles are infective. The A260/A280 ratio is 1.56. The virus is unstable and loses one half its infectivity within 30 seconds in undiluted cucumber sap. The virus is stable in EDTA and salt solutions of 0.03 to 0.3 M. Infectivity is lost quickly in solutions of divalent cations. The thermal inactivation point of PDV after (10 minutes exposure with infectivity stabilized) is 45 to 54°C depending on the isolate.

Both PDV and PNRSV are pollen borne (George and Davidson, 1964) and have no known insect vector (Swenson and Milbrath, 1944). Cole and Mink (1982) have found that PNRSV is carried on the exterior of the pollen grain and they have proposed that pollen germination may not be required for virus transmission.

Megahed and Moore (1967) have reported that up to 70% of the seed from Prunus species infected with PNRSV will carry the virus, and 70 to 80% of the seed from PDV infected trees will carry PDV. In determining the PDV seed transmission rate, Megahed and Moore were not certain that tested seed were from trees infected by PDV alone.

Graft transmission of both PNRSV and PDV has been reported (Fridlund, 1981; Helton and Bolwyn, 1964; Hildebrand et al., 1942; Milbrath, 1959; Thomas, 1940). Helton and Bolwyn (1964) have shown that when bud-shields and bark patches are used as inoculum sources, transmission readily occurs. Fridlund (1968) has reported that air temperature can greatly influence graft transmission. He found that transmission stops immediately when temperatures rise above 38°C and resumes when temperatures fall to 26°C. Fridlund has also found that contact period between scion inoculum and stock receptor affects

trans

when

respe

PNRSV

Gray

P. pu

domes

ceras

L.

are

Cochr

petal

chlor

sever

after

1948)

tree.

syste

a ten

fail

tips

whisp

and D

Which

transmission of PNRSV. Percent transmission was very high (80 and 100%) when the inoculum bud and stock were in contact for 64 and 72 hours, respectively.

Prunus species other than P. cerasus are susceptible to PDV and PNRSV. Some the more important species are: P. fasiculata (Torr.) Gray, P. dulcis (Mill) D.A. Webb, P. persica L. (Batsch), P. mahaleb L., P. pumila L., P. virginiana L., P. americana Marsh, P. bessyi Bailey, P. domestica L., P. avium L., P. pennsylvanica L., P. serrulata Lindl., P. cerasifera Ehrh., P. fruticosa Pall., P. serotina Ehrh. and P. salicina L. Genera othe than Prunus that are susceptible to these two viruses are as follows: Malus, Humulus, Rosa, and Rubus (Fridlund, 1970; Cochran and Hutchins, 1941).

PDV symptoms develop in sour cherry three to four weeks after petal fall. The symptoms emerge as a mottling of the leaves with chlorosis between the leaf veins or a complete yellowing, depending on severity. Normally, the tissues along the larger veins remain green after the interveinal areas have become chlorotic (Berkley and Willison, 1948). After chlorosis develops, the affected leaves are cast from the tree. In some cases green leaves may also be cast. In time the spur systems of infected trees degenerate resulting in yield loss. There is a tendency for lateral leaf buds to grow into shoots or, more often, to fail to develop. The ultimate effect is heavy leaf growth at the branch tips and excessive lengths of barren wood behind the tips producing a whispy or willowy appearance (this symptom may be invalid; S. Morrissey and D. Ramsdell, unpublished data).

PNRSV symptoms appear most strikingly as a delay in foliation, which may be confined to a few branches or present throughout the tree.

Upon
numer
etche
surfa
leaf
the r
or ta
or p
altho
Sever
becom

leaves
sessil
beginn
sympto
and su
in in
foliag
appear

(1946)
records
unusual
symptom
fall.

Upon examination, the first unfolding leaves on infected branches show numerous fine transluscent to brownish spots, arcs or rings having an etched appearance. Sometimes concentric rings are present. The leaf surface of symptomatic leaves is rough and with wavy margins, giving the leaf a crinkled appearance. Later chlorotic and necrotic areas develop; the necrotic tissue falls out causing the leaves to become "shot-holed" or tattered. Often the tip, margin and much of the lamina become wholly or partially necrotic. The remaining tissue is normally colored, although the leaves tend to be smaller and rounder than healthy leaves. Severely affected leaves are often cast. If the etched markings do not become necrotic, they tend to disappear as the season progresses.

On sepals, symptoms appear which are similar to those of the leaves. Petals may show red or pink streaking. The blossoms may be sessile or have shortened pedicels. PNRSV produces symptoms at the beginning of the growing season, but later in the same season these symptoms may disappear; moreover, symptoms may be sparse in the second and succeeding seasons. The severe shock symptoms are seldom recurrent in infected trees. After the initial shock symptoms have developed, foliage tends to become less dense, giving the tree a more open appearance (Berkley and Willison, 1948).

Early season temperatures greatly affect symptom severity. Mills (1946) reported on twenty-five years (1921 to 1946)of New York orchard records. He found that during 8 of the 25 years yellows symptoms were unusually severe. He concluded that the chief factor in determining SCY symptom severity is temperature during the 30 day period following petal fall. An early bloom in a given season may have exposed plants to lower

temperat

also con

appeared

yellows

greenho

to 28°C

no symp

bud i

trees

that

with

tempe

and

37.7

from

trea

was

thr

vir

int

flo

Swe

is

se

po

temperatures following petal fall and so increased symptom severity. He also concluded that prevailing temperatures after yellows symptoms first appeared affected severity.

Keitt and Moore (1943) found from greenhouse experiments that yellows symptoms developed freely on potted 'Montmorency' that were in greenhouses which fluctuated from 12 to 16°C night temperatures and 24 to 28°C day temperatures. At constant temperatures of 20°C or greater no symptoms developed on yellows-infected 'Montmorency'. Moore (1946) bud inoculated potted 'Montmorency' trees with PNRSV and placed the trees in greenhouses held at temperatures from 16 to 28 °C. He observed that PNRSV symptoms were expressed over the entire temperature range, with more rapid symptom development and necrosis occurring at the higher temperatures. Best leaf symptom expression occurred at 20 to 24°C.

Nyland (1960) reported that stone fruit ringspot virus (PNRSV) and PDV were consistently inactivated in cherry by heat treatment at 37.7°C for two weeks. He concluded that the viruses were eliminated from budwood taken from the heat treated plants. If, however, heat treated infected plants were placed at lower temperatures and new growth was allowed to develop, then shock symptoms of ringspot would arise in three to four weeks. This demonstrated that in most heat treatments the virus remained active in some part of the treated plant and then moved into new growth when the heat stress was removed.

Several workers have observed the effect of PNRSV infection on flowering and on percentage fruit set. Way and Gilmer (1962) found in sweet or sour cherry that percent fruit set is reduced when pollination is performed using a SCY infected pollen source, irrespective of the seed parent's disease status. In their study, fruit set from trees pollinated with infected pollen was 25 to 90% that of trees pollinated

with he

with vi

only on

pollen

occurir

the fi

and N

flower

'Montm

from a

free

1.0

'Mont

ency'

infec

respe

poll

days

nurs

of

mad

pro

with healthy pollen. In cases were virus infected trees were pollinated with virus infected pollen, percent fruit set was even lower than when only one parent was infected. They concluded that since the amount of pollen applied to each stigma was likely to be much greater than that occuring under natural pollinating conditions, the percent fruit set in the field may be even lower than they observed. More recently, Vertesy and Nyeki (1974) studied the effect of several ringspot viruses on flowering period and fruit set in two sour cherry cultivars. When 'Montmorency' blossoms infected with PNRSV were pollinated with pollen from a virus-free tree, 2.0% of the fruit reached maturity. When virus-free 'Montmorency' blossoms were pollinated with PNRSV-infected pollen, 1.0 to 3.4% of the blossoms set mature fruit. PNRSV-infected 'Montmorency' also bloomed later and longer than virus-free 'Montmorency'. When healthy 'Pandy-48' was pollinated with either ringspot-infected Germersdorfi-57 or Cigany-7 pollen, fruit set was 28.5 and 2.5% respectively. Ringspot infected 'Pandy-48' had 2.7% fruit set when open pollination occurred. Ringspot virus infected 'Pandy-48' bloomed 3 to 5 days earlier than the virus-free trees.

Control methods for SCY are limited to the use of virus-indexed nursey stock, rouging all infected trees from orchards, and elimination of inoculum sources such as wild Prunus species. Attempts are being made to obtain mild strains of both viruses for use in cross-protection programs but no results have been reported.

CHAPTER ONE

Virus Isolate Collection and Strain Determination

INTRODUCTION

Initial work was begun to: (1) collect isolates of PNRSV and PDV from symptomatic Prunus cerasus L. cv. 'Montmorency' trees, (2) identify the viruses and separate any mixed virus cultures, (3) submit pure isolates of PNRSV and PDV to a differential host range to identify strains of the viruses. In previous work on SCY transmission the virus inoculum used was of uncertain purity and thus, investigators were never clear if the inoculum contained one or both the causal viruses. By collecting virus isolates and testing these isolates for purity, all later inoculation experiments would be conducted with inoculum for only a single virus.

LITERATURE REVIEW

Following reports that Sour Cherry Yellows (SCY) was a bud-transmissable disease of sour cherry, work was done to transmit the suspected virus(es) to other herbaceous hosts. Moore et al. (1948) reported that a virus was transmitted from a SCY-infected sour cherry 'Montmorency' to cucumber (Cucumis sativus L. cv. 'Ohio') by grinding and expressing young expanding cherry leaves that were just beginning to show the initial symptoms of the necrotic ringspot disease. Undiluted expressed juice was used to rub-inoculate young cucumber cotyledons. Transmission was accomplished from trees having necrotic ringspot

disease

ringspot

prune dw

both ro

used t

(Milbra

buds

transm

buds

Buds

gram

cucum

was

cucu

lesi

more

Tit

yel

PNR

les

Du

th

19

m

l

Y

disease symptoms and yellows symptoms or trees having only necrotic ringspot symptoms. Later improvements in technique made transmission of prune dwarf virus (PDV) and necrotic ringspot virus (PNRSV) to cucumber both routine and reliable (Boyle et al., 1954). Sour cherry petals were used to transmit the virus more efficiently than when leaves were used (Milbrath, 1953; McWorter, 1953). Cropley (1966) reported that dormant buds of sweet cherry provided a good source of several mechanically transmitted viruses. Both PNRSV and PDV were transmitted from dormant buds of 15-year-old sweet cherry trees (Davidson and Rundans, 1972). Buds were triturated in a 2.5% nicotine solution at a ratio of 5 ml per gram of bud tissue. The extract was rubbed onto carborundum dusted cucumber cotyledons. Cucumber to cucumber transmission of the viruses was readily accomplished (Moore et al., 1948). Once transmitted to cucumber, PDV and PNRSV could be separated by transferring single lesions to cucumber or by submitting the mixed virus culture to one or more seiving hosts (Fulton, 1957a; Fulton, 1957b; Gilmer, 1961). In Tithonia speciosa Hook., PDV (also known at this time as sour cherry yellows virus (SCYV)) was found to cause a systemic golden mottle while PNRSV never became systemic and induced only small necrotic local lesions on inoculated cotyledons or true leaves. In Cucurbita maxima Duchesne cv. 'Butternut', SCYV (PDV) caused a golden systemic mottle on the first true leaves seven to nine days post-inoculation (PI) (Gilmer, 1961). PNRSV caused chlorotic local lesions (3-5 mm in diameter) on C. maxima cv. 'Butternut' five to seven days post-inoculation, and the lesions often became necrotic. Apical necrosis often occurred when young plants were inoculated.

Numerous other herbaceous hosts have been identified and the symptoms caused by PDV and PNRSV have been described. Fulton (1957a) found that sour cherry virus A (PNRSV) infected 51 of 186 species tested, sour cherry virus B (PDV) infected 86 of 227 species tested, sour cherry virus E (PNRSV) infected 38 of 145 species tested and sour cherry virus G (PNRSV) infected 48 of 150 species tested. Plants that were infected by both PDV and at least one PNRSV isolate were as follows: Zinnia elegans Jacq., Citrullus vulgaris Schrad., Cucumis anguira L., C. melo L., Cucurbita maxima Duch., C. pepo L., Mormordica balsamina L.. Plants infected by only PDV were as follows: Solidago sp., Tithonia speciosa Hook., Nepeta cataria L., Crotalaria spectabilis Roth, Melilotus officinalis (L.) Lam., Plantago virginica L., Browallia elata L. Finally, plants infected by at least one isolate of PNRSV only were as follows: Helianthus annuus L., Cassia tora L. and Cyamopsis tetragonoloba (L.) Taub. There were numerous other species with questionable reactions to inoculation with the various sour cherry viruses. Zinnia elegans Jacq. and Nicotiana tabacum L. were successfully infected with SCY (PDV or PNRSV) (Varney and Moore, 1954). Allen (1963) inoculated numerous plant species with several isolates of PNRSV and found that only Cucumis sativus, Cucurbita maxima, and C. pepo produced symptoms.

Waterworth and Fulton (1964) identified Helianthus annuus, Zinnia elegans, Citrillus vulgaris, Pisum sativum L., Cyamopsis tetragonaloba and Phaseolus vulgaris L. as being susceptible to both PDV and PNRSV. Vigna sinensis Savi (L.) ex Hassk. was susceptible only to PNRSV. Cropley et al. (1964) found that Petunia hybrida Vilm. developed symptoms when inoculated with either PDV or PNRSV. Chenopodium quinoa Willd. and C. amaranticolor Costa & Reyn. were only infected by PNRSV.

Kirkpatrick et al. (1967) extended Fulton's initial host range. Species found to be susceptible to both PDV and PNRSV are as follows. Helianthus sp. (dwarf), Vinca rosea L., Arachis hypogea L., Nicotiana glutinosa L., N. rustica L., N. longiflora L., N. sylvestris L. Species that were only infected by PNRSV are as follows: Chenopodium botrys L., Zinnia linearis L. and Gossypium hirsutum cv. 'Locket'. Species that were infected only by PDV are as follows: Solanum hendersonii, S. miniatum, S. nigrum and Nierembergia hippomanica.

Symptoms caused by 24 isolates of "ringspot virus" from symptomatic cherry trees transmitted to cucumber were found to correlate with symptom development in three species of Prunus (P. avium L. cv. 'Bing', P. persica L. (Batsch) cv. 'J. H. Hale', P. serrulata cv. 'Shirofugen') (Heinis, 1956). Isolates were placed in five severity groups based on symptom expression in cucumber and the Prunus species. The severity groups were as follows: (1) no symptoms, (2) mild symptoms, (3) moderate symptoms, (4) severe symptoms, and (5) necrosis. The author stated the severity of symptoms in cucumber and the Prunus species were correlated with all but a few isolates.

MATERIALS AND METHODS

Isolate Collection and Maintainence

In the summer of 1982, three locations in Michigan were chosen for collecting isolates of PDV and PNRSV. The areas chosen were Van-Buren, Oceana, Grand Traverse and Leelanau counties. These counties provided good coverage of the cherry growing region in Michigan. In VanBuren county, orchards near Paw Paw, Lawrence, and Hartford, Michigan were sampled. In Oceana county, orchards a few miles from Shelby and Hart were sampled. Orchards on the Old Mission pennisula were sampled in Grand Traverse county and finally, several orchards in Leelanau county adjecent to the west arm of Grand Traverse Bay were sampled. Orchards were sampled on one of the following dates: June 1st, 4th and 30th; September 13th and 20th; October 10th, and November 11th in 1982 and February 6th and 9th; June 14th and 18th; July 21st and 22nd in 1983.

Collection of cherry tissue was from trees in orchards that were showing shock symptoms caused by PNRSV. The individual trees were tagged, mapped and then sampled. Symptomatic leaves (just expanding to fully expanded) were sampled randomly on each tree. The leaves were put in plastic bags and immediately placed on ice. The leaf samples were brought back to East Lansing and stored at 4°C. Within two days the leaves were used to inoculate herbaceous host plants. Dormant buds were also collected by randomly cutting branches from the periphery of each tree. The dormant budwood was packaged, placed on ice and taken to East

Lansing where it was placed in cold storage. Seven to ten days and twelve to fourteen days prior to collecting leaf samples, Cucumis sativus L. cv. 'National Pickling' and Cucurbita maxima Duchesne cv. 'Buttercup' seeds were sown so that seedlings could be inoculated immediately after leaf samples were collected or when transfers were to be made. Initially 'Lemon' cucumber was used as recommended by R. W. Fulton. However, 'Lemon' cucumber had relatively low germinaton and seemed quite susceptible to damping-off organisms if germinated at temperatures of 18 to 24°C. During the second summer (1983) 'Lemon' cucumber was replaced by 'National Pickling' cucumber since it appeared to be as sensitive to PNRSV and more sensitive to PDV, while being a more vigorous germinator and less susceptible to damping off. Seeds were sown in VSP soil mix (Michigan Peat Co., Houston, Texas, 77006) or a comparable Michigan State University greenhouse-prepared formulation. The seedlings were maintained in greenhouses where temperatures fluctuated from 18 to 35°C in the spring and summer months and 16 to 29°C in the fall and winter months. Supplemental light was provided by four-tube banks of eight-foot fluorescent cool white 40 watt lights operated at 16 hour daylengths.

When the cotyledons were approximately three-quarters expanded and the first true leaves just beginning to appear, the seedlings were inoculated with triturated cherry leaves or buds taken from cold storage. Partially expanded or newly expanded symptomatic cherry leaves were placed in a cold mortar. Ten to 20 ml of cold 0.03M sodium mono- and dibasic phospate buffer, pH 8.0, amended with 0.14% 2-mercapto-ethanol and 10 mg per liter Polyvinylpyrrolidone (PVP) (inoculation buffer) was added to the mortar before the buds were triturated. A sterile foam rubber sponge was used to rub sap onto carburundum-dusted

(320 mesh) cotyledons. Inoculum was washed off the cotyledons 15 to 60 seconds later with tap water. The plants were observed for ten days post-inoculation for symptom development. Local lesions that appeared were cut out with a razor blade and transferred to healthy cucumber or squash seedlings. At the outset of this work virus cultures were transferred every ten days to two weeks, and after the third transfer the isolates were lost.

The virus cultures were reisolated from the original cherry trees by collecting dormant buds in October, 1982 as previously described. Buds were selected and triturated in a 2.5% Nicotine solution at a rate of 5 ml per gram of buds (Davidson and Rundans, 1972). Upon completing the reisolations, cultures were transferred every four to five days post-inoculation (Loesch and Fulton, 1963). After the third transfer of each culture, cotyledons showing numerous local lesions (15 to 20 per cotyledon) were harvested and lyophilized at -20° C for 24 hours. The lyophylized tissue was placed in 18 x 100 mm screw cap culture tubes which were in turn placed in 25 x 150 tissue culture tubes with calcium chloride layered on the bottom as a desiccant. The large culture tubes were sealed and stored at -20°C. After each culture was transferred eight or nine times in cucumber, either freeze-dried tissue was added to the fresh tissue prior to maceration or freeze-dried tissue alone was used to rejuvenate the cultures.

In order to avoid continual transfers of PNRSV in cucumber, Vinca rosea cv. 'Mixed Colors' was grown from seed for use as a holding host. Vinca was allowed to grow until three to four sets of primary leaves had expanded. Tissue from infected cucumber was triturated and used to sap inoculate Vinca. The transfer of PNRSV from cucumber to Vinca was not

easily accomplished and after repeated attempts to transfer two specific PNRSV isolates, Vinca was abandoned as a holding host and the isolates were maintained in cucumber.

Virus Identification

After the isolated cultures had been established, the virus was identified by one of two serological methods: (1) Ouchterlony immunodiffusion tests or (2) ELISA (enzyme-linked immunosorbent assay). For the initial virus identification, only Ouchterlony immunodiffusion was used. After the isolates were lost and reisolated, Ouchterlony immunodiffusion was used to test for tobacco ringspot virus (TRSV) and tomato ringspot virus (TmRSV) only. ELISA was used to assay for PNRSV, PDV and apple mosaic virus (ApMV) in the reisolated cultures. ApMV antiserum was kindly provided by Dr. D. C. Ramsdell (originally obtained from American Type Culture Collection (ATTC) 12301 Parklawn Drive Rockville, MD 20852). Initial antisera for PNRSV (type strain NRSV-G) and PDV (Fulton's PDV-876) were kindly provided by Dr. R. W. Fulton. TRSV, TmRSV, PDV and PNRSV antiserum was purchased from ATCC. MD 20852.

Ouchterlony Immunodiffusion

To prepare gels for the diffusion tests, 8 g of agarose were suspended in 1000 ml of glass distilled water and then autoclaved for 10 minutes at 120 psi. To the agarose solution, 8.5 g sodium chloride and 1.0 g sodium azide were added. The solution was then poured into 20 x 100 mm plastic petri dishes and allowed to cool. Wells 7 mm in diameter were spaced 3 mm apart; a central well was surrounded by six peripheral wells. The edge of the center well was 5 mm from the edge of the peripheral wells. A Grafar gel cutter was used (Graphar Co., Inc, Detroit, MI). Dilutions of 1:4, 1:8, 1:16 and 1:32 (v/v) were

made for PNRSV and PDV antiserum while TmRSV and TRSV antisera were diluted to 1:16 and 1:32. Antisera were diluted with a 0.85% saline solution. Individual dilutions of antiserum were placed in the center well.

Symptomatic cucumber or squash tissue was macerated in inoculation buffer (as described earlier) and strained through cheese cloth. Samples of the strained extracts were then pipetted into the outer wells. Healthy and diseased herbaceous plant sap controls were used for each antiserum type. The plates were then covered, sealed and allowed to stand for 24 to 48 hours at room temperature. After allowing antibodies and virions time to diffuse, the outer wells were rinsed with glass distilled water to remove any remaining plant extract. An L-Dopa stain was then prepared by adding 0.49 g L-Dopa (Sigma Chemical Co., St. Louis, MO 63178) to 50 ml of a 0.1 M mono- and dibasic Sodium phosphate buffer, pH 7.2. The L-Dopa stain was added to each outer well. Plates were allowed to stand for approximately four hours where upon any remaining stain was rinsed from the wells. The plates were viewed using an indirect light source and precipitin lines were observed and noted.

ELISA Protocol

For ELISA tests, samples of expanding leaves were weighed in amounts ranging from 0.5 to 1.0 g. These were placed in 45 ml plastic centrifuge tubes and diluted at a rate of 1:10 (w/v) with extraction buffer composed of 0.05 M mono- and di-basic potassium phosphate buffered saline solution, adjusted to pH 7.4 and amended with 2.0 % polyvinylpyrolidone, 0.2% ovalbumin and 0.02 % sodium azide (all w/v).

The contents of the tube was then homogenized on ice for 30 to 60 seconds with a Tekmar Tissuemizer with a SDT-182EN shaft (Tekmar Co.,

Cincinnati, OH 45237). The extract stood for 15 to 30 minutes so plant debris would float to the surface. Extracts were always used within four hours of preparation. Microelisa ® substrate plates (Immulon I, flat bottom wells, Dynatech Lab. Inc., Alexandria, VA 22305) were used to conduct the ELISA. Rabbit anti-PNRSV or anti-PDV-igG (coating antibody) was purified by ammonium sulfate precipitation and by passing through a DE-22 cellulose column (Clark and Adams, 1977). Purified gamma globulin was diluted to 1.0 ug/ml with a mono- and di-basic sodium carbonate buffer, pH 9.6. To each of the 96 wells, 200 ul of the coating antibody solution was added; the plate was then sealed in a plastic bag and incubated for 3 to 4 hours at 37°C. After incubation the wells were emptied and washed with a phosphate buffered saline solution, pH 7.4 ammended with 0.5% (v/v) polyethylene sorbitan monolaurate (Tween 20®). The wash solution was allowed to remain in the wells for at least 3 minutes. The wash was done to remove any gamma globulin that remained unattached to the well walls. Test samples were added to the wells in 200 µl aliquots. Samples were taken from the portion of each extract that was free of large leaf fragments. Healthy, diseased and buffer controls were included on each plate. Each test sample was placed on two replicate plates. When loading was complete the plates were again placed in plastic bags, sealed and incubated overnight at 5°C. Following incubation, the wells were washed as described previously; however, four rather than three washes were used to completely remove the viscous plant extract. An antivirus igG alkaline-phosphatase enzyme conjugate stock (Clark and Adams, 1977) was then diluted at a rate of 1:800 (v/v) with extraction buffer and 200 ul aliquots were pipetted into each well. The plates were placed in a plastic bag,

sealed and incubated for three to four hours at 37°C. After incubation, the plates were washed three times as already described. Finally, to each test well was added a 200 ul aliquot of an enzyme substrate solution (freshly prepared by dissolving p-nitrophenyl- phosphate at a rate of 1 mg per ml in a substrate buffer containing 10% diethanolamine (v/v), 0.02 % sodium azide (w/v) and adjusted to pH 9.8 with HCl. The plates stood at room temperature for five to 30 minutes after which the absorbance at 405 nm (A_{405}) was read with either a Microelisa® spectrophotometer (Dynatech. Lab. Inc., Alexandria, VA 22305) or a Bio-Tek EIA Reader, Model EL307 (Bio-Tek Instruments Inc., Burlington, Vermont 05401). Critical absorbance values were calculated by determining the mean and the standard deviation of the healthy controls on a single plate and then summing the mean and three times the standard deviation. A_{405} values greater than or equal to the critical value were considered ELISA-positive and values less than the critical value were considered ELISA-negative.

Herbaceous Host Range

After five individual PNRSV isolates were obtained, seeds of herbaceous host plants for inoculation with PNRSV were sown for use in a host range experiment. The species used in the host range are listed in Table 1. For the initial experiments, seeds were sown at the same time and for later experiments the seeds were sown at appropriate intervals so that all species were simultaneously at an optimal stage for inoculation. Prior to inoculation, isolates were either re-established from freeze-dried tissue or transferred from an existing culture in cucumber. When transferring any culture, sterile disposable plastic gloves were used to avoid contaminating individual isolates. Titer, as gauged by

Table 1. Herbaceous Plant Species Used in Host Range Studies I throught IV to Identify PNRSV Strains in a Collection of Michigan PNRSV Isolates.

Chenopodium amaranticolor Costa & Reyn.

Chenopodium quinoa Willd.

Cucumis sativus L. cv. 'National Pickling'

Cucurbita maxima Duch. cv. 'Buttercup'

Dolichos biflorus L.

Gomphrena globosa L.

Helianthus annuus L.

Lactuca sativa L.

Momordica balsimina L.

Nicotiana tabaccum L. cv. 'Virginia'

Sesbania exaltata (Raf.) Cory

Tithonia speciosa Hook.

Torenia foureneri Linden

Vinca rosea L.

Zinnia elegans Jacq. cv.'Mixed Colors'

---

local lesion number, was increased from transfer to transfer by cutting out individual local lesions and grinding them in a mortar and pestle containing a 5:1 (v/w) ratio of extraction buffer to tissue. The ground tissue was kept on ice while sap inoculations were made. All host range plants were lightly dusted with carborundum (320 mesh) before inoculation. The inoculum was allowed to remain on the leaf surface for 30 to 60 seconds before being rinsed off with tap water.

Each experiment was arranged in a completely randomized design on the greenhouse bench. The host range for each isolate was carried out by choosing two replicate plants for each species and inoculating as already described. For a given isolate, all host range plants were inoculated consecutively before inoculum for another isolate was prepared and used for inoculating its host range. Supplemental lighting was provided by four tube fixtures containing eight foot cool white 40 watt flourescent tubes which were set for a 16 hour daylength. Temperatures were recorded using a hygrothermograph suspended 35 cm above the greenhouse bench. Daily temperature fluctuations during the four experiments are presented in appendix A (Table A1-A4).

The host ranges were examined daily for three weeks post-inoculation and symptoms were noted accordingly. Data were taken on rate of symptom development, local versus systemic infection, characteristic shape of lesions, size and color of lesions, and symptom severity (ie. chlorosis vs. necrosis). The host ranges for each isolate were repeated four times and initiated on the following dates: March 7th, April 26th, September 15th and November 13th, 1983. Information from the four experiments was compiled to form generalized descriptions which are found in Table 4. Photographs of the diagnostic hosts are found in Figure 1, 2, 3 and 4. The unclassified isolates collected in

Michigan were compared to a known severe strain and the type strain and then ranked based on their symptom expression on the various hosts.

Results

Isolate Collection

Two isolates of PDV and ten isolates of PNRSV were collected in thirteen attempts. One PDV isolate was lost and not re-established. Five of the PNRSV isolates were successfully maintained and the remainder were not reisolated, or the source trees were removed from their orchard sites. Isolates were identified as either PNRSV or PDV by precipitin line formation in Ouchterlony immunodiffusion tests against antisera to each of the viruses. The PNRSV isolates were identified as follows: 1A1, Jurczack 2, Jurczack 3, Morrison 2, Morrison 4, LM 3, BBA 4, PKA 1 and HB 4. Isolates that were successfully reisolated were 1A1, Jurczack 2, Jurczack 3, Morrison 2 and Morrison 4. PDV isolates were identified as follows: Meachum PDV and Pugsley PDV. Meachum PDV was maintained while Pugsley PDV was lost and not reisolated. Isolates and their corresponding locations in Michigan are listed in Table 2.

No mixed virus cultures were found when Ouchterlony immunodiffusion tests and ELISA tests were used to identify each virus isolate. Additionally, no apple mosaic ivrus (ApMV) was identified in any isolate.

Virus Identification

Each virus isolate was tested at least twice against PDV, PNRSV, TmRSV, TRSV and ApMV antiserum. The results of Ouchterlony immunodiffusion tests are listed in Table 3. The isolates BBA 4, HB 4,

Table 2: Prunus Necrotic Ringspot and Prune Dwarf Virus Isolate Designations and Locations of Collection Sites.

Isolate Name	City, County in Michigan
1A1	Old Mission Peninsula, Grand Traverse County
BBA 4	Paw Paw, VanBuren County
BBA 5	Paw Paw, VanBuren County
Jurczack 2	Paw Paw, VanBuren County
Jurczack 3	Paw Paw, VanBuren County
HB 4	Hart/Shelby, Oceana County
LM 3	Hart/Shelby, Oceana County
PKA 1	Hart/Shelby, Oceana County
Morrison 2	Lawrence, VanBuren County
Morrison 4	Lawrence, VanBuren County
Meachum PDV	Lawrence, VanBuren County
Pugsley PDV	Paw Paw, VanBuren County

Table 3: Reaction of Virus Isolates When Tested by Ouchterlony immuno-diffusion Using Several Virus Antisera.

Isolate	Antiserum			
	NRSV-G	PDV876	TmRSV	TRSV
BBA 4	+	-	-	-
BBA 5	-	-	+	-
HB 4	+	-	-	-
Jurczack 2	+	-	-	-
Jurczack 3	+	-	-	-
LM 3	+	-	-	-
Morrison 2	+	-	-	-
Morrison 4	+	-	-	-
1A1	+	-	-	-
PKA 1	+	-	-	-
Meachum PDV	-	+	-	-
Pugsley PDV	-	+	-	-

Jurczack 2, Jurczack 3, LM 3, Morrison 2, Morrison 4, PKA 1 and 1A1 reacted only with the NRSV-G antiserum to form a distinct precipitin line, while isolate BBA 5 reacted with TmRSV antiserum and thus was not used in further work. No other isolate tested reacted with more than one antiserum and so, the isolates were assumed to be free of PDV, TmRSV, TRSV and ApMV.

Herbaceous Host Range

The five PNRSV isolates reacted differently in the host range experiments (Table 4). The isolates 1A1 and 107-57 caused symptoms on all species except Lactuca sativa L., Sesbania exaltata (Raf.) Cory, Helianthus annuus L., and Dolichos biflorus L. The Jurczack 3 isolate did not cause symptoms on Gomphrena globosa L., L. sativa , Torenia foureneri Linden, Chenopodium amaranticolor Costa & Reyn., Vinca rosea L., Zinnia elegans Jacq., Nicotiana tabaccum L. cv. 'Virginia', S. exaltata, H. annuus or D. biflorus. The Jurczack 2 and Morrison 4 isolate did not cause symptoms on G. globosa, L. sativa, T. foureneri, N. tabaccum L., S. exaltata, H. annuus or D. biflorus. The Morrison 2 isolate did not cause symptoms on G. globosa, L. sativa, T. speciosa Hook., V. rosea L., Z. elegans, N. tabaccum, S. exaltata, H. annuus or D. biflorus. Finally, the type strain NRSV-G did not cause symptoms on G. globosa, L. sativa, T. foureneri, T. speciosa, V. rosea L., N. tabaccum, S. exaltata, H. annuus or D. biflorus. To directly compare symptoms caused by each isolate, host range species susceptible to all isolates were considered first. Those species are as follows: Cucumis sativus L. 'National Pickling', M. balsimina, C. quinoa Willd., and C. maxima Duch. 'Buttercup'.

Table 4. Description of symptoms on seven herbaceous host species after inoculation with one of five Michigan Prunus necrotic ringspot (PNRSV) isolates (isolates 1A1, Jurczack 2, Jurczack 3, Morrison 2 and Morrison 4) or two known PNRSV strains (severe strain 107-57 from Prosser, WA or R. W. Fulton's PNRSV type-strain NRSV-G).

	Herbaceous Host Species	
Isolate	*Cucumis sativus* L. cv. 'National Pickling'	*Gomphrena globasa* L.
1A1	Small C S (1-3mm) develop on COT 3 DPI & become N S (5-6mm dia.) 5-7 DPI. C S appear on PLF 4 DPI. Terminal D B 10-12 DPI.	Some N LL develop surrounded by red R 7-9 DPI. SYS SYMP shown as slight leaf distortion & red streaks. General C on all PLF (14-20 DPI).
107-57	Numerous C R S develop 2 DPI (3-4mm dia.) & becom N R S on COT 4-8 DPI Terminal DB 10-12 DPI.	Numerous PP LL (4-5 DPI). LL surrounded by red R. SYS SYMP appear as PLF twist & C streaks (10-20 DPI).
Jurczack 2	Light C S (1-3mm dia.) develop 3-4 DPI which in turn expand to form C R S (4-5mm dia.). C R S coalisce to cause overall C. First PLF stunted & may collapse.	No Symptoms
Jurczack 3	Light C S (2-3mm dia.) develop 4 DPI. C S coalesce into large blotches. Terminal stunted & no DB.	No Symptoms
Morrison 2	Light C S (1-3mm dia.) on COT 3 DPI. COT may collapse & first PLF stunted & C. Terminal DB. If no COT collapse C S expand to C R S (4-6mm dia.).	No Symptoms
Morrison 4	Small light C S (1-3mm dia.) develop on COT 3 DPI. C R S develop (5-6mm dia.) & fade while first PLF shows numerous C R S. Terminal stunted; no DB.	No Symptoms
NRSV-G	Numerous C S develop as LL 3 DPI (1-2mm dia.). LL expand (5mm dia.) but first PLF show SYS C S (1-2mm dia.) at 7 DPI. No N. Severe stunting.	No Symptoms

Abbreviations: C = chlorotic/chlorosis, S = spots, N = necrosis/necrotic, LL = local lesion(s), DB = die-back, dia. = diameter, DPI = days post-inoculation, SYS = systemic, COT = cotyledon(s), PLF = primary leaf/leaves, SYMP = symptom(s), PP = pinpoint, R = ring(s).

Table 4. (continued) Herbaceous Host Species

Isolate	Momordica balsamina L.	Torenia fournieri Linden
1A1	First PLF develop some large C S 2-4 DPI (3-4mm dia.). C S become N S 6-7 DPI. No SYS SYMP develop.	C S develop on INOC PLF at 6-8 DPI. SYS SYMP appear as bright C mottle, arcs, rings & streaks at 10-15 DPI.
107-57	Many PP C S (1mm dia.) appear as LL on the first PLF at 3 DPI. C S expand at 4-5 DPI & finally become N R S at 8-9 DPI.	Diffuse C S on inoculated PLF (6-7 DPI). SYS SYMP appear as distinct C mottle with arcs, R S & bands. Some PLF show twisting(10-15 DPI).
Jurczack 2	Numerous C S develop on first PLF at 2-4 DPI (1 mm dia.). LL expand (2-3 mm dia.) & become NL at 4-6 DPI. SYS PLF curl due to aphid infestation.	No Symptoms
Jurczack 3	A few C S develop on first PLF (1mm dia.) & become N R S (4-5mm dia.) at 5-6 DPI.	No Symptoms
Morrison 2	Several C S (1-2mm dia.) develop on first PLF 3 DPI. C S expand (2-3mm dia.) & become N at 4-6 DPI. Some C S expanded further (5-6mm dia.) & finally fade out.	SYS C develops at 5-7 DPI. DPI. Some PLF develop complete C while others show vein clearing 12-15 DPI. No. LL appear.
Morrison 4	Numerous light C S (1-2mm dia.) develop on first PLF & expand to 2-4mm dia.. C S become N from center outward at 4-6 DPI.	No Symptoms
NRSV-G	Numerous C LL on first PLF at 3 DPI. Some LL (2-3mm dia.)expand slightly & finally fade while others expand & become N S at 4-6 DPI (5-6mm dia.).	No Symptoms

Table 4. (continued) Herbaceous Host Species

Isolate	Tithonia speciosa Hook. cv.'Mixed Colors'	Cheonopodium amranticolor Costa & Reyn.
1A1	Several N S (1-2mm dia.) develop on inoculated PLF at 3-4 DPI. N S expand slightly. SYS vein-clearing at 8-10 DPI.	Numerous large C R S (4-5 mm dia.) appear as LL at 3 DPI. C R S become more C but do not expand (4-7 DPI). LL become N at 15-20 DPI.
107-57	Light N S show as LL (1-2mm dia.) at 4 DPI. N S expand to 2-3mm dia. at 5-6 DPI. SYS vein learing on PLF at 8 DPI.	Numerous PP C S develop at 3 DPI as LL. C S expand (2-3 mm dia.) & finally become N S at 4-8 DPI. The N S do not expand. No SYS SYMP.
Jurczack 2	Numerous PP N LL appear (1mm dia.) at 3 DPI. LL expand (1-2mm dia.) at 5-7 DPI. No SYS SYMP.	Numerous light C S (1-2mm dia.) appear as LL at 5 DPI. Numerous SYS PP C S (1mm dia.) at 5-6 DPI. LL and SYS fade at 10-15 DPI.
Jurczack 3	Numerous PP N LL (1mm dia.) appear at 3 DPI. The LL enlarge (2-3mm dia.) & no SYS SYMP were observed.	No Symptoms
Morrison 2	No Symptoms	A few C R S (4-5mm dia.) appear as LL at 5 DPI. At 7-9 DPI C R S fade & SYS PP C S (1mm dia.) appear. SYS SYMP fade at 1215 DPI.
Morrison 4	Several N S appear as LL (2-3mm dia.) at 3 DPI. N S did not expand No SYS SYMP observed.	Several light C R S (4-5mm dia.) develop as LL at 3 DPI. C R S expanded (6-8mm dia.) & finally faded out at 9-12 DPI.
NRSV-G	No Symptoms	PP C S (1mm dia.) appear at 4-5 DPI. SYS mottle appears at 10-14 DPI. The LL fade out at 9-10 DPI. SYS mottle fades at 1821 DPI.

Table 4. (continued) Herbaceous Host Species

Isolate	Chenopodium quinoa Willd.	Vinca rosea L.
1A1	Some large diffuse C R S (67mm dia.) at 3 DPI. C R S expand(710mm dia. & become distinct at 5-6 DPI. New PLF show distor tion & C mottle at 69 DPI.	Diffuse PP C LL (1mm dia.) develop at 4-5 DPI. SYS C streaks & PLF twisting ap- pear at 9-14 DPI.
107-57	Many C S develop (2-3mm dia.), expand (5-6mm dia.) & some fade locally at 3-7 DPI. A few C S become N S at 8-10 DPI. No SYS SYMP.	No LL appear. SYS SYMP appear as severe PLF distortion & C blotches at 7-14 DPI.
Jurczack 2	C S develop as LL (34mm dia.) at 3 DPI. C S change to C R S (67mm dia.) at 4-5 DPI. C R S fade out at 7-12 DPI. No SYS SYMP.	No Symptoms
Jurczack 3	Numerous light C S appear as LL (2-3mm dia.) at 3 DPI. C S intensify but do not expand at 45 DPI & fade out at 911 DPI. No SYS SYMP.	No Symptoms
Morrison 2	Diffuse C S develop as LL (23mm dia.)at 3 DPI. LL fade out at 5-10 DPI. No SYS SYMP.	No Symptoms
Morrison 4	Numerous PP C LL (1mm dia.) develop at 3 DPI. LL expand to 2-3mm dia. & fade out at 5-9 DPI. No SYS SYMP.	No Symptoms
NRSV-G	Numerous C S appear as LL (3-4mm dia.) at 3 DPI. LL expand (5-7mm dia.) & fade out at 5-10 DPI. No SYS SYMP.	No Symptoms

Table 4. (continued) Herbaceous Host Species

Isolate	*Zinnia elegans* L. cv. 'Mixed Colors'	*Nicotiana tabaccum* L. cv.'Virginia'
1A1	No LL. PLF severely distorted & show C at 8-10 DPI. PLF C develops from leaf baseout to leaf tip. Vein clearing observed at 12-21 DPI.	Numerous sunken PP LL appear at 4-5 DPI. LL do not expand. SYS light C mosaic appears at 6-10 DPI.
107-57	C S appear as LL (4-5 mm dia.) at 3 DPI. PLF showed SYM vein clearing at 10-20 DPI.	No LL appear. SYS mosaic & slight PLF distortion appears at 5-10 DPI.
Jurczack 2	No Symptoms	No Symptoms
Jurczack 3	No Symptoms	No Symptoms
Morrison 2	No Symptoms	No Symptoms
Morrison 4	No Symptoms	No Symptoms
NRSV-G	Light C blotches (5-8 mm dia.) appear as LL 4 DPI. C blotches diffuse to 10-12mm dia. at 14 DPI & fade out at 15-21 DPI. No SYS SYMP.	No Symptoms

Table 4. (continued) Herbaceous Host Species

Isolate	*Cucurbita maxima* Dusch. cv. 'Buttercup'
1A1	N R S appear as LL (2-3mm dia.) on COT at 3 DPI. N R S expand (4-5mm dia.) & some coalesce at 7 DPI. LL appear sunken. No SYS SYMP.
107-57	Many C S appear as LL (2-3mm dia.) at 3 DPI. C S become N S, expand (5-6mm dia.) & some coalesce at 5-7 DPI. COT collapse. No SYS SYMP.
Jurczack 2	Many sunken C S appear as LL (1-2mm dia.) with N centers at 3-4 DPI. Tissue between LL becomes C at 5-7 DPI. LL do not expand. No SYS SYMP.
Jurczack 3	C S appear (1-2mm dia.) as LL at 4 DPI. C S become sunken & show N centers at 7 DPI. LL do not expand noticeably. No SYS SYMP.
Morrison 2	Light C S (3-4mm dia.) appear at 3 DPI. C S become N S (4-5mm dia.) at 6-10 DPI. No SYS SYMP. COT become C at 1521 DPI.
Morrison 4	Diffuse C S (2-3mm dia.) appear as LL at 3 DPI. C S expand slightly (3-4 mm dia.) & become sunken. LL become N S (45mm dia.)at 15-21 DPI.
NRSV-6	Light C S appear as LL (3-4mm dia.) at 6-7 DPI. LL not sunken & no N develops. Tissue between LL becomes C at 10-12 DPI.

When C. sativus was inoculated with the 107-57 severe strain chlorotic ringspots appeared that were 3 to 4 mm in diameter (dia.) at two days post-inoculation (PI). The ringspots became completely necrotic in four to eight days PI. Lesions did not significantly increase in size. Tip die-back occurred at eight to ten days PI. The 1A1 isolate caused small chlorotic spots (1-3 mm dia.) to appear three days PI as local lesions on the cucumber cotyledons. The spots expanded (5-6 mm dia.) and became necrotic at five to seven days PI. Chlorotic spots appeared on the first partially expanded primary leaf at four days PI. Tip die-back occurred at ten to twelve days PI. The Jurczack 3 isolate caused light chlorotic spots (2-3 mm in dia.) on C. sativus L. cotyledons at four days PI which expanded considerably over the following days until the lesions coalesced to form large chlorotic blotches on the cotyledons. There was no tip die-back but terminal growth was stunted and internodes were compressed. The Jurczack 2 isolate also caused light chlorotic spots (1-3 mm dia.) to develop three to four days PI which in turn expanded concentrically to form chlorotic ringspots (4-5 mm dia.). The chlorotic ringspots coalesced to cause an overall yellowing of the cotyledons. There was no tip die-back and terminal stunting was similar to that of Jurczack 3. The Morrison 4 isolate caused small chlorotic spots (1-3mm dia.) on the C. sativus cotyledons three days PI which later expanded (5-6 mm dia.) and formed chlorotic ringspots, which finally faded. The first primary leaf showed numerous chlorotic ringspots. There was terminal and leaf stunting but no tip die-back. The Morrison 2 isolate caused C. sativus cotyledons to form light chlorotic spots (1-3 mm dia.) three days PI. In two experiments the cotyledons collapsed and the tips died back while in two other

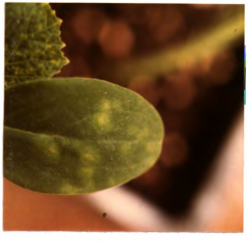
experiments the chlorotic spots expanded (4-6 mm dia.) to form chlorotic ringspots. Terminal growth was also stunted. The type-strain NRSV-G caused numerous chlorotic spots (1-2 mm dia.) to develop three days PI. The local lesions expanded (5 mm dia.) but no ringspots developed. The first primary leaf showed chlorotic spots (1-2mm dia.) at seven days PI and there was no necrosis or tip die-back. Terminal growth and leaves were stunted (Figure 1).

When the first pair of primary leaves of M. balsamina were inoculated with isolate 107-57, numerous chlorotic spots (< 1 mm dia.) appeared three days PI that expanded (2-3 mm dia.) at four to five days PI and eventually became completely necrotic eight to nine days PI. No systemic symptoms were observed. Isolate 1A1 caused large chlorotic spots (3-4 mm dia.) to develop at two to four days PI. The chlorotic spots became completely necrotic six to seven days PI. Lesions did not expand further and there were no systemic symptoms observed. The Jurczack 3 isolate caused scattered chlorotic spots to develop on the first primary leaves (< 1 mm dia.). The chlorotic spots expanded to 4-5 mm dia. and became necrotic five to six days PI. Jurczack 2 caused numerous small chlorotic spots (< 1 mm dia.) to form two to four days PI. The local lesions enlarged (2-3 mm dia.) and became completely necrotic four to six days PI. There was slight leaf curling on emerging leaves but this was also observed on buffer inoculated controls. M. balsimina leaves inoculated with the Morrison 4 isolate developed numerous light chlorotic spots (1-2 mm dia.) which enlarged (2-4 mm dia.) and became necrotic from the lesions' centers outward at four to six days PI. No systemic symptoms were observed. The Morrison 2 isolate caused chlorotic spots (2-3 mm dia.) to develop at three days PI which later enlarged and some became necrotic from the center outward

Figure 1. Symptoms on Cucumis sativus cv. 'National Pickling' plants inoculated with: 107-57 (known severe strain from Prosser, Washington; the Michigan isolates 1A1, Jurczack 2, Morrison 4; and NRSV-G (R. W. Fulton's type strain).

107-57

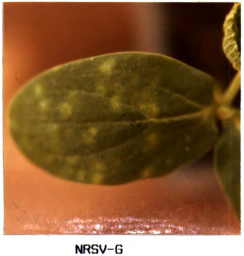
1A1



Jurczack 2

Jurczack 3

Morrison 2



NRSV-G

Figure 1.

from four to six days PI. Some of the spots enlarged further (5-6 mm dia.) and finally faded completely. No systemic symptoms other than a slight leaf curling occurred. M. balsimina leaves inoculated with NRSV-G developed numerous chlorotic local lesions approximately 1 mm in dia. at three days PI. Some local lesions expanded to 2 to 3 mm in dia. and faded out at four to six day PI. Some lesions continued to expand (4-6 mm dia.) and became necrotic at four to six days PI (Figure 2).

Chenopodium quinoa seedlings having three to four sets of leaves were inoculated with the various isolates. Numerous chlorotic spots (2-3 mm dia.) appeared at four days PI with the severe strain 107-57 which enlarged to 5-6 mm dia. at six to seven days PI. Some spots faded while others became necrotic at eight to ten days PI. No systemic symptoms were observed. The isolate 1A1 caused light chlorotic spots (4-5 mm dia.) to appear at three days PI. At five to six days PI the spots enlarged forming distinct ringspots. Newly emerging leaves were distorted and puckered, showing a chlorotic mottle at six to nine days PI. New leaves did not expand fully. The Jurczack 3 isolate caused numerous light chlorotic spots at three days PI (2-3 mm dia.) which became distinct at four to five days PI. The spots then faded over the next three to four days. The chlorotic spots did not expand and no systemic symptoms were observed. The Jurczack 2 isolate when inoculated to C. quinoa caused chlorotic spots to develop locally at three days PI. Chlorotic ringspots then developed as the chlorotic spots expanded (5-6 mm dia.) over the following two days. At seven to twelve days PI the chlorotic ringspots faded completely. No systemic symtpoms appeared. The Morrison 4 isolate caused the formation of numerous pinpoint chlorotic local lesions (< 1 mm dia.) at three days PI. Lesions enlarged

Figure 2. Symptoms on Momordica balsimina L. plants inoculated with: 107-57 (known severe strain from Prosser, Washington; the Michigan isolates 1A1, Jurczack 2, Morrison 4; and NRSV-6 (R. W. Fulton's type strain).

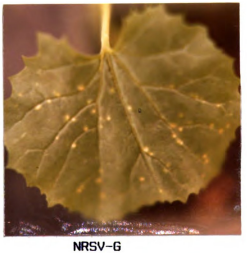
107-57

1A1

Jurczack 2

Jurczack 3

Morrison 2



NRSV-G

Figure 2.

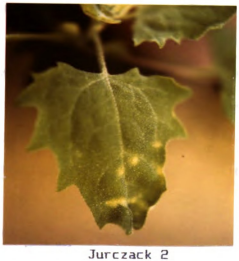
(2-3 mm dia.) and then faded out over the next five to nine days PI. No systemic symptoms appeared. The Morrison 2 isolate caused very diffuse chlorotic spots to develop at three days PI which then faded completely at five to seven days PI. Finally, PNRSV-G caused numerous local chlorotic spots (3-4 mm dia.) at three days PI. Spots expanded to 5-7 mm dia. and then faded from five to ten days PI. Local lesions finally faded completely and systemic symptoms appeared (Figure 3).

The cotyledons of Cucurbita maxima Duch. cv. 'Buttercup' were inoculated when they were approximately 3/4 expanded. Symptoms caused by 107-57 appeared as numerous chlorotic spots (2-3 mm dia.) three days PI (2-3 mm dia.). The spots became sunken and necrotic at five to seven days PI. The lesions enlarged (5-6 mm dia.) and necrosis followed until necrotic spots coalesced to form large necrotic areas. In some cases the cotyledons collapsed. The first primary leaf expanded normally and showed no symptoms. The isolate 1A1 caused sunken necrotic ringspots (3 mm dia.) to form three days PI. The necrotic areas expanded to up to 6 mm in diameter at seven days PI and some lesions coalesced to form large necrotic blotches. The Jurczack 3 isolate caused chlorotic spots to appear four days PI which became sunken and at seven days PI the lesions showed necrotic centers. The spots did not enlarge and no further necrosis occurred. No systemic symptoms were observed. Jurczack 2 caused sunken chlorotic spots (3-4 mm dia.) at three to four days PI which had necrotic centers 1-2 mm in diameter. The areas between individual local lesions became chlorotic at five to seven days PI. There was no enlargement and no further necrosis. No systemic symptoms were observed. C. maxima cotyledons developed diffuse chlorotic spots at three days PI when inoculated with the Morrison 4 isolate. The chlorotic spots enlarged to 3-4 mm in diameter and became sunken at five to

Figure 3. Symptoms on Chenopodium quiona Willd. plants inouclated with: 107-57 (known severe strain from Prosser, Washington; the Michigan isolates 1A1, Jurczack 2, Morrison 4; and NRSV-G (R. W. Fulton's type strain).

107-57

1A1



Jurczack 2

Jurczack 3

Morrison 2

NRSV-G

Figure 3.

seven days PI. The lesions became completely necrotic at fifteen to twenty days PI. No systemic symptoms appeared. The Morrison 2 isolate caused light chlorotic spots at three to four days PI (3-4 mm dia.). The chlorotic spots became completely necrotic at six to ten days PI. No systemic symptoms were observed. The type strain PNRSV-G caused very light chlorotic spots to appear at six to seven days PI (3-4 mm dia.). The lesions became sunken and there was no necrosis of the lesions. Tissue between lesions became yellow at ten to twelve days PI. There were no systemic symptoms observed (Figure 4).

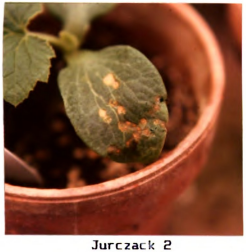
The range of symptoms that developed on the various hosts indicated that strains of PNRSV were present in the isolate collection. Each herbaceous host was considered individually and the symptoms caused by each isolate were given a severity ranking from zero to seven, zero being no symptom expression and seven being most severe. In cases where two isolates had the same rank, the highest rank was six rather than seven. For example, two isolates had equal rank of four so the highest rank was then six. The rankings are listed in Table 5. The summations of the rankings which appear at the bottom of the table were used as final rankings for the isolates.

In ranking the isolates, several assumptions were made concerning symptom severities. (1) Chlorotic spots, necrotic spots, chlorotic ringspots, necrotic ringspots and chlorotic blotch sizes were positively related to isolate severity. (2) Local lesions and systemic symptoms together on the same host were considered more severe than local lesions or systemic symptoms alone on the same host. (3) Terminal die-back was considered to be more severe than terminal stunting. (4) Necrosis was considered to be more severe than chlorosis. (5) The rate of symptom

Figure 4. Symptoms on Curcurbita maxima Dusch. cv. 'Buttercup' plants inoculated with: 107-57 (known severe strain from Prosser, Washington; the Michigan isolates 1A1, Jurczack 2, Morrison 4; and NRSV-G (R. W. Fulton's type strain).

107-57

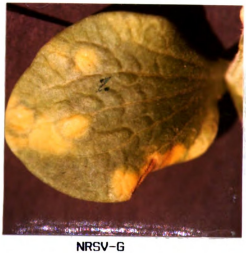
1A1



Jurczack 2

Jurczack 3

Morrison 2



NRSV-G

Figure 4.

Table 5: Severity Ranking of Five Michigan PNRSV isolates, One Prosser, WA severe PNRSV strain, and the PNRSV Type Strain Based on the Reaction of 11 Herbaceous Hosts.

Host	PNRSV Isolate						
	1A1	107-57	Jurc. 2	Jurc. 3	Morr. 2	Morr. 4	NRSV-6
Cucumis sativus cv. 'National Pickling'	5	6	4	4	3	1	2
Gomphrena globosa	1	2	0	0	0	0	0
Momordica balsimia	6	5	5	1	2	4	3
Tourenia foureneri	2	2	0	0	1	0	0
Tithonia speciosa	4	5	2	3	0	1	0
Chenopodium amaranticolor	5	4	3	0	3	1	2
C. quinoa	7	6	5	3	1	2	4
Vinca rosea	2	1	0	0	0	0	0
Zinnia elegans cv. 'Mixed Colors'	2	3	0	0	0	0	1
Nicotiana tabaccum cv. 'Virginia'	2	1	0	0	0	0	0
Cucurbita maxima cv.'Buttercup'	6	7	4	5	3	2	1
Total	42	42	23	16	13	11	13

For each host the strains were ranked from 1 to 7, 7 being most severe. If two isolates were ranked equally, then the severity ranking would begin at one and end with six. A zero indicates no symptoms developed.

dev

num

chl

mor

tio

as f

Morr

isol

and

acro

or n

wher

seve

and

host

mina

isol

the

five

symp

rang

seve

NRS

seva

was

mos

and

development was positively related to severity. (6) Local lesion number was positively related to severity. (7) The intensity of chlorosis was positively related to severity. (8) The degree of morphological distortion was positively related to severity. (9) Duration of symptoms were positively related to severity.

The ranking totals of each isolate in the host range study were as follows: 1A1 = 42, 107-57 = 42, Jurczack 2 = 23, Jurczack 3 = 16, Morrison 2 = 13, Morrison 4 = 11, NRSV-G = 13. From this ranking,the isolates have been grouped into three levels of severity. Isolate 1A1 and 107-57 (the known severe strain) caused the most severe symptoms across the host range. Isolate 1A1 or 107-57 were ranked either highest or next highest for each host except in the case of Mormordica balsimina where symptoms caused by 107-57 and Jurczack 2 were considered equal in severity. The Jurczack 2 isolate was ranked considerably lower than 1A1 and 107-57. Jurczack 2 caused symptoms on six of the eleven herbaceous hosts, and was ranked from second most severe in the case of M. balsimina to fourth most severe in the case of I. speciosa. The Jurczack 3 isolate was next in the severity ranking and did not differ greatly from the remaining three isolates. The Jurczack 3 isolate caused symptoms on five of eleven hosts, however, the Morrison 2 isolate alone caused symptoms on Zinnia elagans. The ranking of the Morrison 2 isolate ranged from third most severe in Chenopodium amaranticolor to least severe in Torenia fourieneri and Chenopodium quinoa. Ranking of the NRSV-G type strain ranged from fourth most severe in C. quinoa to least severe in Z. elegans and C. maxima. Finally, the Morrison 4 isolate was ranked least severe overall with individual ranks ranging from third most severe in M. balsimina to least severe in C. sativus, I. speciosa and C. amaranticolor.

The five severity ranking totals ranged from 42 to 11. The difference in ranking between the Jurczack 3, Morrison 2, Morrison 4 isolates and the NRSV-G type strain were not sufficent to assign them to three different severity levels. Therefore, these four isolates are grouped into the least severe level. The Jurczack 2 isolate had a total ranking that indicate it is more severe than the Jurczack 3, Morrison 2, Morrison 4 isolates and the NRSV-G type strain. Finally, the total rankings of 1A1 and 107-57 indicate that these two isolates are the most severe of the seven isolates tested.

DISCUSSION

In this initial study, three objectives were pursued: (1) the collection of isolates of PNRSV and PDV from symptomatic sour cherry trees (*P. cerasus* L.), (2) the identification of the viruses and separation of mixed cultures, and (3) the determination of PNRSV strains using differential host ranges.

Isolate Collection

Isolates of PNRSV and PDV were successfully collected over a 13 month period. However, when the initial isolations were made, the number of serial transfers from cucumber to cucumber were insufficient (specifically with PNRSV isolates) to maintain the virus cultures in an infectious condition. Waterworth and Fulton (1964) reported that to insure virulent inoculum from *C. sativus* L. cv. 'Lemon', transfers of PNRSV were made two or three times a week. Following re-isolation of the lost PNRSV cultures, transfers were made every four or five days and stock cultures were maintained in lyophilized cucumber cotyledons that had developed 15 to 20 local lesions per cotyledon.

The PDV Pugsley isolate was lost probably for the same reason that the PNRSV isolates were lost. The PDV Meachum isolate, however, retained its infectivity when maintained for long periods (2-3 weeks) in *C. sativus* L. cv. 'National Pickling'.

The limited number of virus cultures isolated in the 13 collection attempts and the relatively few isolates of PDV collected may have been due to any one of several factors. PNRSV may have been

present in higher frequency than PDV in the orchards sampled. The sampling method used may have been biased so that only PNRSV infected trees were sampled. The time of the growing season when collections were made could have been suboptimal for isolating PNRSV and even more so for PDV. The method used to transmit the viruses to cucumber or squash may not have been effective in transferring the virus(es) that was actually present in sour cherry tissue.

Several workers have surveyed sour cherry orchards for the incidence of PDV and PNRSV. S. Morrissey and D. Ramsdell (personal communication) have noted that PNRSV appears with considerably higher frequency than PDV in Michigan sour cherry orchards that have been assayed by ELISA for these two ilar viruses. Willison et al. (1948) reported that in sour cherry orchards, the incidence of PNRSV was considerably greater than the incidence of yellows (PDV). In one 12-year-old orchard, initial necrotic ringspot incidence was approximately 54% in 1940 and increased to approximately 98% in 1945. During the same period for the same orchard, the initial incidence of yellows was approximately 3% and increased to approximately 28%. There was no mention of doubly infected plants. These estimates were made by visual inspection and so were likely to contain a considerable amount of error. Gerginova (1980) reported that in a seven season study (1969-1975) on three orchards, the initial incidence of PNRSV was 8.2, 8.1 and 1.8%, while the incidence during the seventh season was 68.2, 30.3, and 21.4%, respectively. The initial incidence of PDV in the same three orchards was 2.1, 2.4, and 0.8%, while incidence during the seventh season was 35.3, 14.6, and 17.3%, respectively. He concluded that PNRSV spread more rapidly than PDV.

In the 13 tissue collections, trees were chosen based on conspicuous ringspot, leaf tatter and shot-hole symptoms on expanding and fully expanded leaves. These symptoms are associated with PNRSV (Nyland et al., 1976) and not necessarily with PDV infection. This may explain the failure to isolate PDV except from trees that were chosen not because of symptomlolgy but rather because the trees had tested ELISA positive for PDV only.

Finally, transmission of virus was almost certainly reduced by using leaf tissue that had matured. Since the first group of tissue collections was made from June through August, 1982, most of the tissue collected was in a mature condition. As cherry leaves mature, the relative amounts of tannins, phenolic and polyphenolic compounds increase (Cadman, 1959 and Cropley, 1964). These classes of compounds have been shown to reduce virus infectivity (Bawden and Kleczkowski, 1945; Hampton and Fulton, 1951 and Mink, 1965). The combination of declining virus titer in aging leaves and the concomitant rise in inhibitory compounds may explain why the summer isolation attempts met with only limited success.

Future isolate collection could be improved by first using serological assays for virus detection in combination with tree inspection for virus-induced symptoms during a growing season. Dormant budwood could then be collected during the winter months and buds could be forced in a greenhouse at temperatures of 18 to 24°C to provide expanding leaves and flower petals for inoculum. Alternatively, the dormant buds could be used directly as a source of inoculum (Davidson and Rundans, 1972). This approach would allow for virus isolation at times other than during the hectic spring weeks when flowering and initial leafing out occurs. Collected dormant budwood can be stored

under refrigeration and high relative humidity for several months and thus provide a reserve of virus if re-isolation would be required.

Virus Identification

In the isolate collections that were made in 1982, ten PNRSV isolates and one PDV isolate were identified by Ouchterlony tests. One isolate identified as TmRSV was discarded. None of the isolate collections were mixtures of PDV, TmRSV, TRSV, ApMV or PNRSV. The lack of mixed cultures, again may have been due to choosing trees with distinct symptoms associated with PNRSV. The samples taken had no or very little PDV present, so when inoculations to cucumber and squash where made only PNRSV had sufficent titre to affect transmission. In using the Ouchterlony test and ELISA, no isolates ever reacted with more than one antiserum. In the one-way Ouchterlony test with one antibody there were no spur formations between the unknown samples and the known diseased control. Any spur formation between such wells would have indicated some unique serological differences (one or more antigenic determinents not shared between isolates). This, however, does not prove that the viruses were serologically identical, since neither the antigens nor heterologous antibodies were in proper proportions for determining relatedness between strains. Additionally, all strains would have had to been tested against each antibody to determine relatedness.

Herbaceous Host Range

The results from the host range studies have indicated that there were differences between the isolates based on the generalized descriptions. In addition to differences between isolates there were also some differences between replications of the individuals treated alike. For

example, symptoms caused by the isolate Jurczack 2 on C. sativus cv. 'National Pickling' varied from development of local lesions and eventual overall chlorosis to collapse of cotyledons at 6 to 8 days PI. C. sativus reacted in two ways when inoculated with the Morrison 2 isolate. First, local lesions appeared, followed by either collapse of cotyledons and stunted primary leaves or expansion of chlorotic spots on cotyledons to form chlorotic ringspots. The cotyledonary collapse occurred once (replication 4). When M. balsimina was inoculated with the Jurczack 2 isolate, a systemic leaf curl appeared at 10 to 15 days PI in the third replication. The cause of leaf curl appeared to be a green peach aphid infestation. The Jurczack 2 isolate caused only a few local lesions on M. balsimina in replication two. T. fourneri was infected by the Morrison 2 isolate two of four times (replications one and four). In both cases, when infection occurred similar symptoms appeared. C. amaranticolor showed symptoms when inoculated with the 1A1, 107-57 and Morrison 4 isolates in three, two and two of the four replications respectively, and the symptoms were similar for each replication. V. rosea showed symptoms when inoculated with 1A1 and 107-57 in the same two replications (one and four). In the two replications that showed symptoms, the plants were growing vigorously and had much larger leaves than the plants in the two replications that did not show symptoms. Z. elegans cv. 'Mixed Colors' showed symptoms in two of the four replications (three and four) when inoculated with the type strain NRSV-G. N. tabacum cv. 'Virginia' developed symptoms when inoculated with the isolates 1A1 and 107-57 in two of four replications (one and four). Symptoms on C. maxima cv. 'Buttercup' were consistent except in the case of the Jurczack 2 isolate when the number of chlorotic spots was consi-

derably less in the second replication and as a result the inter-lesion chlorosis described in Table 4 was slower to develop in replication two.

The preceding variations in the host range could have been caused by a number of factors. In the experiments, there were temperature variations from 20 to 27°C, 16 to 35°C, 16 to 37°C and 16 to 27°C in replications one, two, three and four respectively (Appendix A). The high temperature extremes in replications two and three undoubtedly affected virus replication and thus symptom expression would have varied as well (Kassanis, 1957).

Virus titer in the inoculum probably varied among the isolates at the time of initiation of a given replication. Additionally, titer for a single isolate probably differed from one replication to the next. Since the virus isolates were cultured in C. sativus cv. 'National Pickling' seedlings, attaining seven cultures that were relatively synchronized was not always possible. As a result, some isolates may have been at a peak of titer while others were declining from peak titer. In an attempt to minimize the asynchrony, all the isolates were initiated from lyophylized cucumber tissue and on the same day (four to five days PI) equal numbers of local lesions were removed from infected plants. The lesions were placed in nearly equal volumes of buffer and extracted. If one or more isolates did not transfer from lyophylized tissue, more of the same tissue was used to reinitiate the isolate when the remainder of the isolates were transferred in live cucumber for the first time. Variations in titer were always present and this fact is acknowledged and has been taken into consideration in compiling the results of the severity rankings.

The varied reactions of the 11 hosts to the isolates used in the host range studies were similar in their symptomology. Tithonia speciosa cv. 'Mixed Colors' produced reactions that ranged from no symptoms to necrotic spots appearing as local lesions on inoculated primary leaves, followed in some cases by systemic vein clearing on other primary leaves. Fulton (1957a) found that T. speciosa developed no symptoms when inoculated with PNRSV-G. In this study PNRSV-G did not cause symptoms on T. speciosa. Kirkpatrick et al. (1967) reported that T. speciosa developed no symptoms when inoculated with PNRSV, while Gilmer (1961) showed that PNRSV would cause small necrotic local lesions on inoculated cotyledons or true leaves. Zinnia elegans inoculated with the various PNRSV isolates showed reactions ranging from no symptoms to chlorotic spots (4-5 mm dia.) appearing as local lesions which in some cases were followed by systemic vein clearing. My findings agree with those of Fulton (1957a) and Waterworth and Fulton (1964) that NRSV-G caused only local infection in Z. elegans. Varney and Moore (1952) reported symptoms on Z. elegans that ranged from a faint temporary mottle to a striking permanent yellow and white mottle. Their descriptions also concur with what was observed in my study. Kirkpatrick et al. (1964) reported that C. amaranticolor developed symptoms after inoculation with PNRSV that ranged from systemic chlorotic lesions to necrotic local lesions followed by systemic chlorotic lesions. In the current host range study, C. amaranticolor was found to react to NRSV by producing a wide range of symptoms, but systemic symptoms were not observed in any case. From these reports, it appears that there is no single reaction common to PNRSV isolates. The range in symptoms is rather wide.

The overall severity ranking (as decribed earlier) may or may not be correlated to the reaction of various isolates in Prunus cerasus L. cv. 'Montmorency'. Heinis (1956) described a "ringspot virus" that was transmitted from cherry trees to cucumber. Symptoms caused by various isolates on cucumber were compared to symptoms on P. avium cv. 'Bing', P. persica cv. 'J. H. Hale', and P. serrulata cv. 'Shirofugen' that were bud-inoculated with the same isolates. It was concluded that the symptom severity on cucumber apparently was correlated with the degree of symptom severity on the three Prunus species with few exceptions. In his experiment, however, Heinis did not complete Koch's postulates and so the bud source which was used may have contained more than one virus and likewise more than one virus strain.

In my work I did not correlate symptom expression on the host range species to that in P. cerasus, so the severity ranking that has been determined must at this point be resticted to how the virus isolates reacted within the host range. It may be dangerous to assume a direct relationship between a virus isolate's reaction in herbaceous host range and its reaction in P. cerasus.

CHAPTER TWO

Propagation of Virus-free *Prunus* Plant Material

INTRODUCTION

The goal for the second stage of my research was to clonally propagate *Prunus cerasus* L. 'Montmorency' and 'Meteor' by micro-propagation and by heel-cuttings and to clonally micro-propagate *Prunus cerasus* x *Prunus fruticosa* (clone 173/9). The propagation was done to produce uniform, virus-free clones in numbers that would allow for direct comparison of inoculation methods and PNRSV strains .

LITERATURE REVIEW

Sour cherry may be asexually propagated by several methods. Budding involves grafting a bud of a desired scion cultivar onto a rootstock. Upon healing of the bud graft union the top portion of the rootstock is removed to leave the scion as the sole actively growing shoot. One disadvantage to budding is that the important *Prunus* rootstocks are seed-propagated and thus are not genetically uniform. Sour cherry may also be propagated by stem cuttings. Heel cuttings (Hartman and Kester, 1975) of *P. cerasus* cv. 'Meteor' will root to a moderate degree when treated with 2000 ppm indole-3-butyric acid (IBA) (R. Perry, personal communication). 'Montmorency', however, has been difficult to propagate in this manner. Finally, sour cherry along with several other species of *Prunus* have been micro-propagated from shoot tips.

Micro-propagtion of Prunus species

The following Prunus species have been successfully micro-propagated: P.accolade (Boxus and Quoirin, 1974), P. avium (Snir, 1982; Feucht and Dausend, 1976; Dunstan, 1981), P. cerasus L. (Constantine and Abott, 1978; Popov et al, 1976), P. insistia (Hammerschlag, 1980), P. psuedocerasus (Feucht, 1976), P. salicinia (Rosati, 1980), and P. serrulata (Boxus and Quoirin, 1974).

Prunus tissue used as explants

Constantine and Abott (1978) reported that apical meristems or shoot tip explants of P. cerasus cv. 'Montmorency' were used to initiate shoot cultures. Shoot tips were found to establish more easily than meristems but subsequent growth after sub-culturing was similar for both tissue types. Rosati et al. (1980) also micro-propagated japanese plum (P. salicinia Lindl. cv. 'Calita') from shoot tips.

Dormant buds of P. avium L. have been used to establish shoot cultlures. Snir (1982) initiated cultures of the cultivars: 'Black Tartarian', 'Bing', 'Sam', 'Royal Ann', 'Burlat', 'Renier' and 'Early Ruby' by first surface disinfesting dormant buds and then dissecting the buds to remove to sterile primordia.

P. accolade and P. serrulata have also been micro-propagated from dormant sterile buds (Boxus and Quoirin, 1974). Only buds in "deep dormancy" were found to be able to differentiate shoots. The number of normal plants that were obtained from dormant buds was postively associated with the duration of the rest period.

Establishment Media

Constantine and Abott (1978) established explants on a Linsmaier

and Skoog basal medium (1965) modified with 0.5 uM (0.113 mg/l) 6-benzylaminopurine (BAP). From this medium, 80% of the explants eventually proliferated shoots.

Snir (1982) established dormant buds of P. avium on media described by Knop and by Tabachnik and Kester (1977) with the additional ammendments 1.0 mg/l BAP.

Rosati et al. (1980) established P. salicinia cv. 'Calita' shoots tips on a medium consisting of the Murashige and Skoog (M/S) mineral salts (Murashige and Skoog, 1962) with NaEDTA and $FeSO_4$ replaced by 20 mg/l FeNaEDTA plus 3% sucrose and 0.75% agar. The pH was adjusted to 5.9 with 0.1 N KOH before autoclaving.

Boxus and Quoirin (1974) established dormant bud explants of P. accolade and P. serrulata on media consisting of the M/S macro-nutrients and the Heller micro-nutrients and Jacquiot vitamin mixture (Gauthret, 1959), 0.1 ug/ml GA_3 (from 90% GA_3 stock), 1.0 ug/ml BAP, 2.0 x 0.02 ug/ml 2,4-Dichlorophenoxyacetic acid (2,4-D), 20.0 g/l sucrose, 8.0 g/l agar and pH adjusted to 5.0.

Shoot Proliferation Media

Popov et al. (1976) placed established explants of P. cerasus cv. 'Shubinka' on the medium described in the preceeding section with additional amendments. Addition of 0.1 to 0.5 mg/l kinetin had little effect on shoot proliferation and at concentrations of 10.0 mg/l proliferation was depressed. Addition of BAP at concentrations of 0.1 to 0.5 mg/l stimulated shoot proliferation. BAP concentrations of 1.0 mg/l inhibited shoot proliferation. When shoots were subcultured to fresh BAP media, additional shoot growth and proliferation from axillary buds was observed.

Snir (1982) found that *P. avium* cultivars proliferated shoots on three different media. The three media consisted of the establishment medium described by Tabachik and Kester (1977) ammended with either 1.0, 1.0 or 2.0 mg/l BAP and either 0.1, 1.0 or 1.0 mg/l IBA respectively. GA_3 was added at a rate of 0.5 mg/l to all three media. For the cultivars 'Sam' and 'Bing', the best axillary shoot proliferation occurred when sodium dikegulac was added to the medium at a rate of 500 to 1000 mg/l.

Rosati *et al.* (1980) placed established shoots of *P. salicinia* cv. 'Calita' on media consisting of M/S salts (1962) with NaEDTA plus 0.4 mg/l thiamine HCl, 100 mg/l myo-inositol, 1.0 mg/l BAP (as ammonium salt), 3% sucrose, and 0.75% agar. On this medium, dense masses of 15 to 20 shoots, originating from the initial explant, were formed after seven to eight weeks and after which subcultures were made. Additional subculturing followed at three to five week intervals, giving a proliferation rate of 10:1 to 20:1.

Boxus and Quoirin (1974) placed established explants onto proliferation media consisting of M/S macro-nutrients ,the Heller micro-nutrients and the Jacquiot vitamin mixture (Gauthret, 1959), 0.1 ug/ml GA_3, 0.02 ug/ml of 2,4-D, 20 g/l sucrose, 8.0 g/l agar and pH adjusted to 5.0.

Rooting Shoots in Culture

Popov *et al.* induced root formation on shoots of *P. cerasus* cv. 'Shubinka' excised from proliferation cultures by first soaking the cut ends of the shoots in a 50.0 mg/l IBA solution for 18 hours. The treated shoots were then placed on a medium containing macro-nutrients described by White (1963), the Heller micro-nutrients (1953), 2%

sucrose, 0.5 mg/l pyridoxine, 0.5 mg/l thiamine, 0.5 mg/l nicotinic acid, 1.0 mg/l ascorbic and 0.7% agar. The shoots first developed callus at the cut ends and eventually formed roots. Up to 60% of the treated shoots developed roots after two to three weeks and the roots grew and branched actively. Popov et al. noted that treatment of shoots that were less than 5 mm in length with IBA resulted in limited root formation (10-20%).

Snir (1982) rooted cultivars of P. avium on a medium consisting of the modified M/S salts (1964) and 0.5 mg/l napthalene acetic acid (NAA). By first wounding the base of the shoots the rate of rooting and the number of roots per shoot increased from 68% with 7.3 + 0.7 roots per shoot in the unwounded shoots to 80% rooting with 11.5 + 1.0 in the wounded shoots 14 days after being placed on the medium. Most cultivars eventually reached 100% rooting.

Feucht and Dausend (1976) placed surface sterilized 5 mm internode segments of P.avium on a modified M/S medium (Murashige and Skoog, 1962). The pH was adjusted to 5.3 prior to autoclaving. P. avium was found to root best when 1.0 mg/l NAA and 1.0 mg/l BAP or abscisic (ABA) was added to the basal medium. The rate of root initiation, however, was only 10%. Dunstan (1981) found that P. avium cv. 'Mazzard' (F12/1) rooted best when the proliferation medium contained 1.12 mg/l BAP and rooting medium contained 1.50 mg/l NAA. However, at 1.50 mg/l NAA, some roots were fused or associated with callus and plantlets which had such roots survived at lower rates. When final plantlet survival was considered, the optimal system was a rooting medium with an NAA concentration of 0.75 mg/l and a shoot proliferation medium ammended with 1.12 mg/l BAP. In addition, rooting was optimized when the M/S salt concentrations were reduced to 1/4 or 1/2 full

strength and when sucrose was increased from 14 to 42 g/l. Rooting normally occurred in four days and roots were from 0.6 to 1.2 cm in length.

When 5 mm internode segments of P. pseudocerasus were placed on a modified M/S medium, pH 5.3 and ABA and IBA added at a rate of 1.0 mg/l and 10.0 mg/l, respectively, 70% of the internode segments initiated roots (Feucht and Dausend, 1976). However, most roots were aerial and grew away from the media.

Boxus and Quoirin (1974) rooted P. accolade and P. serrulata on medium consisting of the M/S macro-nutrients (Murashige and Skoog, 1962), the Heller micro-nutrients and the Jacquiot vitamin mixture [(Gauthret, 1959), 0.2 ug/ml GA_3 (from 90% GA_3 stock), 1.0 ug/ml BAP, 2 x 0.02 ug/ml 2,4-D, 20 g/l sucrose, 8 g/l agar, 10^{-6} g/ml IBA and ph 5.0]. With this medium, root formation occurred on 25% of the shoots within 3 to 5 weeks.

MATERIALS AND METHODS

Two approaches were taken to produce plants for the inoculation experiments. Micro-propagation was attempted with 'Montmorency', 'Meteor' and a German rootstock, P. cerasus x P. fruticosa (clone 173/9). 'Meteor' was also propagated by vegetative heel-cuttings.

Micro-propagation

Plant material growing on a modified Linsmaier and Skoog (1965) shoot proliferation media (J. Liu, unpublished results) was provided by Dr. Ronald Perry and M. A. Polenick, Department of Horticulture, Michigan State University. The constituents of the medium are listed in Table 6. Plantlets were maintained in either two ounce screw-cap glass jars or in 25 X 150 mm siliconized culture tubes. Cultures were held in slant racks under cool white flourescent lights at approximately 34 uE/m sec. All cultures were maintained at 26 +/- 2 C. When shoots reached 2 to 5 cm in length, axillary and terminal shoots were subcultured individually. Subculturing was done every 2 to 4 weeks and after 8 weeks in the case of 'Montmorency'.

Shoots of 'Meteor' from field and greenhouse grown trees were established in culture. Actively growing shoots 3 to 10 cm in length were collected and placed in plastic bags on ice to maintain moisture and reduce respiration. Shoots were prepared for disinfestation by excising all fully expanded leaves and removing all stipules at the

Table 6: Shoot Proliferation Media used in Micropropagation of *Prunus cerasus* L. cv. 'Meteor' and *P. cerasus* x *P. fruticosa* (clone 173/9).

Macro-nutrient	mg/L
NH_4NO_3	1650.0
KNO_3	1900.0
$CaCl_2$ $2H_2O$	440.0
$MgSO_4$ $7H_2O$	370.0
KH_2PO_4	170.0
Na_2EDTA $2H_2PO$	37.25
$FeSO_4$ $7H_2O$	27.85
NaH_2PO_4 H_2O	170.0[a]
Micro-nutrients	
H_3BO_3	6.2
$MNSO_4$ $4H_2O$	22.3
$ZnSO_4$ $7H_2O$	8.6
KI	0.83
Na_2MoO_4 $2H_2O$	0.25
$CuSO_4$ $5H_2O$	0.025
$CoCl_2$ $6H_2O$	0.025
Organic Amendments	
Sucrose	30.0 g/L
myo-Inositol	100.0 mg/L
Adenine sulfate	80.0 mg/L
Agar	8.0 g/L
Thiamine HCl	0.4 mg/L
Growth Regulators	
6-benzylamino purine (BAP)	3.0 mg/L

pH adjusted to 5.8 prior to autoclaving

a = modification of L/S medium by J. Liu (1982)

base of each petiole. Most unexpanded leaves were removed and shoots were placed in tap water until the all the shoots were similarly prepared.

Four surface sterilization protocols were compared. For method I, shoots were collected on June 3, 1983 from actively growing 'Meteor' trees that had tested ELISA negative for PNRSV, PDV and ApMV. These shoots were taken from refrigeration on June 7, 1983 and placed in culture. Shoots were placed in a 10% bleach solution (full strength bleach is 5.25% by weight sodium hypochlorite) plus several drops of Tween-20 [Polyoxyethelene (20) sorbitan monolaurate] in an autoclaved bowl under a sterile laminar air flow hood. After 10 minutes, the bleach solution was poured off and shoots were given four one minute washes with sterile, distilled and deionized water. The shoots were left then in a fourth wash. Remaining unexpanded leaves and/or stipules were removed aseptically and the cut end of each shoot was recut to remove oxidized tissue. Shoots 3 to 6 cm in length were placed lengthwise on proliferation media in plastic petri plates (five shoots per plate). After five to ten days, shoots showing no obvious bacterial or fungal infection were transferred to either two ounce glass jars or glass culture tubes containing 20 ml of proliferation media.

Shoots treated by method II were taken from 3-year-old virus-free 'Meteor' trees actively growing in the MSU plant science greenhouse on June 11, 1983. Shoots were placed in a dilute solution of AlconoxR detergent (less than 1 g/L) and agitated for five minutes. Shoots were then quickly immersed in 95% ethanol and placed in a 10% bleach solution containing a few drops of Tween-20 for 20 minutes. Shoots were washed and plated as described in method one. Method III was identical to method two except that a 15% bleach solution was used. In method IV

shoots were collected from eight year old virus-free 'Meteor' at Hilltop Orchards, Lawrence, Michigan on June 23, 1983 and placed in culture on June 25, 1983. Shoots prepared as described previously were placed in an Alconox solution (less than 1 g/l) and agitated with a magnetic stirer for five minutes. After a two second dip in 95 % ethanol, the shoots were placed in a vaccuum flask containing a 15 % bleach solution plus Tween-20 (2-3 drops/l). Following a two minute vaccuum infiltration, the shoots were allowed to soak in the bleach solution for an additional ten minutes. Washing and plating was done as previously described. Clone 173/9 was established into culture from shoots collected from previously micro-propagated greenhouse-grown plants. Disinfesting method IV was utilized with clone 173/9 and no data were recorded for sterilization efficiency.

Subculturing was done every four to six weeks until sufficent numbers of cultures were produced. Shoots originating from terminal or axillary meristems were used for further subculturing. Adventitious shoots were avoided to maintain clonal integrity.

For rooting, proliferated shoots were excised and placed on a medium consisting of two thirds strength macro- and micronutrients described by Linsmaier and Skoog (1965) and additional micronutrients as described by Gamborg and Witter (1975). The constituents of the media are listed in Table 7. Each shoot that was 2 cm or greater in length was placed in two ounce sterile glass screw cap jars containing 20 ml of the rooting medium. The culture environment for rooting was identical to that described for shoot proliferation. Over a two week period, cultures with at least two well formed roots (greater than 2 cm in length) were transplanted. All agar was removed from the roots by for-

Table 7: Rooting Media used in Micro-propagation of Prunus cerasus L. cv. 'Meteor' and P. cerasus x P. fruticosa (clone 173/9).

CONSTITUENT	
Macroelements	**mg/L**
NH_4NO_3	550.0
KNO_3	633.3
$CaCl_2\ 2H_2O$	146.65
$MgSO_4\ 7H_2O$	123.3
KH_2PO_4	56.65
Na_2EDTA	37.25
$FeSO_4\ 7H_2O$	27.85
$NaH_2PO_4\ H_2O$	170.0[a]
Micro-nutrients	
H_3BO_3	4.66
$MnSO_4\ H_2O$	14.10
$ZnSO_4\ 7H_2O$	4.20
KI	0.227
$Na_2MoO_4\ 2H_2O$	0.250
$CuSO_4\ 5H_2O$	0.025
$CoCl_2\ 6H_2O$	0.025
Organic Amendments	
Sucrose	30.0 g/L
myo-Inositol	100.0 mg/L
Agar	7.0 g/L
Thiamine HCl	0.4 mg/L
Growth Regulators	
Indole-3-acetic acid	3.0 mg/L

pH adjusted to 5.8 prior to autoclaving

a= modification of L/S medium be J. Liu (1982)

ceps and soaking in tap water. The plantlets were dipped into a Captan and Thiram fungicide mixture (1:1, one teaspoon per gallon) and transplanted into sterilized Jiffy-7 peat pellets.

Plants were gradually acclimated inside plastic bags and treated twice weekly with the fungicide mixture. As the relative humidity around the plants was lowered by gradually opening the plastic bags, plants were less subject to fungal infestation. Plants were fertilized with a 0.1% KNO_3 solution after seven days in the peat pellets and once a week for two additional weeks. After approximately three weeks, the nearly acclimated plants were taken to the greenhouse where the plastic bags were left on for an additional week. The plants were transplanted into six inch clay pots containing a 3:3:3:3:2 (sphagnum peat moss : sand : perlite : vermiculite : loam) mixture and were fertilized weekly with a solution of Peter's Soluble Fertilizer (20-20-20) and once every four weeks with 330 Fe iron chelate (Ciba Geigy Co., Greensboro, NC). Every three months Peter's Soluble Trace Element Mixture was applied at one third the recommended strength for perennial shrubs.

The potted plants were grown under either flourescent light or high pressure sodium lights with daylengths of 16 hours. Despite the regular fertilization schedule and supplemental lighting, the plants periodically set terminal buds. To insure continued growth, a 500 ppm GA_3 solution was applied at intervals ranging from seven to 14 days.

Propagation by Heel-Cuttings

'Meteor' branches which had set terminal buds and had indexed negative for PNRSV, PDV and Apple Mosaic Virus (ApMV) were taken on June 17, 1983 from trees at Hilltop Orchards, Lawrence, Michigan. Branches were stored at approximately 4°C for two days. Cuttings were prepared by

removing shoots of the current year's growth while leaving a heel of one year old tissue from the branch. Leaves on the individual cuttings were either removed completely or clipped in half perpendicular to the midrib so that three or four half-leaves remained. Cuttings were then dipped into a 2000 ppm indole-3-butyric acid (IBA) solution for five seconds and immediately stuck into sterilized wooden flats containing perlite underlain with coarse gravel. The cuttings were stuck only deep enough to cover the heel with one to two cm of perlite, and wire was suspended over the flats to keep the cuttings upright. The flats were placed on a mist bench which maintained relative humidity at 70 to 90 % with two to three second bursts at one minute intervals from 6 am to 8 pm and from 1 am to 2 am in each 24 hour cycle. After six weeks under mist cuttings having formed at least one root greater than one cm in length were transplanted into the potting mixture previously described for micro-propagated plants. A dilute solution of Peter's 20-20-20 soluble fertilizer was applied at the end of two weeks. Rooted cuttings which did not break bud during or immediately after rooting were treated with 500 ppm GA_3 to induce bud break. The GA treatment was continued weekly until buds broke, and in some cases the bud scale was punctured to better expose the meristem to the hormone. Three weeks after transplanting, the rooted cuttings were placed on the same watering, fertilizing and GA schedule as was described for the micro-propagated plants.

RESULTS

Explant Establishment

The results of the four surface disinfestation methods are presented in Table 8. In method one, after ten days in petri plates, 11 of 33 shoots were not visibly contaminated with fungi or bacteria. The remaining 22 shoots were either contaminated or dead. In method two, after ten days, 25 of 40 shoots had no visible contamination and were placed on proliferation media; one shoot showed no visible contamination but was dead. Of the 35 shoots that were treated by method three, 28 shoots were not visibly contaminated after 10 days and four shoots died. In method four, 24 of 31 shoots showed no visible contamination after ten days and one shoot died.

Shoot Proliferation

When 'Montmorency' was cultured on proliferation media, no shoots developed. The cultures remained as a rosette, and after two subcultures to fresh media the rosettes began to yellow and die. The 'Montmorency' cultures were discarded after the second subculture.

'Meteor' cultures provided by R. Perry were subcultured and shoot proliferation continued for an additional three to four months. After a total of eight months in culture, 'Meteor' continued proliferating shoots; however, the newly emerged shoots developed a water-soaked, vitreous appearance. These shoots did not initiate roots when placed on rooting medium and eventually died.

Table 8. Results of four methods used to surface disinfest shoots of Prunus cerasus L. cv. 'Meteor' which were placed into aseptic tissue culture.

	Disinfestation Method			
	Method I[a]	Method II	Method III	Method IV
Total shoots treated	33	40	35	31
Number of dead shoots	--[b]	1	4	5
Number of contaminated shoots	22	18	8	6
Number of sterile shoots	11	21	23	20
Percent sterile shoots	33%	52.2	65.7	64.4

[a]Method I: shoots were soaked for 10 minutes in a 10% bleach solution; Method II: shoots were soaked for 5 minutes in a dilute Alconox solution, dipped into 95% ethanol and soaked for 20 minutes in a 10% bleach solution; Method III: same as method II except the bleach concentration was 15%; Method IV: shoots were soaked in a dilute Alconox solution, given a two second 95% ethanol dip and vaccuum infilitrated in a 15% bleach solution for two minutes then soaked for an additional 10 minutes in the bleach solution.

[b]No distinction was made between dead shoots and contaminated shoots for disinfestation Method I. Dead shoots were scored as contaminated.

The cultures of clone 173/9 also continued to proliferate shoots for eight to nine months. After eight months the proliferated shoots became brittle when handled during subculturing, but rooting still occurred when shoots were placed on the rooting medium.

In some cultures of 'Meteor', the leaves which came in contact with the proliferation medium developed adventitious shoots. Adventitious lines were not maintained, since it has been shown in other species (Solanum and Lycopersicon spp.) that plants derived from adventitious shoots have a higher frequency of mutations and chromosomal aberrations than shoots arising from apical or axillary meristems.

No conspicuous morphological abnormalities were seen in the other micro-propagated plants of 'Meteor' or clone 173/9. On several occassions plants that had been transplanted and placed in the greenhouse developed as a rosette with no main stem. However, application of 500 ppm GA_3 induced the rosetted plants to expand internodally and develop a main stem with actively growing terminal shoots.

DISCUSSION

The results from the four different surface disinfestation methods indicate that method III. and method IV. are the most effective. However, each method was used to treat only one group of shoots, and there may have been considerable variation in the degree to which the shoots were infested with surface pathogens. Also, since the methods were performed one after another, the worker's proficiency in handling the shoots may have improved with each new method so that the percent sterile shoots were a result of improved general technique, rather than an experimental modification. The shoot proliferation and rooting media used in this micropropagation system worked well with both P. cerasus cv. 'Meteor' and with the rootstock P. fruticosa x P.cerasus (clone 173/9). 'Montmorency' cultures remained in a rosette form and did not develop elongated shoots. This observation is similar to that of Popov et al. (1976). The addition of BAP to their proliferation medium at a concentration of 1.0 mg/l depressed the development of P. cerasus cv. 'Shubinka'. Constantine and Abbott (1978) also found that if the BA concentration was reduced from approximately 1.2 mg/L to 0.113 mg/L (5.0 to 0.5 uM), 80% of the Montmorency explants grew at the reduced concentration. The level of BAP used in my medium to culture 'Montmorency' was 3.0 mg/l.

Propagation by heel-cuttings may be of questionable value in the case of P. cerasus cv. 'Meteor'. Cuttings rooted very slowly and were

subject to algal and fungal infestations on the mist benches. Only 45 % of the cuttings resulted in plants that were sufficiently vigorous for use in the inoculation experiments. For many of the plants, repeated treatment with GA_3 was required for shoot growth.

CHAPTER THREE

Inoculation experiments with Prunus Necrotic Ringspot Virus (PNRSV) on *Prunus cerasus* L. 'Meteor' and *P. cerasus* X *P. fruticosa* (clone 173/9)

Introduction

In the previous two chapters, work has been described involving first, the collection of PNRSV isolates and the identification of strains within the group of isolates, and second, the propagation of virus-free clonal material. With this preliminary work completed, the final stage of this work was begun. The goals set were: (1) To test four inoculation methods alone and in combination in order to find the most effective method of infecting sour cherry plants with strains of PNRSV, (2) to determine the amount of time required after inoculation until all infected plants could be detected by enzyme-linked immunosorbent assay (ELISA), (3) to determine if strain differences affected transmission rates. The information gained upon realizing these goals would be valuable in continuing the work to identify both PNRSV and PDV heritable resistance to both PNRSV and PDV within the *Prunus* gene pool. Additionally, studies to determine the mode of inheritance of resistance genes will be much more precise.

Literature Review

Mechanical transmission of PNRSV to *Prunus* has been accomplished by using a number of inoculation methods. The methods range from graft

inoculation between and within species of Prunus to sap inoculation from herbaceous hosts (Cucumis sativus L. or Nicotiana tabaccum L.) to Prunus spp.

Bud inoculation was the earliest inoculation method reported and the method most commonly used to transmit PNRSV (Keitt and Clayton, 1939; Hildebrand, 1942; Moore, 1945 and Berkeley, 1947). In this method, dormant budwood is collected from a tree known to be infected with PNRSV. Plants are inoculated with infected budwood by chip budding, T-budding or budding in other ways that insure good contact of vascular tissues. The buds can be allowed to callus and develop a graft union with the recipient trees; or buds can be removed after a given period of time before complete healing in occurs (Fridlund, 1968).

Keitt and Clayton (1943) reported the results of several inoculation experiments with sour cherry yellows (PDV) which strongly suggested that cherry yellows was bud transmissable. In 1938, 24 three-year-old 'Montmorency' trees were bud inoculated with one or more virus-infected buds. None of the virus-infected buds produced shoots although union of tissues between the inserted bud-piece and the budded tree occurred in many cases. Twenty of the twenty-four inoculated trees showed symptoms in 1939 and four trees were symptomless. In 1940, the four previously symptomless trees showed symptoms. An experiment begun in 1939 included 52 trees that were bud inoculated with yellows infected 'Montmorency' buds. In 1940, 40 trees were diseased, six showed doubtful symptoms and six were symptomless. Keitt and Clayton noted that in all the cases where graft union (callusing) was observed between budwood and recipient, the disease was successfully transmitted. At the time of Keitt and Clayton's work "yellows infected" cherry trees were not neccesarily infected by PDV alone; PNRSV co-infection was possible and likely.

Hildebrand (1942) described a method for rapid transmission of PNRSV and SCY. Peach trees were inoculated first by chip budding one or two diseased buds on to a plant and then pruning the plant back to one dormant bud above the point of inoculation. He found that symptoms were produced on new growth from the dormant bud within 14 days after inoculation. Berkeley and Willison (1948) used the double budding technique to transmit PNRSV and yellows (PDV). One or more virus-infected buds were inserted into a Prunus mahaleb seedling, and above the inoculum buds a healthy bud of 'Montmorency' was inserted. PNRSV and PDV were transmitted to the 'Montmorency' bud but percentage infection was not reported.

More recently, Fridlund (1967, 1968) reported on the effects of time and temperature on graft transmission of PNRSV. Potted seedlings of Prunus tomentosa Thunb. were chip-bud inoculated at bud break with a single bud from P. domestica L. cv. 'Italian Prune' infected with PNRSV. The rate of graft transmission of PNRSV accelerated progressively as the environmental temperature was increased from 18 to 30°C. Further increases in temperature caused the rate of transmission to decrease progressively until transmission ceased at 38°C. Fridlund concluded that 30°C was the approximate optimum temperature for the most rapid graft transmission of PNRSV in P. tomentosa and a minimum of approximately 58 hr were required for 100% transmission to occur. Fridlund (1968) reported that graft transmission of PNRSV stopped immediately when the test trees were exposed to 38°C, and when test trees were returned to 26°C transmission resumed immediately. At 26°C approximately 20, 35, 75, 90 and 90% transmission was acheived when inoculum-receptor contact period was 48, 56, 64, 72 and 80 hr respectively. If the time the plants were at 38°C was subtracted from the total inoculum-

receptor contact period, the transmission rates were found to be similar to the rates when inoculated plants were constantly maintained at 26°C.

Transmission of PNRSV by sap inoculation has been accomplished when crude extract of young infected leaves of Prunus avium L. (triturated in 0.05 M phophate buffer, pH 7.8 or 0.01 M sodium diethyldithiocarbamate (DIECA)) was sap inoculated to P. pennsylvanica L. Also, PNRSV was transmitted from infected leaves of Nicotiana tabaccum L. cv. 'White Burley' that were triturated in the same two buffers just described (Cropley, 1960); percent transmission was not reported. In 1948, Moore et al. reported that a virus disease was transmitted from 'Montmorency' to cucumbers (Cucumis sativus L. cv. 'Ohio'). Transmission was accomplished by grinding very young cherry leaves that were just beginning to show the initial symptoms of PNRSV and then the undiluted, expressed juice was rubbed onto the carborundum dusted leaves of young cucumber plants. Others have also reported transmission of prunus necrotic ringspot from sour cherry to cucumber (Boyle et al., 1954). Transmission from cucumber back to 'Montmorency' was accomplished by placing small pieces of infected cucumber leaf under the bark of greenhouse grown cherry trees (Boyle et al., 1954). Symptoms of PNRSV developed on one of six bark flap inoculated trees. In another experiment, one of three sour cherry trees developed symptoms when barkflap inoculated with necrotic ringspot infected cucumber. R. W. Fulton (unpublished data) has also successfully transmitted PNRSV from infected cucumber back to species of Prunus by bark flap inoculation.

There have also been reports of relatively novel methods for transmitting woody plant viruses (Cropley, 1964; Linder, 1959). Cropley reported that apple chlorotic leaf spot virus was transmitted from

Chenopodium amaranticolor Costa & Reyn. to Malus pumila Mill. cv. 'Spy 227' by an inarch graft between the two plants. Seven 'Spy 227' plants were inarched to the stems of infected C. amaranticolor and after 2 to 4 months, four of the inarched 'Spy 227' plants developed symptoms typical of apple chlorotic leafspot virus. Virus was recovered from three plants by back inoculation to Chenopodium spp. A fifth apple plant developed symptoms the following year.

Lindner et al. (1959) reported that by using an air brush to apply inoculum, a 10 to 20 fold increase in efficiency of TMV transmission was achieved over conventional rub inoculation. Variability between replicates was also decreased by the air brush method. Inoculations with PNRSV yielded 16 times as many lesions on cucumber cotyledons with the air brush than did rub inoculations. When the inoculum was prepared from PNRSV-infected sour cherry petals, cucumber cotyledons that were airbrush inoculated developed 26 times more lesions per cucumber cotyledon than those that were rub-inoculated with a portion of the same inoculum.

MATERIALS AND METHODS

INOCULUM

Inoculum for each inoculation experiment was prepared from either: (1) virus infected cotyledons of Cucumis sativus L. cv. 'National Pickling', (2) from dormant buds of PNRSV infected Prunus cerasus L. cv. 'Montmorency' trees, or (3) partially purified virus that had been propagated in Chenopodium quinoa Willd.

Inoculum from Cucumis sativus L. cv. 'National Pickling'

To prepare inoculum from cucumber, each PNRSV strain was rejuvinated from lyophilized virus-infected cucumber tissue by first rehydrating the tissue in cold 0.03 M sodium mono- and dibasic phosphate buffer (inoculation buffer), pH 8.0, amended with 0.02 M 2-Mercaptoethanol and 10 mg/L polyvinylpyrrolidone (PVP). The tissue was allowed to rehydrate for at least ten minutes before it was triturated. Carborundum dusted young cucumber cotyledons were rub-inoculated as described previously in chapter one (pages 2-3). Local lesions were individually cut out four to five days post inoculation and used to transfer virus to additional cucumber plants. Two such transfers were made so that local lesions numbers increased to 15 to 20 per cotyledon. Upon initiation of the experiment, whole cotyledons were removed and triturated in cold inoculation buffer (5:1, ml of buffer per gram of tissue). Up to five inoculations were made with one inoculum preparation. Additional inoculum was prepared for the remaining inoculations.

Inoculum from PNRSV infected 'Montmorency'

Dormant buds were collected from the original trees infected with either PNRSV strain 1A1 or Jurczack 2. Each bud stick collected was ELISA tested to determine if PNRSV alone was infecting the bud wood. One bud from the terminal, middle and base of each stick was sampled, weighed and diluted with extraction buffer (described in chapter one) at a rate of 20 ml per gram of tissue, and crushed in a seed crushing plate. A 100 ul aliquot was added to each test well that had been coated with either anti-PNRSV, PDV or ApMV gamma globulin and the plates were incubated overnight at 2°C. The remainder of the procedure was the same as described in chapter one. The tested buds sticks were suitably identified, placed in moist sphagnum peat moss and stored at 2°C until needed for inoculations.

INOCULATION METHODS

Leaf-Rub Inoculation

Leaf-rub inoculations on cherry plants were completed by dusting several expanding leaves with carborundum adsorbing infectious cucumber sap onto a sterile foam rubber sponge blotting excess sap off the sponge and uniformly rubbing the upper surfaces of three leaves (6-8 strokes per leaf). Within 90 seconds after the last leaf was inoculated, inoculum was rinsed off with tap water. All the plants for a given treatment were inoculated consecutively. Upon completion of the first treatment, the second treatment was applied to the next group of plants.

Bark-Flap Inoculation

Bark-flap inoculations were completed by first cutting a small piece of cucumber cotyledon (approximately 0.5 x 1.0 cm) containing at least one local lesion. On the stem of the test plant, a flap of bark was cut back with a razor blade, and the cucumber tissue was immediately inserted under the flap. The bark-flap was then pressed firmly down over the cucumber tissue and an adhesive, latex bandage (Sealtex Co., Clearwater, FL 33515) was wrapped over the bark-flap both to hold it in place and to maintain high moisture around the inoculation site. The latex bandage was left in place throughout the experiment.

Bud Inoculation

Either a chip-bud graft or a side veneer graft was utilized to inoculate the test plants. The method of executing the chip bud graft and the side veneer graft are described by Hartman and Kester (1975). After each chip bud or "scion piece" was secured by budding rubbers, Parafilm® (American Can Company, Dixie/Marathon, Greenwich, CT 06830) was wrapped over the budding rubber to seal the grafted area. After 7 to 10 days the Parafilm® was removed while the budding rubbers were removed after 3 to 4 weeks. When chip buds were used to inoculate test plants, the top of each plant was pruned back leaving 5 to 7 buds above the inoculum source. This was done to encourage new growth and rapid symptom development. No pruning followed the side-veneer graft inoculations. In all experiments, a 500 ppm GA_3 solution was applied routinely to maintain new shoot growth.

Root-rub Inoculation

Inoculum for root-rub inoculations was prepared in the same manner described for leaf-rub inoculations. To inoculate the cherry

plants, individuals were removed from their pots; soil was shaken and washed off the root system and the roots were bloted dry between paper towels. The roots were then lightly dusted with carborundum (320 mesh). Using a sterile foam rubber sponge, the inoculum was rubbed onto as much of the root system as possible. The roots were washed immediately after inoculation and the plants were transplanted to fresh sterilized soil mix. In all experiments except number one, root-inoculated plants were placed under shading to prevent water stress. Root-rub inoculation apparently damaged the root systems so that severe wilting occurred if plants were exposed to full sunlight. Root-rub inoculations were always carried out last a treatment; that is, if a combination of inoculations were done on a given group of plants, leaf rub and bark flap inoculations were completed before the root inoculations.

Inoculation Experiments

Experiment I

The experiment was arranged in a completely randomized design with five treatments and ten replications per treatment. Fifty-five heel cutting propagated 'Meteor' plants were selected for uniformity, divided into five groups of ten and one group of five and on November 19, 1983 then placed in darkness for 48 hours. As stated in chapter 2, all cherry plants used in the following experiments tested ELISA negative for the presense of PNRSV, PDV and ApMV. Individual plants were removed from darkness on November, 21; the leaves were dusted with carborundum and then rub inoculated with cucumber sap infected with one of the following viruses: (1) PNRSV strain 1A1, (2) PNRSV strain Jurczack 2, (3) PNRSV strain 107-57, (4) PNRSV strain CH-42 partially

purified (mild strain from Prosser, Washington), (5) Control, healthy 'National Pickling' cucumber. The purification protocol for strain CH-42 is outlined in Figure 5 (W. Howell, IAREC, Washington State University, Prosser, WN, personal communication). The last group of five plants was inoculated with the PNRSV strain Jurczack 2 by the bark flap method. The inoculated plants were then randomized on a greenhouse bench under high pressure sodium lamps set for 16 hr daylengths. The plants in experiment I were ELISA tested on the following dates to determine if virus infection had occurred : January 6, February 7, March 12, April 23, and October 24, 1984. For ELISAs performed prior to May 1, 1984 A_{405nm} (A405) values were read from a Microelisa ® spectophotometer (Dynatech. Lab. Inc., Alexandria, VA 22305). On sample dates after May 1, 1984 A_{405} readings were taken with a EIA® reader, model 307 (Bio-Tek Instruments, Inc., 1 Mills St., Burlington, VT 05401). For all ELISA tests after May 1, absorbances were recorded to three decimal points.

When sampling for ELISA tests, young, expanding leaves were preferentially collected, and if no expanding leaves were present, the youngest leaves were sampled. Preparation of leaf tissue has been described in chapter one. On October 24, 1984, dormant buds were collected from the test plants rather than leaf tissue in all seven experiments. Sampled bud tissue was weighed and 20 ml of extraction buffer was added per gram of tissue. The remainder of the procedure was followed as described in chapter one except that substrate was allowed to incubate for 30 min in all ELISA tests prior to reading absorbance values. When determining ELISA positives, absorbance values for the healthy control plants were averaged and standard deviations calculated. Critical values used were the sum of the healthy mean absorbance plus

Figure 5. Partial purification protocol used to prepare inoculum from *Chenopodium quinoa* Willd. systemically infected with the mild Prosser, WA PNRSV strain CH-42.

Partial Pruification for PNRSV (strain CH-42) from Systemically Infected Chenopodium quinoa Willd.

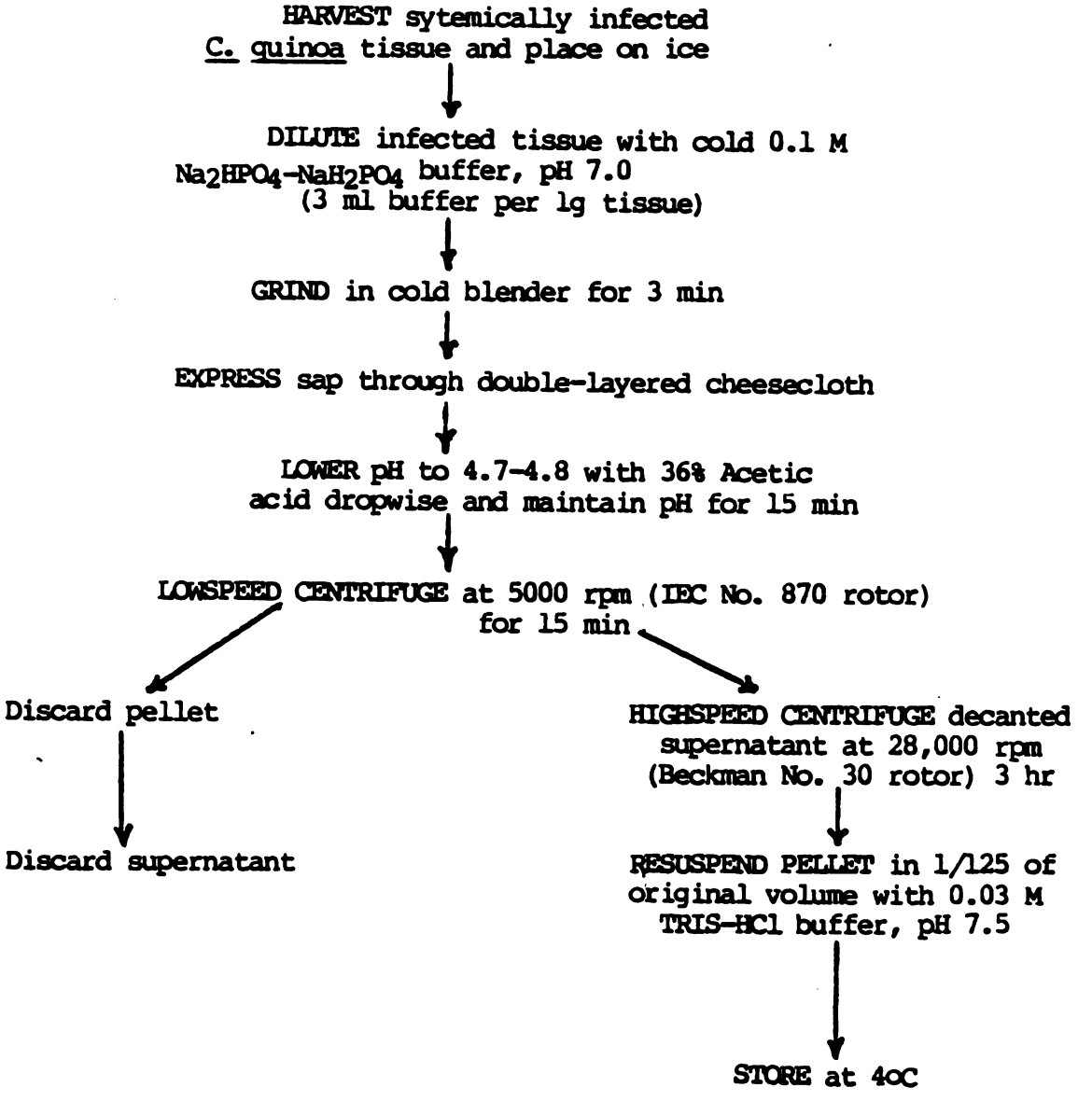


Figure 5.

three times the standard deviation from the healthy mean. Absorbances above the critical value were considered ELISA positive and any absorbance equal or less than the critical values was considered negative. This amended ELISA procedure was used in all other experiments.

Experiment II

This experiment was arranged in a completely randomized design with five treatments and ten replications per treatment. To initiate experiment II, 50 heel-cutting propagated 'Meteor' plants were selected for uniformity, placed randomly in groups of ten and given a dark treatment 48 hr prior to inoculation. A single PNRSV strain, Jurczack 2, was utilized in this experiment. The virus was propagated in cucumber as already described, and when the virus cultures were at peak titer (3 to 4 days post inoculation) five treatments were applied to the five groups of plants. The treatments consisted of individual inoculation methods and combinations of the methods as follows: (1) Leaf-rub inoculation (LF), (2) Bark-flap inoculation (BF), (3) Leaf-rub and bark-flap inoculation (LF/BF), (4) Leaf-rub, bark-flap and root-rub inoculation (LF/BF/R), (5) Healthy control (leaf and bark-flap inoculation with healthy 'National Pickling' cucumber). A given treatment was applied to all replicates before the next treatment was applied. After all treatments were applied, plants were randomized as described in experiment I.

All inoculations were made on February 21, 1984 and plants within the experiment were ELISA-tested on April 3, April 18, May 1, June 5 and October 24, 1984. Expanding or youngest leaves were sampled and prepared as described previously. Bud samples were taken on April 18 and October 24, 1984 and prepared as described in experiment I.

Experiment III

The experiment was arranged in a completely randomized design with four treatments and ten replications per treatment. Forty micro-propagated 'Meteor' were selected for uniformity, randomly placed into four groups of ten and given a 48 hr dark treatment. The PNRSV strains 1A1, Jurczack 2, and NRSV-G were propagated in cucumber prior to initiating the experiment. The three PNRSV strains and healthy cucumber control were the treatments and were inoculated to the respective groups of ten cherry plants by the leaf-rub and bark-flap combination. For a given strain all plants were first leaf-rub inoculated after which all the plants were bark-flap inoculated. For each strain, all ten replicates were inoculated before moving on to the next strain. Upon completing inoculations, the plants were randomized on a greenhouse bench and given supplemental lighting by four-tube banks of eight-foot cool white flourescent light for 16 hr each day. The experiment was initiated on March 5, 1984 and the plants were ELISA tested for on the following dates: April 23, May 7, June 5 and October 24, 1984.

Experiment IV

The experiment was arranged in a completely randomized design with four treatments and 20 replications per treatment. In this experiment, 80 micro-propagated clone 173/9 plants were chosen for uniformity, placed randomly in four groups of 20 and given a 48 hr dark treatment. The inoculation method used in this experiment was the leaf-rub and bark-flap combination. The four treatments were: (1) PNRSV strains 1A1, (2) PNRSV strain Jurczack 2, (3)PNRSV strain NRSV-G and (4) healthy cucumber as a control. The experiment was initiated on May 3, 1984 and the plants were ELISA tested on the following dates: June 5,

July 24, August 13, and October 24, 1984. Leaf tissue was sampled on the first three dates and dormant bud tissue was sampled on October 24, 1984. This experiment is a repetition of experiment except that heel-cutting propagated plant were used.

Experiment V

The experiment was executed in a completely randomized design with two treatments and 30 replications per treatment. For experiment five, 60 heel-cutting propagated 'Meteor' plants were selected for uniformity, randomly placed in two groups of 30 and given a 48 hr dark treatment. The bud inoculation method was used to inoculate the treatments: (1) PNRSV strains 1A1 and (2) Jurczack 2. Following the bud inoculation, all plants were placed randomly in a mist chamber to maintain high relative humidity around the bud-scion graft. After seven days the plants were removed from mist and randomized on greenhouse benches as described for all other experiments. Supplemental light was provided by four-tube banks of eight-foot cool white flourescent lights for 16 hours each day. The experiment was initiated on June 12, 1984 and plants were ELISA tested for PNRSV on the following dates: July 24, August 13, September 19, and October 24, 1984. In this experiment healthy control plants from experiments three and four were sampled for the ELISA test to conserve plant material for experiment number seven.

Experiment VI

The experiment was arranged in a completely randomized design with five treatments and 30 replications per treatment. The 150 micropropagated plants of P. cerasus x P. fruticosa (clone 173/9) were selected for uniformity, randomly placed in five groups of 30 and given a 48 hr dark treatment. The leaf-rub and bark-flap combination and bud

inoculation methods were used. The PNRSV strains 1A1 and Jurczack 2 were either propagated in cucumber or budwood was utilized as described previously. The treatments were: (1) strain 1A1, L/BF; (2) strain 1A1, bud; (3) strain Jurcack 2, L/BF; (4) strain Jurczack 2, bud; and 5) healthy control, L/BF (healthy 'National Pickling' cucumber). In this experiment, the leaf-rub and bark-flap inoculations were done on June 28, 1984 while the bud inoculations were done the following day on June 29, 1984. The experiment was ELISA-tested for PNRSV on the following dates: August 13, September 3 and October 24, 1984.

Experiment VII

The experiment was executed in a completely randomized design with 30 replications per treatment. Sixty heel-cutting propagated 'Meteor' were selected for uniformity, randomly placed in two groups of 30 and given a 48 hour dark treatment. Following the dark treatment, one group was bud inoculated with PNRSV strain 1A1 while the second group was bud inoculated with PNRSV strain Jurczack 2. No control plants were inoculated with healthy buds, however, healthy control plants from experiment III and IV were used during all virus assays. The experiment was initiated on June 30, 1984 and the plants were ELISA-tested for PNRSV infection on August 14, September 19 and October 24, 1984.

RESULTS

Experiment I

On January 6, 1984, six weeks after inoculation, plants were assayed by ELISA for PNRSV infection. Based on the critical value (healthy mean plus three standard deviations) all plants inoculated by the leaf-rub and root-rub L/R combination with either PNRSV strain 1A1, Jurczack 2 or 107-57, tested ELISA negative (Table 9). All plants inoculated by the bark flap method (BF) with PNRSV strain Jurczack 2, tested ELISA negative. The absorbance values for each replicate on each test date are shown in Appendix B (Table B1).

For the second ELISA on February 7, the five treatments 1A1(L/R) Jurczack 2(L/R), 107-57(L/R), CH-42(L/R), and Jurczack 2(BF) gave positive ELISA tests on zero plants in ten, zero plants in ten, two plants in ten (20%), zero plants in ten and one plant in five (20%) respectively. From the March 12 ELISA, only treatment Jurczack 2(BF) resulted in three plants in ten (60%) testing ELISA-positive. On April 23, 1984 one plant in ten (10%) treated with 107-57 (L/R), one plant in ten (10%) treated with CH-42(L/R) and one plant in five (20%) treated with Jurczack 2(BF) tested ELISA-positive. Finally, from the ELISA conducted on October 24, 1984, Jurczack 2 (BF) tested positive for four plants in ten (40%). Table 10 shows the percentage of plants testing ELISA-positive on at least one of the test dates. Absorbance values for each test date are listed in Appendix B (Table B1).

Table 9. Percentages of 'Meteor' sour cherry plants which tested ELISA-positive in experiment I on five sample dates. Plants were inoculated with one of four PNRSV strains.[a]

	PNRSV Strain (Inocultion Method)					
Date	1A1 (L/R)[b]	Jurc2 (L/R)	107-57 (L/R)	CH-42 (L/R)	Jurc2 (BF)	Control (L)
Jan. 6, 1984	0[c]	0	0	0	0	0
Feb. 7	0	20	0	0	20	0
March 12	0	0	0	0	60	0
April 23	0	0	10	10	20	0
Oct. 24	0	0	0	0	80	0

[a] Virus strains: 1A1=PNRSV severe strain 1A1, Jurc2=PNRSV intermediate strain Jurczack 2, CH-42=partially purified Prosser, WA mild strain CH-42, Control=extraction buffer (0.03 M mono- and di-basic sodium phosphate with 0.02 M 2-ME, pH 8.0).

[b] Inoculation methods: L=leaf-rub, L/R=leaf-rub and root-rub combintion, and BF=Bark flap inoculation.

[c] Ten plants per treatment were inoculated on Nov. 21, 1983, except for the treatment Jurc2 (BF) where 5 plants were used.

Table 10. The cumulative percentages of 'Meteor' sour cherry plants from experiment I which tested ELISA-positive on at least one of five sample dates. Plants were inoculated with one of four PNRSV strain.[a]

	PNRSV Strain (Inocultion Method)					
Date	1A1 (L/R)[b]	Jurc2 (L/R)	107-57 (L/R)	CH-42 (L/R)	Jurc2 (BF)	Control (L)
Jan. 6, 1984	0[c]	0	0	0	0	0
Feb. 7	0	20	0	0	20	0
March 12	0	20	0	0	60	0
April 23	0	20	10	10	80	0
Oct. 24	0	20	10	10	100	0

[a] Virus strains: 1A1=PNRSV severe strain 1A1, Jurc2=PNRSV intermediate strain Jurczack 2, CH-42=partially purified Prosser, WA mild strain CH-42, Control=extraction buffer (0.03 M mono- and di-basic sodium phosphate with 0.02 M 2-ME, pH 8.0).

[b] Inoculation methods: L=leaf-rub, L/R=leaf-rub and root-rub combintion, and BF=Bark flap inoculation.

[c] Ten plants per treatment were inoculated on Nov. 21, 1983, except for the treatment Jurc2 (BF) where 5 plants were used.

Experiment II

Plants in experiment two were first assayed by ELISA on April 3, 1984, and the treatment L/BF had zero plants in ten test ELISA-positve while the treatments L, BF and L/BF/R had one plant in ten (10%), three plants in ten (30%) and one plant in ten (10%), respectively test ELISA positive.

On April 18, the treatments L, L/BF and L/BF/R had one plant in ten (10%), six plants in ten (60%) and two plants in ten (20%), respectively, test ELISA-positive. Table 11 shows the percentage of plants that tested ELISA-positive for each treatment at each test date. Table 12 shows the percentage of plants that tested ELISA-positive on at least one test date.

From the ELISA conducted on May 1, June 5, and October 24, 1984, no plants tested ELISA-positive.

At ten days to three weeks post inoculation, conspicuous leaf symptoms developed on inoculated plants that closely resembled foliar field symptoms caused by PNRSV infection. The symptoms appeared sporadically on each of the treatments which included leaf rub inoculations. Data for each ELISA test are listed in Appendix B (Table B2).

Experiment III

The plants in experiment three were ELISA tested for PNRSV infection on April 23, 1984. Based on the critical A405 value (healthy mean plus three standard deviations) the treatments Jurczack2 and NRSV-G resulted in two plants in ten (20%) and one plant in ten (10%), testing ELISA positive, respectively (Table 13).

On May 7, both treatment strain 1A1 and Jurczack2 had zero plants in ten test ELISA-positive. The treatment strain NRSV-G had one plant

Table 11. The percentages of 'Meteor' sour cherry plants which tested ELISA-positive in experiment II on five sample dates. Plants were inoculated with PNRSV strain Jurczack 2 by one of four methods.[a]

Date	Inoculation Method				
	L[b]	BF	L/BF	L/BF/R	Control
April 3, 1984	10[c]	30	0	10	0
April 18	10	0	60	20	0
May 1	0	0	0	0	0
June 5	0	0	0	0	0
Oct. 24	0	0	0	0	0

[a] Plants were inoculated on February 21, 1984.

[b] Inoculation methods: L=leaf-rub, BF=bark-flap, L/BF=leaf-rub/bark-flap combination, and L/BF/R=leaf-rub/bark-flap/root-rub combination. PNRSV-infected cucumber was used as inoculum with each method.

[c] Ten plants per treatment were inoculated for each treatment.

Table 12. The cumulative percentages of 'Meteor' sour cherry plants which tested ELISA-positive in experiment II on at least one of five sample dates. Plants were inoculated with PNRSV strain Jurczack 2 by one of four methods.[a]

Date	Inoculation Method[b]				
	L	BF	L/BF	L/BF/R	Control
April 3, 1984	10[c]	30	0	10	0
April 18	20	30	60	30	0
May 1	20	30	60	30	0
June 5	20	30	60	30	0
Oct. 24	20	30	60	30	0

[a] Plants were inoculated on February 21, 1984.

[b] Inoculation methods: L=leaf-rub, BF=bark-flap, L/BF=leaf-rub/bark-combination, and L/BF/R=leaf-rub/bark-flap/root-rub combination. PNRSV-infected cucumber was used as inoculum with each method.

[c] Ten plants per treatment were inoculated for each treatment.

Table 13. The percentage of 'Meteor' sour cherry plants which tested ELISA-positive in experiment III on four dates. Three PNRSV strains were inoculated by the leaf-rub/bark-flap combination.[a]

Date	PNRSV Strains[b]			
	1A1	Jurc2	NRSV-G	Control
April 23, 1984	0[c]	20	10	0
May 7	0	0	10	0
June 5	0	0	0	0
Oct. 24	40	10	0	0

[a] Inoculum consisted of PNRSV-infected cucumber.

[b] Virus strains: 1A1=severe PNRSV strain 1A1, Jurc2=intermediate PNRSV strain Jurczack 2, NRSV-G=R. W. Fulton's PNRSV type strain G, Control= healthy cucumber.

[c] Ten sour cherry plants were inoculated per treatment on March 5, 1984.

in ten (10%) test ELISA-positive. On the third test date, June 5, no plants tested ELISA-positive.

From the last ELISA-test on October 24, the treatment strain 1A1 and Jurczack2 had four plants in ten (40%) and one plant in ten (10%) test ELISA positive,respectively. Table 13 shows the percentage of plants testing ELISA positive for each treatment on each test date. Table 14 shows the cumulative percentage of plants that tested ELISA positive on at least one date. Absorbance values for each ELISA test are listed in Appendix B (Table B3).

Experiment IV

The first ELISA-test was conducted on June 5, 1984. Of the plants treated with 1A1, Jurczack2 and NRSV-G one plant in twenty (5%), five plants in twenty (25%) and zero plants in twenty, respectively, tested ELISA positive. The second ELISA-test conducted on July 24 indicated that for the treatment strains 1A1, Jurczack2 and NRSV-G, one plant in twenty (5%), two plants in twenty (10%) and nine plants in twenty (45%) tested ELISA-positive, respectively. The third ELISA-test on August 13 showed that the treatments 1A1 and Jurczack2 and NRSV-G had one plant in twenty (5%) and three plants in twenty (15%) and zero plants in twenty respectively test ELISA-positive.

On October 24, a fourth ELISA was conducted and the treatments 1A1 and Jurczack2 both had five plants in twenty (25%) test ELISA positive while the treatment NRSV-G had zero plants in twenty test ELISA positive. Table 15 shows the percentage of plants that tested ELISA positive for each treatment on each test date. Table 16 shows the cumulative percentage of plants testing ELISA-positive on at least one test date.

Table 14. The cumulative percentage of 'Meteor' sour cherry plants which tested ELISA-positive in experiment III on at least one of four dates. Three PNRSV strains were inoculated by the leaf-rub/bark-flap combination.[a]

	PNRSV Strains[b]			
Date	1A1	Jurc2	NRSV-G	Control
April 23, 1984	0[c]	20	10	0
May 7	0	20	20	0
June 5	0	20	20	0
Oct. 24	40	30	20	0

[a] Inoculum consisted of PNRSV-infected cucumber.

[b] Virus strains: 1A1=severe PNRSV strain 1A1, Jurc2=intermediate PNRSV strain Jurczack 2, NRSV-G=R. W. Fulton's PNRSV type strain G, Control= healthy cucumber.

[c] Ten sour cherry plants were inoculatled per treatment on March 5, 1984.

Table 15. The percentage of 'Meteor' sour cherry plants which tested ELISA-positive in experiment IV on four dates. Three PNRSV strains were inoculated separately to plants by the leaf-rub/bark-flap combination.[a]

Date	PNRSV Strain[b]			
	1A1	Jurc2	NRSV-G	Control
June 5, 1984	5[c]	25	0	0
July 24	5	10	45	0
Sept. 13	5	15	0	0
Oct. 24	25	25	0	0

[a] Inoculum was from PNRSV-infected cucumber.

[b] Virus strains: 1A1=severe PNRSV strain 1A1, Jurc2=intermediate PNRSV strain Jurczack 2, NRSV-G=R. W. Fulton's type virus strain G, and Control=healthy cucumber sap.

[c] Twenty sour cherry plants per treatment were inoculated on May 3, 1984.

Table 16. The cumulative percentage of 'Meteor' sour cherry plants which tested ELISA-positive in experiment IV on at least one of four dates. Three PNRSV strains were inoculated seperately to plants by the leaf-rub/bark-flap combination.[a]

Date	PNRSV Strain[b]			
	1A1	Jurc2	NRSV-G	Control
June 5, 1984	5[c]	25	0	0
July 24	10	35	45	0
Sept. 13	10	40	45	0
Oct. 24	30	55	45	0

[a] Inoculum was from PNRSV-infected cucumber.

[b] Virus strains: 1A1=severe PNRSV strain 1A1, Jurc2=intermediate PNRSV strain Jurczack 2, NRSV-G=R. W. Fulton's type virus strain G, and Control=healthy cucumber sap.

[c] Twenty sour cherry plants per treatment were inoculated on May 3,1984.

Chi-square analyses were conducted to compare treatments in a pair-wise fashion (Tables 17 & 18). In the first analysis (Summation index Chi-square) a summation index was calculated by summing the number of ELISA positives over the four ELISA sample dates. In each treatment comparison the observed summation index did not differ significantly from the expected 1:1 ratio. In a second Chi-square analysis (Cummulative index Chi-square) a cumulative index was caluculated for each treatment as the sum of the 'Meteor' plants that ELISA-positive on at least one ELISA sample date. In each pair-wise treatment comparison the observed cumulative indices did not differ significantly from the expected 1:1 ratio. Absorbance values for each test date are listed in Appendix B (Table B4).

Experiment V

The first ELISA was conducted on July 24, 1984. For the treatments strain 1A1, bud (1A1,B) and Jurczack 2, bud (Jurc2,B) eight plants in 30 (26.7%) and three plants in 30 (10%), respectively, tested ELISA positive. On August 13, the treatments 1A1,B and Jurc2,B had 28 plants in 30 (93.3%) and 10 plants in 30 (33.3%), respectively, test ELISA positive.

The ELISA conducted on September 19 indicated that the treatments 1A1,B and Jurc2,B had 29 plants in 30 (96.7%) and 22 plants in 30 (73.3%), respectively, test ELISA-positive. Table 19 shows the percentage of plants that tested ELISA-positive for each treatment of each test date. The cumulative percentage of plants testing ELISA-positive on at least one date was the exactly the same as shown in Table 19.

A summation index Chi-square analysis was conducted to compare treatments strain 1A1,B with strain Jurc2,B. The observed summation

TABLE 17.--Chi square analysis of pairwise comparisons between treatments in Experiment IV. The number of infected 'Meteor' plants for each treatment was summed over the four sample dates. A significant X^2 indicates that the paired treatments caused unequal rates of PNRSV infection

Treatment Comparison	Observed[a]	Expected[b]	X^2	P
1 A 1/Jurc 2[c]	+ 8:15	(1:1)	2.197	.1 - .5
	-12:5			
1 A 1/NRSV-G	+ 8:9	(1:1)	0.00	1.00
	-12:11			
Jurc 2/NRSV-G	+15:9	(1:1)	1.265	.1 - .5
	- 5:11			

[a]Observed values are for ELISA positives (+) and ELISA negatives (-) for the compared treatments summed over the sampling dates.

[b]Null hypothesis: The percent of infected (and healthy) plants are equal between the compared treatments.

[c]All treatments were inoculated by the leaf-rub/bark flap combination on May 3, 1984.

TABLE 18.--Chi-square analysis of pair-wise comparisons between treatments in Experiment IV. A cumulative index was calculated as the sum of all 'Meteor' plants that tested positive on at least one sample date. A significant χ^2 indicated that the cumulative indices for the paired treatments were unequal

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
1 A 1: Jurc 2[c]	+ 6:11	1:1	1.637	.1-.5
	-14:19			
1 A 1: NRSV-G	+ 6:9	1:1	0.427	.5-.9
	-14:11			
Jurc 2:NRSV-G	+11:9	1:1	0.100	.5-.9
	- 9:11			

[a]Observed values are for ELISA positives (+) and ELISA negatives (-) for the compared treatments.

[b]Null hypothesis: the percent PNRSV infected (and healthy) 'Meteor' plants are equal for compared treatments.

[c]All treatments were inoculated by the bud method.

Table 19. The percentage of 'Meteor' sour cherry plants which tested ELISA-positive in experiment V on four dates. The PNRSV severe strain 1A1 and the intermediate strain Jurczack 2 (Jurc2) were inoculated seperately to plants by the bud method.[a]

Date	PNRSV Strain		
	1A1	Jurc2	Control
July 7, 1984	26.7	10.0	0[b]
August 8	93.3	66.7	0
Sept. 9	96.7	70.0	0
Oct. 24	96.7	73.3	0

[a] Thirty 'Meteor' plants were inoculated with dormant virus-infected sour cherry buds on June 12, 1984.

[b] Control plants consisted of 10 and 20 control plants from experiments III and IV respectively.

indices differed significantly from the expected 1:1 ratio. The treatment strain 1A1,B caused 94 of of a possible 120 ELISA-positives while treatment Jurc2,B caused 65 of a possible 120 ELISA-positives (Table 20).

For the cumulative index Chi-square analysis, the comparison of the observed cumulative indices for treatments strain 1A1,B and strain Jurc2,B differed significantly from the expected 1:1 ratio (P=0.05-0.01) (Table 21). The treatment strain 1A1,B caused 29 of 30 plants to test ELISA positive on at least one sample date while treatment Jurc2,B caused 22 plants to test ELISA-positive on at least one sample date. The absorbance values for each date are listed in Appendix B (Table B5).

Experiment VI

The plants in experiment VI were first ELISA-tested for PNRSV infection on August 13, 1984 and for both treatments 1A1,leaf-rub/bark flap (1A1,L/BF) and Jurczack 2, leaf-rub/bark-flap (Jurc2,L/BF), all plants tested ELISA negative. The treatments strain 1A1,B and Jurc2,B had ten plants in 30 (33%) and 12 plants in 30 (40%), respectively, test ELISA-positive. On September 3, 1984, the treatment 1A1,L/BF and Jurc2,L/BF had two plants in 30 (6.7%) and six plants in 30 (20%), respectively, tested ELISA-positive. The treatments 1A1,B and Jurc2,B had 20 plants in 30 (66.7%) and 23 plants in 30 (76.7%), respectively, tested ELISA-positive.

On October 24, 1984 the treatments 1A1,L/BF and Jurc2,L/BF had eight plants in 30 (26.7%) and nine plants in 30 (30%), respectively, test ELISA-positive. For the treatments 1A1,B and Jurc2,B 23 plants in 30 (76.7%) and 24 plants in 30 (80%), respectively, tested ELISA positive. Table 22 shows the percentage of plants that tested ELISA positive for each treatment on each test date. The cumulative

TABLE 20 .--Chi square analysis of pair-wise comparisons between treatments in Experiment V. The number of infected 'Meteor' plants for each treatment was summed over the four sample dates. A significant χ^2 indicated that the paired treatments caused unequal rates of PNRSV infection

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
1 A 1 B/Jurc 2B[a]	+94:65 -26:55	1:1	14.610	<.001

[a]Observed values are for ELISA positives (+) and ELISA negatives (-) for the compared treatments summed over the sampling dates.

[b]Null hypothesis: The percent of infected (and healthy) plants are equal between the compared treatments.

[c]Treatment PNRSV strain 1 A 1 (1 A 1) and strain Jurczack 2 (Jurc 2) were inoculated by the bud method on June 12, 1984.

TABLE 21.--Chi-square analysis of pair-wise comparisons between treatments in Experiment V. A cumulative index was calculated as the sum of all 'Meteor' plants that tested positive on at least one sample date. A significant χ^2 indicated that the cumulative indices for the paired treatments were unequal

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
1 A 1: Jurc 2	+29:22 - 1:8	1:1	4.706	.05-.01

[a]Observed values were for ELISA positives (+) and ELISA negatives (-) for the compared treatments.

[b]Null hypothesis: The percent PNRSV infected (and healthy) 'Meteor' plants are equal for compared treatments.

[c]All treatments were inoculated by the bud method.

Table 22. The percentage of *Prunus cerasus* X *P. fruticosa* (clone 173/9) plants which tested ELISA-positive in experiment VI on three dates. The PNRSV severe strain 1A1 and the intermediate PNRSV strain Jurczack 2 (Jurc2) were used to inoculate plants by the bud method (B) or the leaf-rub/bark-flap method (L/BF).[a]

Date	PNRSV Strain				
	1A1 (L/BF)	1A1 (B)	Jurc2 (L/BF)	Jurc 2 (B)	Control (L)
August 13, 1984	0	33.3	0.0	40.0	0.0[b]
Sept. 3	6.7	66.7	20.0	76.7	0.0
Oct. 24	26.7	76.7	30.0	80.0	0.0

[a] Thirty 'Meteor' plants per treatment were inoculated on June 28, 1984 with either PNRSV-infected cucumber or cherry buds.

[b] Control plants were inoculated by the leaf-rub/bark-flap method using virus extraction buffer and healthy cucumber respectively.

percentages of plants testing ELISA-positive on at least one test date were the same as those shown in Table 22 except for the treatment isolate Jurc2,L/BF tested on October 24, 1984 where the cumulative percentage was 33.3%. Absorbance values for each test date are listed in Appendix B (Table B6).

A summation Chi-square analysis was done to compare treatments in a pairwise fashion (Table 23). For the paired comparisons between treatment strain 1A1,L/BF and strain 1A1,B and between treatments Jurc2,L/BF and Jurc2,B the observed sums of ELISA-positives differed significantly from the expected 1:1 ratio ($P = < 0.001$ and $P = < 0.001$, respectively). For the paired comparisons between treatment strain 1A1,L/BF and Jurc2,L/BF and between treatments strain 1A1,B and strain Jurc2,B, the observed sums did not differ significantly from the expected 1:1 ratio (P=0.5-0.1 and P=0.5-0.1, respectively).

From the cumulative index Chi-square analysis (Table 24), the pairwise comparisons of the cumulative indices between treatments strain 1A1,L/BF and 1A1,B and between treatments strain Jurc2,L/BF and strain Jurc2,B differed significantly from the expected 1:1 ratio ($P = < 0.001$ and P=0.01-0.001, respectively). For the pair-wise comparisons of the observed cumulative indices between treatments strain 1A1,L/BF and strain Jurc2,B, the observed cumulative indices did not differ significantly from the expected 1:1 ratio (P=0.9-0.5 and P=0.9-0.5, respectively). Absorbance values for each test date are listed in Appendix B (Table B6).

TABLE 23 .--Chi-square analysis of pair-wise comparisons between treatments in Experiment VI. The number of infected 'Meteor' plants for each treatment was summed over the three sample dates. A significant χ^2 indicated that the paired treatments caused unequal rates of PNRSV infection

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
(1 A 1L/BF)/1 A 1 B[c]	+10:53	1:1	43.077	<.001
	-80:27			
(Jurc 2L/BF)/Jurc 2B	+15:59	1:1	42.430	<.001
	-75:21			
(1 A 1L/BF)/ (Jurc 2L/BF)	+10:15	1:1	0.7432	.1-.5
	-80:75			
1 A 1B/Jurc 2B	+53:59	1:1	0.5909	.1-.5
	-27:21			

[a]Observed values are for ELISA positives (+) and ELISA negatives (-) for the compared treatments summed over the sampling dates.

[b]Null hypothesis: The percent of infected (and healthy) plants are equal between the compared treatments.

[c]Treatments: 1 A 1 L/BF = PNRSV strain 1 A 1 inoculated by the leafrub/barkflap combination, Jurc 1 L/BF = PNRSV strain Jurczack inoculated by the leafrub/ bark flap combination, 1 A 1 B = PNRSV strain 1 A 1 inoculated by the bud method, Jurc 2B = PNRSV strain Jurczack 2 inoculated by the bud method on June 28, 1984.

TABLE 24.--Chi-square analysis of pair-wise comparisons between treatments in Experiment VI. A cumulative index was calculated as the sum of all 'Meteor' plants that tested positive on at least one sample date. A significant χ^2 indicated that the cumulative indices for the paired treatments were unequal

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
1 A 1 L/BF: 1 A 1 B[c]	+ 8:24	1:1	15.067	<.001
	-22:6			
Jurc 2 L/BF: Jurc 2B	+11:24	1:1	9.874	.01-.001
	-19:6			
1 A 1 L/BF: Jurc 2 L/BP	+ 8:11	1:1	0.308	.5-.9
	-22:19			
1 A 1 B: Jurc 2 B	+24:24	1:1	0.109	.5-.9
	- 6:6			

[a]Observed values were for ELISA positives (+) and ELISA negative (-) for the compared treatments.

[b]Null hypothesis: the percent PNRSV infected (and healthy) 'Meteor' plants are equal for compared treatments.

[c]Treatments were inoculated by either the leafrub/barkflap (L/BF) combination or the bud (B) method.

Experiment VII

The first ELISA test was conducted on August 13, 1984 and the treatments 1A1,B and Jurc2,B had 19 plants in 30 (63.3%) and 28 plants in 30 test ELISA-positive, respectively.

On September 3, 1984 the treatment strain 1A1,B had 23 plants in 30 (80%) while the treatment strain Jurc2,B had 29 plants in 30 (96.7%) test ELISA-positive.

Finally, on October 24, 1984, the treatments 1A1,B and Jurc2,B had 26 plants in 30 (83.7%) and 29 of 30 (96.7%) plants test positive, respectively. Table 25 shows the cumulative percentage of plants testing ELISA-positive on at least one test date. A summation index Chi-square analysis was done to compare the treatments strain 1A1,B and Jurc 2,B in a pair-wise fashion (Table 26). The observed summation indices differed significantly from the expected 1:1 ratio ($P = < 0.001$). When all three ELISA tests are considered there was 90 possible ELISA positives. For treatment strain Jurc2,B 86 of the possible 90 were ELISA-positive while for treatment strain 1A1,B 67 of the possible 90 tested ELISA-positives.

No Chi-square analysis was conducted using the cumulative indices since the smallest expected classes were less than five.

Absorbance values for each date are listed in Appendix B (Table B7).

Table 25. The cumulative percentage of 'Meteor' sour cherry plants[a] which tested ELISA-positive in experiment VII on at least one of three dates. The PNRSV severe strain 1A1 and the intermediate strain Jurczack 2 (Jurc2) were inoculated to plants by the bud method (B) on June 30, 1984.

	PNRSV Strain		
Date	1A1	Jurc2	Control
August 13, 1984	63.3	93.3	0.0[b]
Sept. 3	80.0	96.7	0.0
Oct. 24	86.7	96.7	0.0

Thirty plants per treatment were inoculated with PNRSV-infected cherry buds.

Control plants were inoculated with virus extraction buffer and healthy cucumber using the leaf-rub/bark-flap combination.

TABLE 26.--Chi-square analysis of pair-wise comparisons between treatments in Experiment VII. The number of infected clone 173/9 plants for each treatment was summed over the four sample dates. A significant χ^2 indicated that the paired treatments caused unequal rates of PNRSV infection.

Treatment Comparison	Observed[a]	Expected[b]	χ^2	P
1 A 1 B/Jurc 2 B[c]	+62:86	1:1	14.118	<.001

[a]Observed values are for ELISA positives (+) and ELISA negatives (-) for the compared treatments summed over the sampling dates.

[b]Null hypothesis: The percent of infected (and healthy) plants are equal between the compared treatments.

[c]Both treatments were inoculated by the bud method on June 30, 1984.

DISCUSSION

For this third and final section section three goals were set: (1) to test four inoculation methods separately and in combinations to determine which most effectively transmitted PNRSV to cherry plants (2) to determine the length of the incubation period required after inoculation so that all infected plants could be detected by ELISA, (3) to determine if strain differences caused changes in efficiency of inoculation methods.

Critical ELISA Absorbance Values

In the preceeding results section, the critical value used was calculated by summing the healthy mean absorbance value for a given ELISA test on a given day and three standard deviations from the healthy mean. This calculated value was chosen at the outset of this work and used consistently through all experiments. As indicated in the appendices some absorbances, particularly in experiments one through four, were only slightly larger than the corresponding critical values when compared, but none-the-less were scored as positive. In the later experiments, when bud inoculation experiments were used, absorbance values for a majority of the plants tested were distinctly greater than critical values.

Apparently the use of a more conservative critical value for scoring plants as ELISA positive or negative would show clearly which inoculation method was most effective.

In the appendices a second and usually more conservative critical value has been calculated for each ELISA test. The critical value is twice the healthy mean A405nm value. Since the primary objective was to identify an effective reliable inoculation method, the use of such a conservative value allows for marginally effective methods to be disqualified quickly. The conservative critical values point out which inoculation method or methods caused the most rapid increase in virus titre and thus the greatest A405nm value.

A conservative critical value would naturally lead to mis-scoring some plants as negative or healthy when in fact such plants could be infected but show very low virus titre. At the same time, however, by use of higher critical values, healthy plants with spuriously high A405nm values would not be as likely to be scored as positive or virus infected.

With the current work and objective in mind, conservative results are preferrable since inoculations have been done with cultivars known to be susceptible to PNRSV. In future work when plant material with unknown reaction to PNRSV is screened, careful examination of ELISA values will be needed so that tolerant individuals (a genotype that allows virus infection but only at low virus titres) will not be over-looked.

Inoculation methods

Leaf rub inoculation appears to be ineffective for transmitting PNRSV, since the last three of five ELISA tests from experiment two indicated that no plants given the leaf rub treatment tested positive.

Leaf-rub/root-rub inoculation did not effectively transmit PNRSV. When inoculated plants were tested by ELISA at intervals beginning six weeks post-inoculation and ending eleven months post-inoculation, the

results were inconsistent and in any given test ELISA positives only reached 20% (based on the critical value of healthy mean + three standard deviations).

The leaf-rub/bark-flap/root-rub combination tested in experiment two is not a suitable method for mechanically transmitting PNRSV, since no plants tested ELISA positive in the last three sampling dates (critical value of healthy mean plus three standard deviations). The bark flap method which was first tested in experiment one appeared to effectively transmit PNRSV. By the last sampling date on October 24, 1984, four of five inoculated plants tested ELISA positive. These results agreed with those reported by Boyle et al. (1954) and with unpublished results of Fulton. However, in experiment two, plants inoculated by the bark flap method had no ELISA positives in the final three of five test dates. If in experiment one, a more conservative critical value (twice the healthy mean value) had been used initially, the results would have been zero percent positives rather than 80%. The method apppears thus to be unacceptable as an inoculation method for use in a program to screen for virus resistance.

The combination of leaf-rub/bark-flap inoculation (L/BF) resulted in no ELISA positives on the final three sampling dates in experiment two, however, on April 18, 1984 six of ten plants tested ELISA positive. In experiment six L/BF inoculations caused eight of 30 (26%) and nine of 30 (30%) plants to test ELISA positive on the final test date. Although the percent positives were increasing with each test the effectiveness of the method is not acceptable for use in a screening program.

Finally, bud inoculation which was first tested in experiment five appears highly effective in transmitting PNRSV. The results agree with reports by Hildebrand (1942) and Fridlund (1967, 1968). In experi-

ments V and VI, although different genotypes were used, the results from sample date one may indicate that there is a lag period during which virus titre builds to detectable levels. In experiment VII the lag period (indicated by low number of positives on the first sampling date) was not as apparent as it was in the previous experiments.

Bud inoculation did not result in 100% ELISA positives on a given date and in fact infection in experiment V for PNRSV strain Jurczack 2 was 73.3%. To attain 100% infection in inoculations, two virus-infected buds rather than one could be used to insure transmission.

PNRSV strain effects

The effectiveness of the bud and leafrub/bark-flap (L/BF) inoculation method when different virus strains were used was determined in experiments IV through VII only. The L/BF method was equally effective in infecting PNRSV strains 1A1, Jurczack 2 and NRSV-G. The bud inoculation method was more effective with strain 1A1 than with strain Jurczack 2 in experiment V while in experiment VI, the bud method was equally effective with 1A1 and Jurczack2. Finally, in experiment VII bud inoculation was more effective with Jurczack 2 than with 1A1. From these experiments it is not clear if one strain causes higher infection rates. The inconsistent infection rates for each virus strain between experiments may have been due to the bud wood that was used to inoculate plants. The bud wood infected by each of the PNRSV strains both came from 'Montmorency'. However tree age was not the same, geographical location and the location of the wood on the trees all differed.

Additionally, since Prunus cerasus L. cv. 'Meteor' was used in experiments V and VI while in experiment VII, P. cerasus x P. fruticosa (clone 173/9) was used in experiment VII, so direct comparisons of the results between experiments V, VI and VII are not possible.

Sampling dates

The bud inoculation method appears to have a considerable lag period between the time of inoculation and the time when maximum % infection is acheived. In experiment V, % ELISA positives was nearly maximized for treatment strain 1A1,B by the August 13, 1984 sample date while treatment strain Jurc 2,B showed progressive increases from August 24 through October 24, 1984.

In experiment VI, the bud inoculations with strains 1A1 and Jurc 2 showed increasing percentage of ELISA positives from the first to the last sample date. The rate of increase in percentage of ELISA positives however was small between the September 3 and the October 24, 1984 sample date.

For experiment VII, the treatment strain Jurc 2,B caused nearly maximum percentage infection on the first sample date August 13, 1984 while the treatment strain 1A1,B caused percentage infection that progressively increased from August 13, 1984 to October 24, 1984. It is not clear when the optimum time is for ELISA testing plants that have been inoculated with PNRSV by the bud method. However, sampling will be ineffective if carried out before a two to three month lag period has elapsed.

The preceeding statement may not hold if young seedling plants are inoculated with viruliferous buds while the seedlings are actively growing. The lag period may be reduced to one month or less. The plant

material used in this current work were routinely treated with GA3 to prolong the period of active growth. Certainly, artificial induction of meristematic activity will influence the rate at which virus particles replicate and most likely slow the infection process.

Figure A1. Temmperature (degrees F) fluctuations during host range experiment I. Temperatures were taken at 3:00 pm and 3:00 am each day during the experiment.

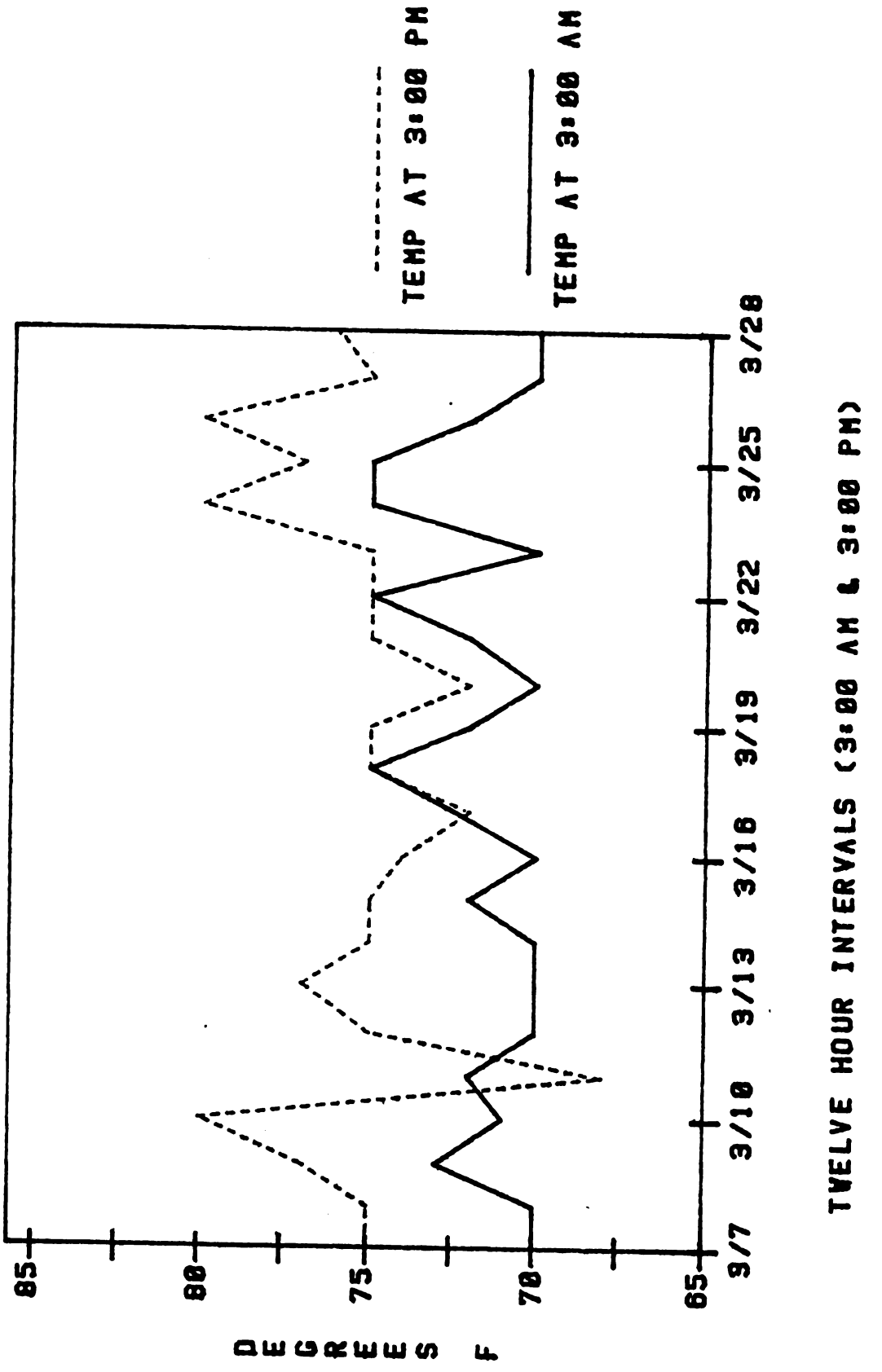

TEMP AT 3:00 PM
TEMP AT 3:00 AM
DEGREES F
85
80
75
70
65
3/7
3/10
3/13
3/16
3/19
3/22
3/25
3/28
TWELVE HOUR INTERVALS (3:00 AM & 3:00 PM)

Figure A1

Figure A2. Temmperature (degrees F) fluctuations during host range experiment II. Temperatures were taken at 3:00 pm and 3:00 am each day during the experiment.

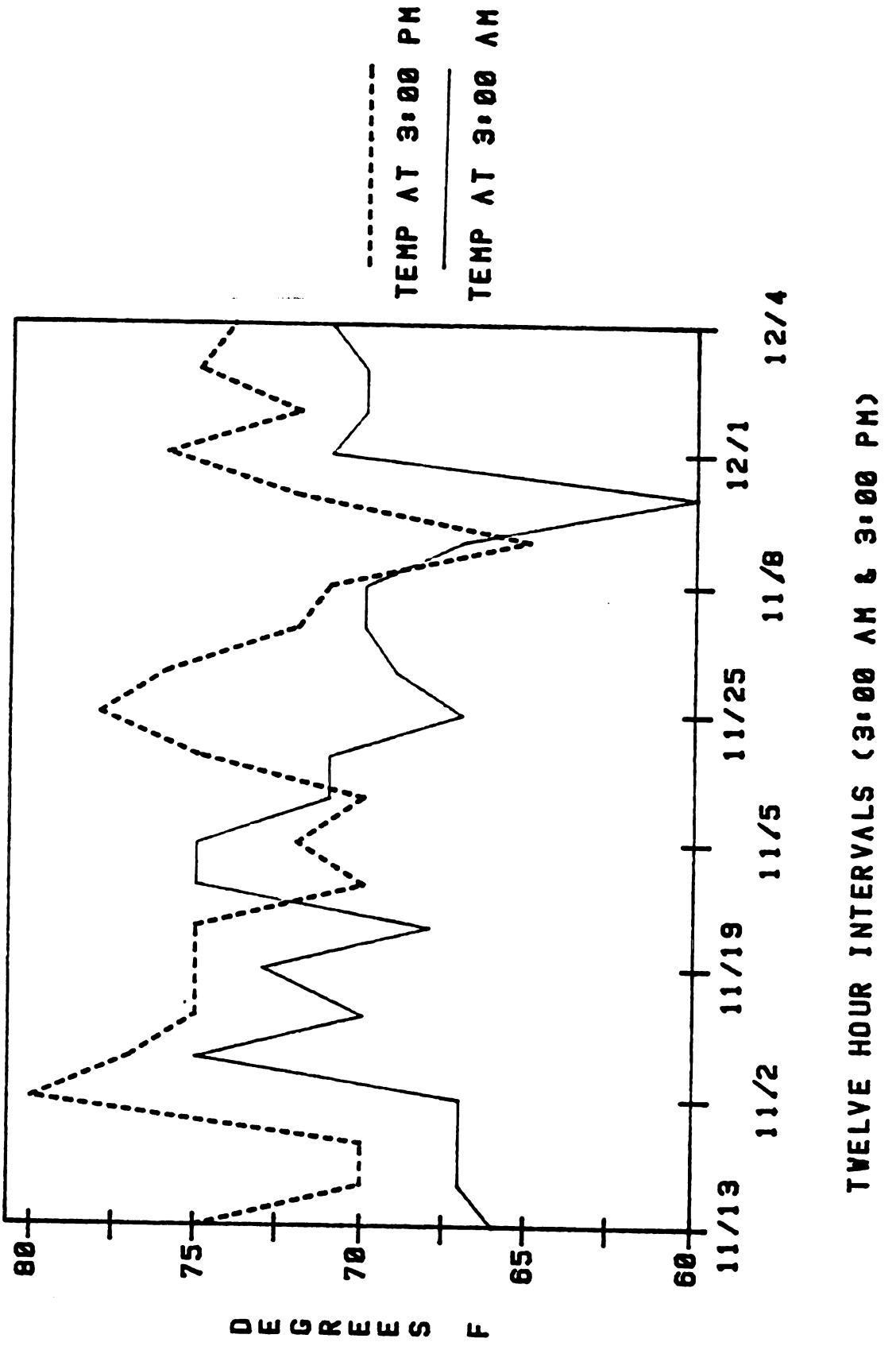

TEMP AT 3:00 PM
TEMP AT 3:00 AM
DEGREES F
80
75
70
65
60
11/13
11/2
11/19
11/5
11/25
11/8
12/1
12/4
TWELVE HOUR INTERVALS (3:00 AM & 3:00 PM)

Figure A2

Figure A3. Temmperature (degrees F) fluctuations during host range experiment III. Temperatures were taken at 3:00 pm and 3:00 am each day during the experiment.

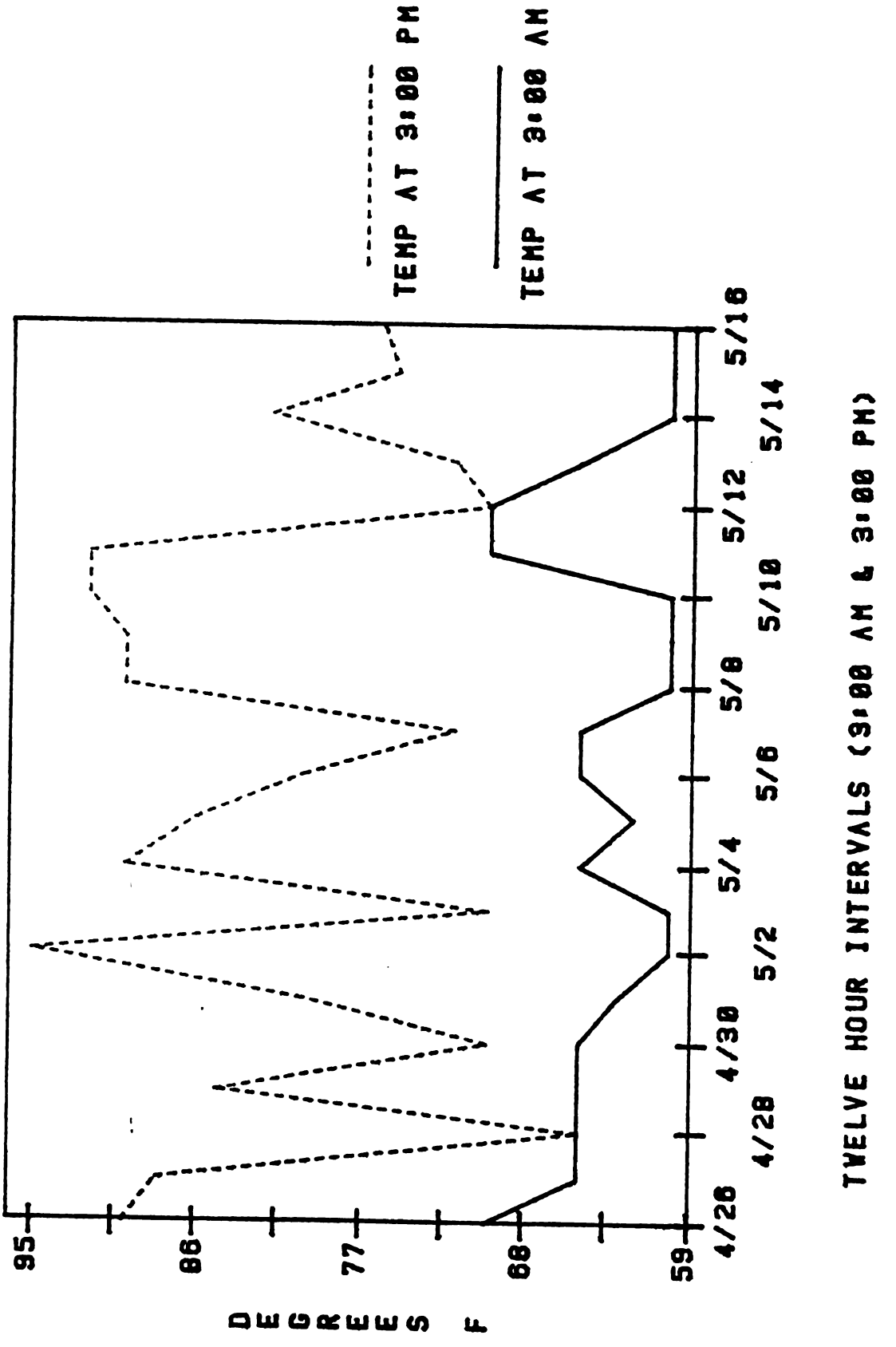

TEMP AT 3:00 PM
TEMP AT 3:00 AM
95
86
77
68
59
DEGREES F
4/26
4/28
4/30
5/2
5/4
5/6
5/8
5/10
5/12
5/14
5/16
TWELVE HOUR INTERVALS (3:00 AM & 3:00 PM)

Figure A3

Figure A4. Temmperature (degrees F) fluctuations during host range experiment IV. Temperatures were taken at 3:00 pm and 3:00 am each day during the experiment.

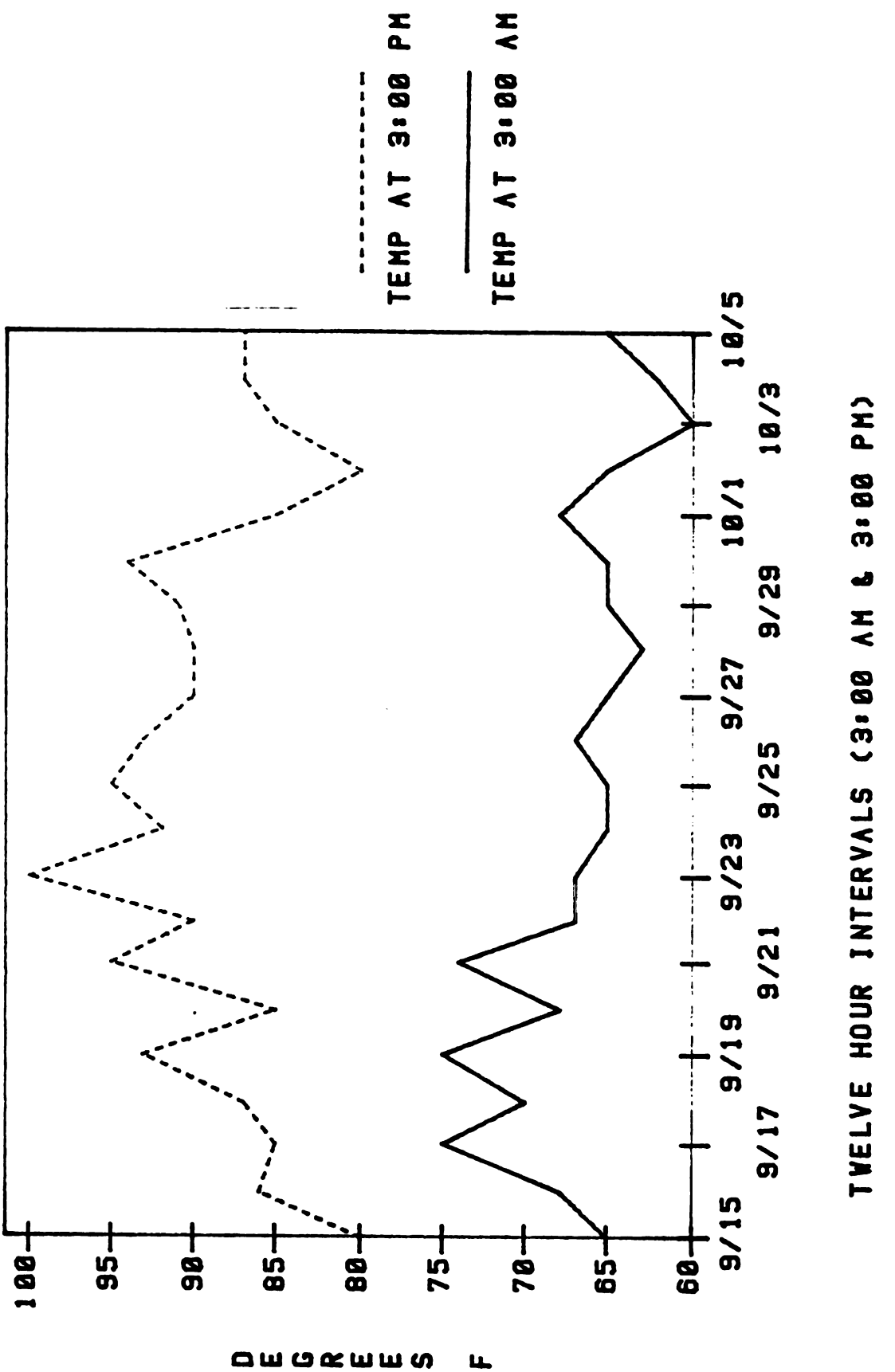

TEMP AT 3:00 PM
TEMP AT 3:00 AM
DEGREES F
100
95
90
85
80
75
70
65
60
9/15
9/17
9/19
9/21
9/23
9/25
9/27
9/29
10/1
10/3
10/5
TWELVE HOUR INTERVALS (3:00 AM & 3:00 PM)

Figure A4

APPENDIX B

TABLE B-1.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on five dates for inoculation Experiment I. Inoculations were made on November 21, 1983, and the leafrub/rootrub (L/R) or barkflap (BF) method was used

Treatment (Strain, Method)	Plant No.	1984 Sample Dates of ELISA Test				
		1/6	2/7	3/12	4/23	10/24
Control[b] L/R	1	0.38	0.19	0.26	0.42	0.31
	2	0.20	0.15	0.31	0.36	0.21
	3	0.21	0.17	0.37	0.42	0.25
	4	0.25	0.19	0.42	0.31	0.18
	5	0.26	0.14	0.28	0.30	0.23
	6	0.25	0.17	0.39	0.32	0.16
	7	0.15	0.24	0.32	0.30	0.19
	8	0.25	0.15	0.42	0.28	0.23
	9	0.26	0.15	0.28	0.37	0.24
	10	0.23	0.14	0.27	0.35	0.23
1 A 1 L/R	1	0.26	0.15	0.39	0.33	0.18
	2	0.37	0.20	0.44	0.30	0.27
	3	0.27	0.17	0.50	0.32	0.21
	4	0.29	0.19	0.33	0.23	0.24
	5	0.30	0.17	0.41	0.23	0.34
	6	0.27	0.18	0.31	0.42	0.24
	7	0.22	0.21	0.29	0.34	0.17
	8	0.26	0.23	0.30	0.33	0.16
	9	0.22	0.22	0.29	0.32	0.21
	10	0.26	0.17	0.40	9.31	0.21
Jurc 1 L/R	1	0.20	0.18	0.39	0.40	0.22
	2	*[c]	0.18	*	0.40	0.26
	3	*	0.19	0.34	0.41	0.31
	4	0.26	0.27	0.41	0.38	0.17
	5	0.19	0.32	0.38	0.27	0.28
	6	0.27	0.23	0.47	0.36	0.19
	7	0.20	0.23	0.39	0.29	0.29
	8	0.22	0.14	0.30	0.35	0.34
	9	0.22	0.16	0.40	0.33	0.26
	10	0.28	0.26	0.43	0.37	0.31

TABLE B-1.--Continued

Treatment (Strain, Method)	Plant No.	1984 Sample Dates of ELISA Test				
		1/6	2/7	3/12	4/25	10/24
107-57 L/R	1	0.21	0.22	0.44	0.31	0.32
	2	0.22	0.25	0.36	0.32	0.21
	3	0.30	0.19	0.37	0.35	0.28
	4	0.29	0.18	0.38	0.54	0.19
	5	0.23	0.19	0.32	0.44	0.23
	6	0.22	*	0.43	0.32	0.32
	7	*	0.19	0.30	0.37	0.18
	8	0.28	0.19	0.22	0.28	0.27
	9	0.26	0.18	0.43	0.34	0.18
	10	0.23	0.19	0.33	0.35	0.30
CH-42 L/R	1	0.24	0.19	0.30	0.28	0.37
	2	0.23	0.17	0.33	0.50	0.17
	3	0.21	0.21	*	0.39	9.34
	4	0.29	0.22	0.35	0.32	0.33
	5	*	0.24	0.30	0.24	0.26
	6	0.24	0.24	0.31	0.26	0.34
	7	0.28	0.24	0.24	0.35	0.15
	8	0.21	0.22	0.28	0.30	0.19
	9	0.30	0.21	0.36	0.24	0.34
	10	0.21	0.23	0.24	0.29	0.35
Jure 2 BF	1	0.37	0.24	0.48	0.33	0.42
	2	0.25	0.21	0.45	0.54	0.30
	3	0.21	0.26	0.32	0.32	0.40
	4	0.25	0.27	0.44	0.16	0.45
	5	0.36	0.24	0.53	0.29	0.35
Critical ELISA Values						
$\bar{x}_{\text{healthy } A_{405nm}}$ + 3 Std. Dev.		0.42	0.26	0.52	0.49	0.35
$2\ \bar{x}_{\text{healthy } A_{405nm}}$		0.45	0.34	0.66	0.69	0.45

[a]Each A_{405nm} value represents the mean of two replicate wells per plant.

[b]Control = healthy plants inoculated with extraction buffer by the leafrub method, 1A1=PNRSV strain Plot 1A1, Jurc 2=PNRSV strain Jurczack 2, 107-57=PNRSV severe strain 107-52 (Prosser, Washington, CH 42=PNRSV mild strain CH-42 (Prosser, Washington).

[c]* indicates missing A_{405nm} value.

TABLE B-2.--Mean A_{405nm} values[a] for each plant was ELISA tested for PNRSV on five dates for inoculation Experiment II. Plants were inoculated with PNRSV strain Jurczack 2 on February 21, 1984.

Treatment (Inoculation Method)	Plant	1984 Sample Date of ELISA Test				
		4/3[c]	4/18	5/1	6/5	10/24
Control[b]	1	0.38	0.30	0.165	0.250	0.247
	2	0.30	0.20	0.119	0.161	0.260
	3	0.36	0.35	0.96	0.125	0.303
	4	0.25	0.24	0.126	0.172	0.236
	5	0.31	0.25	0.191	0.295	0.327
	6	0.28	0.26	0.176	0.268	0.242
	7	0.41	0.29	0.99	0.130	0.234
	8	0.29	0.22	0.122	0.171	0.246
	10	0.34	0.28	0.194	0.303	0.200
L/BF/R	1	*[d]	0.47	0.198	0.322	0.239
	2	0.27	0.52	0.159	0.214	0.272
	3	0.40	0.25	0.163	0.246	0.234
	4	0.33	0.34	0.137	0.201	0.295
	5	0.33	0.27	0.181	0.267	0.227
	6	0.30	0.30	0.181	0.284	0.314
	7	0.54	0.17	0.146	0.275	0.256
	8	0.29	0.37	0.143	0.174	0.261
	9	0.26	0.38	0.139	0.197	0.204
	10	0.34	0.29	0.116	0.157	0.264
BF	1	0.40	0.33	0.148	0.221	0.323
	2	0.30	0.25	0.135	0.193	0.222
	3	0.33	0.32	0.140	0.220	0.228
	4	0.30	0.29	0.252	0.397	0.259
	5	0.39	0.28	0.130	0.204	0.279
	6	0.36	0.24	0.173	0.265	0.245
	7	0.45	0.37	0.129	0.187	0.241
	8	0.52	0.19	0.107	0.143	0.297
	9	0.51	0.29	0.116	0.157	0.289
	10	0.47	0.24	0.189	0.304	0.242
L/BF	1	0.34	0.36	0.173	0.266	0.162
	2	0.33	0.35	0.163	0.219	0.287
	3	*	0.44	0.132	0.193	0.304
	4	0.24	0.24	0.108	0.218	0.250
	5	0.47	0.30	0.135	0.197	0.214
	6	0.31	0.42	0.161	0.246	0.281
	7	0.42	0.45	0.173	0.250	0.212
	8	0.32	0.40	0.194	0.302	0.325
	9	0.27	0.49	0.188	0.301	0.251
	10	0.38	0.47	0.144	0.212	0.248

TABLE B-2.--Continued

Treatment (Inoculation Method)	Plant	1984 Sample Date of ELISA Test				
		4/3[c]	4/18	5/1	6/5	10/24
LF	1	0.42	0.29	0.126	0.180	0.236
	2	0.36	0.25	0.156	0.220	0.270
	3	0.30	0.21	0.109	0.149	0.239
	4	0.49	0.39	0.181	0.275	0.312
	5	0.39	0.37	0.155	0.241	0.273
	6	0.38	0.15	0.238	0.378	0.311
	7	0.36	0.23	0.188	0.282	0.284
	8	0.37	0.33	0.103	0.124	0.240
	9	0.28	0.22	0.142	0.210	0.248
	10	0.34	0.41	0.165	0.246	0.306
Critical ELISA Values						
$\bar{x}_{healthy}$ + 3std. dev.		0.47	0.40	0.253	0.410	0.366
$2\bar{x}_{healthy}$		0.65	0.52	0.288	0.420	0.501

[a]Each A_{405nm} value represents the mean of two replicate wells per plant.

[b]Control = healthy plants. Leafrub/barkflap inoculated with extraction buffer and healthy cucumber respectively, L/BF/R = leafrub/barkflap/rootrub inoculation combination, BF = barkflap inoculation, L/BF = leafrub/barkflap inoculation combination, L = leafrub inoculation.

[c]On April 3 and 18 A_{405nm} values were recorded from a Microelisa® spectrophotometer (accurate to two decimals) and on May 1, June 5, and October 25, 1984, A_{405} values were recorded from a Bio-Tek® EIA reader, model 307 (accurate to three decimal places).

[d]*indicates missing A_{405nm} value.

TABLE B-3.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on four dates for inoculation Experiment III. Plants were inoculated with three PNRSV strains on March 5, 1984

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		4/23[c]	5/7	6/5	10/24
Control[b]	1	0.15	0.209	0.135	0.255
	2	0.14	0.178	0.119	0.185
	3	0.18	0.236	0.99	0.231
	4	0.12	0.222	0.133	0.178
	5	0.15	0.164	0.229	0.116
	6	0.11	0.179	0.137	0.167
	7	0.15	0.184	0.136	0.216
	8	0.18	0.230	0.110	0.235
	9	0.15	0.166	0.80	0.199
	10	0.12	0.213	0.147	0.188
1 A 1	1	0.16	0.231	0.152	0.320
	2	0.16	0.200	0.153	0.310
	3	0.14	0.190	0.197	0.231
	4	0.21	0.165	0.199	0.298
	5	0.17	0.259	0.141	0.248
	6	0.16	0.205	0.180	0.276
	7	0.19	0.166	0.177	0.210
	8	0.16	0.140	0.120	0.356
	9	0.17	0.179	0.111	0.423
	10	0.15	0.230	0.125	0.330
Jurc 2	1	0.20	0.139	0.211	0.185
	2	0.18	0.170	0.140	0.251
	3	0.18	0.203	0.200	0.324
	4	0.21	0.200	0.184	0.266
	5	0.22	0.174	0.180	0.212
	6	0.15	0.161	0.157	0.208
	7	0.16	0.253	0.220	0.251
	8	0.17	0.196	0.190	0.188
	9	0.20	0.201	0.220	0.270
	10	0.26	0.240	0.227	0.276
NRSV-G	1	0.14	0.263	0.180	0.219
	2	0.15	0.212	0.202	0.285
	3	0.16	0.189	0.175	0.208
	4	0.12	0.304	0.202	0.248
	5	0.17	0.191	0.149	0.200
	6	0.16	0.243	0.230	0.236
	7	0.14	0.234	0.188	0.237
	8	0.19	0.193	0.166	0.151

TABLE B-3.--Continued

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		4/23[c]	5/7	6/5	10/24
NRSV-G	9	0.45	0.157	0.195	0.214
Continued	10	0.15	0.185	0.217	0.293
Critical ELISA Values					
$\bar{x}_{healthy}$ + 3 Std. dev.		0.22	0.279	0.251	0.317
$2\bar{x}_{healthy}$		0.30	0.396	0.266	0.394

[a]Each A_{405} value represents the mean of two replicate wells per plant.

[b]Control: Healthy plants were inoculated with extraction buffer and healthy cucumber by the leafrub/barkflap combination, respectively, 1 A 1 = PNRSV strain Plot 1A1, Jurc 2 = PNRSV strain Jurczack 2, NRSV-G = Fulton's type PNRSV strain G.

[c]On sample date, April 23, 1984, A_{405nm} values were recorded from a Microelisa® spectrophotometer (accurate to two decimal places) while on sample dates May 7, June 5, and October 24, A_{405nm} values were recorded from a Bio-Tek® EIA reader, model 307 (accurate to three decimal places).

TABLE B-4.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on four dates for inoculation Experiment IV. Plants were inoculated by the leafrub/barkflap combination on May 3, 1984

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		6/5	7/24	8/13	10/24
Control[b]	1	0.20[c]	0.161	0.130	0.225
	2	0.165	0.177	0.120	0.196
	3	0.16	0.163	0.153	0.220
	4	0.17	0.195	0.134	0.153
	5	0.17	0.199	0.111	0.203
	6	0.14	0.185	0.137	0.191
	7	0.14	0.191	0.161	0.173
	8	0.12	0.192	0.173	0.184
	9	0.20	0.198	0.163	0.217
	10	0.18	0.174	0.125	0.231
	11	0.19	0.148	0.131	0.229
	12	0.20	0.163	0.121	0.221
	13	0.18	0.209	0.110	0.281
	14	0.18	0.222	0.144	0.163
	15	0.19	0.205	0.150	0.193
	16	0.15	0.185	0.221	0.269
	17	0.18	0.184	0.165	0.284
	18	0.22	0.180	0.173	0.109
	19	0.22	0.164	0.174	0.127
	20	0.20	0.217	0.221	0.164
1 A 1	1	0.40	0.179	0.473	0.605
	2	0.18	0.260	0.136	0.198
	3	0.19	0.237	0.174	0.208
	4	0.17	0.160	0.244	0.290
	5	0.11	0.147	0.193	0.394
	6	0.20	0.188	0.190	0.268
	7	0.20	0.175	0.157	0.290
	8	0.17	0.156	0.159	0.312
	9	0.18	0.172	0.222	0.206
	10	0.18	0.221	0.152	0.244
	11	0.16	0.168	0.210	0.427
	12	0.15	0.141	0.219	0.367
	13	0.23	0.173	0.181	0.321
	14	0.23	0.184	0.131	0.184
	15	0.20	0.198	0.159	0.344
	16	0.17	0.146	0.190	0.313
	17	0.14	0.174	0.201	0.263
	18	0.17	0.184	0.239	0.234
	19	0.14	0.223	0.201	0.340
	20	0.18	0.149	0.215	0.174

TABLE B-4.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on four dates for inoculation Experiment IV. Plants were inoculated by the leafrub/barkflap combination on May 3, 1984

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		6/5	7/24	8/13	10/24
Control[b]	1	0.20[c]	0.161	0.130	0.225
	2	0.165	0.177	0.120	0.196
	3	0.16	0.163	0.153	0.220
	4	0.17	0.195	0.134	0.153
	5	0.17	0.199	0.111	0.203
	6	0.14	0.185	0.137	0.191
	7	0.14	0.191	0.161	0.173
	8	0.12	0.192	0.173	0.184
	9	0.20	0.198	0.163	0.217
	10	0.18	0.174	0.125	0.231
	11	0.19	0.148	0.131	0.229
	12	0.20	0.163	0.121	0.221
	13	0.18	0.209	0.110	0.281
	14	0.18	0.222	0.144	0.163
	15	0.19	0.205	0.150	0.193
	16	0.15	0.185	0.221	0.269
	17	0.18	0.184	0.165	0.284
	18	0.22	0.180	0.173	0.109
	19	0.22	0.164	0.174	0.127
	20	0.20	0.217	0.221	0.164
1 A 1	1	0.40	0.179	0.473	0.605
	2	0.18	0.260	0.136	0.198
	3	0.19	0.237	0.174	0.208
	4	0.17	0.160	0.244	0.290
	5	0.11	0.147	0.193	0.394
	6	0.20	0.188	0.190	0.268
	7	0.20	0.175	0.157	0.290
	8	0.17	0.156	0.159	0.312
	9	0.18	0.172	0.222	0.206
	10	0.18	0.221	0.152	0.244
	11	0.16	0.168	0.210	0.427
	12	0.15	0.141	0.219	0.367
	13	0.23	0.173	0.181	0.321
	14	0.23	0.184	0.131	0.184
	15	0.20	0.198	0.159	0.344
	16	0.17	0.146	0.190	0.313
	17	0.14	0.174	0.201	0.263
	18	0.17	0.184	0.239	0.234
	19	0.14	0.223	0.201	0.340
	20	0.18	0.149	0.215	0.174

TABLE B-4.--Continued

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		6/5	7/24	8/13	10/24
Jurc 2	1	0.42	0.239	0.323	0.692
	2	0.31	0.106	0.218	0.229
	3	0.18	0.92	0.181	0.306
	4	0.18	0.212	0.156	0.353
	5	0.17	0.159	0.241	0.157
	6	0.15	0.189	0.191	0.192
	7	0.13	0.168	0.161	0.288
	8	0.17	0.162	0.162	0.273
	9	0.10	0.262	0.214	0.197
	10	0.18	0.192	0.164	0.194
	11	0.18	0.151	0.203	0.399
	12	0.29	0.221	0.211	0.193
	13	0.43	0.172	0.334	0.713
	14	0.32	0.143	0.179	0.328
	15	0.16	0.156	0.190	0.353
	16	0.19	0.181	0.148	0.206
	17	0.20	0.244	0.251	0.263
	18	0.16	0.202	0.211	0.174
	19	0.17	0.219	0.143	0.245
	20	0.13	0.291	0.178	0.291
NRSV-G	1	0.13	0.272	0.190	0.255
	2	0.15	0.269	0.189	0.314
	3	0.18	0.202	0.176	0.141
	4	0.17	0.177	0.205	0.177
	5	0.14	0.183	0.195	0.227
	6	0.17	0.180	0.201	0.204
	7	0.21	0.188	0.199	0.243
	8	0.23	0.225	0.183	0.274
	9	0.17	0.195	0.193	0.165
	10	0.16	0.262	0.146	0.185
	11	0.17	0.320	0.131	0.269
	12	0.16	0.306	0.168	0.196
	13	0.19	0.259	0.161	0.135
	14	0.15	0.158	0.234	0.222
	15	0.16	0.231	0.154	0.320
	16	0.19	0.307	0.223	0.206
	17	0.18	0.192	0.206	0.339
	18	0.14	0.195	0.217	0.228
	19	0.20	0.347	0.206	0.221
	20	0.12	0.251	0.171	0.211

TABLE B-4.--Continued

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		6/5	7/24	8/13	10/24
Critical Values					
$\bar{x}_{healthy}$ + 3 std. dev.		0.26	0.245	0.246	0.342
$2\bar{x}_{healthy}$		0.36	0.372	0.302	0.404

[a]Each A_{405nm} value represents the mean of two replicate wells per plant.

[b]Control: healthy plants were inoculated with extraction buffer and healthy cucumber by the leafrub and bark-flap methods, respectively.

[c]On sample date, June 5, A_{405nm} values were recorded from a Microelisa®, EIA reader, model 307 (accurate to three decimal places).

TABLE B-5.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on four dates for inoculation Experiment V. Plants were bud inoculated with PNRSV strains 1 A 1 and Jurczack 2 (Jurc 2) on June 12, 1984

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		7/24	8/13	9/19	10/24
Control[b]	1	0.206	0.198	0.146	0.284
	2	0.117	0.104	0.210	0.269
	3	0.139	0.175	0.240	0.109
	4	0.230	0.201	0.195	0.109
	5	0.119	0.093	0.148	0.188
	6	0.135	0.128	0.117	0.164
	7	0.127	0.077	0.152	0.281
	8	0.108	0.077	0.183	0.225
	9	0.163	0.124	0.135	0.193
	10	0.164	0.123	0.204	0.163
	11	0.191	0.111	0.219	0.173
	12	0.206	0.290	0.206	0.191
	13	0.156	0.123	0.248	0.217
	14	0.155	0.130	0.234	0.184
	15	0.204	0.120	0.178	0.221
	16	0.146	0.122	0.301	0.199
	17	0.167	0.132	0.220	0.220
	18	0.167	0.098	0.107	0.196
	19	0.189	0.121	0.244	0.203
	20	0.095	0.111	0.259	0.153
	21	0.209	0.111	0.195	0.216
	22	0.152	0.122	0.228	0.167
	23	0.235	0.136	0.104	0.231
	24	0.199	0.106	0.269	0.235
	25	0.125	0.982	0.155	0.255
	26	0.149	0.131	0.203	0.229
	27	0.109	0.111	0.277	0.231
	28	0.142	0.092	0.246	0.222
	29	0.143	0.201	0.207	0.116
	30	0.206	0.097	0.235	0.178
Jurc 2	1	0.130	0.754	1.487	1.999
	2	0.169	0.510	1.503	0.959
	3	0.108	0.111	0.151	0.091
	4	1.999	1.999	1.999	1.999
	5	0.101	0.650	1.708	1.258
	6	0.122	0.845	0.407	1.999
	7	0.110	0.433	1.999	1.999
	8	0.096	0.119	0.220	0.097

TABLE B-5.--Continued

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		7/24	8/13	9/19	10/24
Jurc 2	9	0.109	0.334	1.999	1.999
	10	0.095	0.141	0.165	0.094
	11	0.109	0.476	0.944	1,999
	12	0.150	0.324	0.752	0.591
	13	0.098	1.204	0.981	1.999
	14	0.096	0.120	0.205	0.484
	15	0.098	0.535	1.999	1.999
	16	1.999	1.999	1.999	1.999
	17	0.089	0.105	0.204	0.097
	18	0.103	0.291	0.889	1.132
	19	0.118	0.111	0.213	0.090
	20	0.099	0.569	0.831	1.511
	21	0.191	1.999	1.999	1.999
	22	0.098	0.469	1.808	1.999
	23	0.525	1.834	1.999	1.999
	24	0.090	1.000	1.999	1.999
	25	0.100	0.108	0.236	0.092
	26	0.096	0.115	0.104	0.101
	27	0.146	0.982	1.999	1.999
	28	0.109	0.208	0.500	1.372
	29	0.106	0.335	1.999	1.999
	30	0.095	0.130	0.200	0.105
1 A 1	1	0.105	1.940	1.999	1.999
	2	0.105	0.812	1.999	1.999
	3	0.103	0.569	1.170	1.012
	4	0.428	0.464	1.010	0.751
	5	0.107	0.819	1.907	1.999
	6	1.999	1.999	1.999	1.999
	7	0.096	0.739	1.342	1.693
	8	0.111	0.489	1.999	1.999
	9	0.084	0.140	0.512	0.610
	10	1.999	1.999	1.999	1.999
	11	0.101	0.350	0.722	1.445
	12	0.102	0.313	0.869	1.999
	13	0.109	0.339	1.460	1.999
	14	0.095	1.088	1.999	1.999
	15	0.102	0.692	1.805	0.961
	16	0.113	0.693	1.702	1.999
	17	0.095	1.088	1.999	1.999
	18	0.095	0.429	1.999	1.999
	19	0.732	1.668	0.817	0.990
	20	0.099	0.444	1.778	0.879

TABLE B-5.--Continued

Treatment (Strain)	Plant	1984 Sample Date of ELISA Tests			
		7/24	8/13	9/19	10/24
1 A 1 Continued	21	0.106	0.141	0.136	0.096
	22	1.827	1.999	1.999	1.999
	23	1.999	1.806	0.585	0.479
	24	0.091	0.333	1.731	1.748
	25	1.999	1.971	0.869	1.999
	26	0.100	0.862	0.816	1.175
	27	0.596	1.792	1.999	1.999
	28	0.116	0.644	1.999	1.999
	29	0.107	0.588	1.999	1.999
	30	0.106	0. 632	1.180	1.999
Critical Value:					
$\bar{x}_{healthy}$ + 3 std. dev.		0.176	0.222	0.356	0.336
$2\bar{x}_{healthy}$		0.324	0.244	0.404	0.402

[a]Each A_{405} value represents the mean of two replicate wells per plant.

[b]Control = healthy plants inoculated with extraction buffer and healthy cucumber by the leafrub and barkflap combination, respectively. The control plants are from the previous inoculation Experiments III and IV.

TABLE B-6.--Mean A_{405nm} values[a] for each plant that was ELISA tested for PNRSV on three dates for inoculation Experiment VI. Plants were either leafrub/barkflap (L/BF) or bud (B) inoculated with PNRSV strain 1A1 or Jurczack 2 (Jurc 2) on June 28, 2984, and June 29, 1984, respectively

Treatment (Strain, inoculation method)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
Control[b] L/BF	1	0.219	0.115	0.184
	2	0.107	0.079	0.194
	3	0.224	0.156	0.208
	4	0.089	0.223	0.107
	5	0.104	0.216	0.118
	6	0.123	0.121	0.158
	7	0.153	0.121	0.221
	8	0.155	0.099	0.173
	9	0.122	0.081	0.210
	10	0.123	0.150	0.215
	11	0.132	0.082	0.116
	12	0.142	0.230	0.150
	13	0.170	0.207	0.156
	14	0.131	0.209	0.184
	15	0.206	0.131	0.191
	16	0.108	0.190	0.216
	17	0.199	0.106	0.127
	18	0.169	0.237	0.196
	19	0.233	0.160	0.214
	20	0.137	0.096	0.171
	21	0.243	0.223	0.217
	22	0.099	0.126	0.162
	23	0.109	0.116	0.122
	24	0.276	0.094	0.135
	25	0.107	0.189	0.141
	26	0.147	0.171	0.237
	27	0.248	0.150	0.169
	28	0.097	0.110	0.218
	29	0.119	0.140	0.162
	30	0.118	0.151	0.129
1 A 1 L/BF	1	0.270	0.231	0.179
	2	0.121	0.109	0.223
	3	0.217	0.228	0.215
	4	0.149	0.160	0.104
	5	0.278	0.229	0.170
	6	0.191	0.109	0.331

TABLE B-6.--Continued

Treatment (Strain, inoculation method)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
1 A 1 B	21	0.255	0.212	0.249
	22	0.388	1.839	1.999
	23	0.226	0.121	0.298
	24	0.322	1.289	1.999
	25	0.152	0.604	0.485
	26	0.163	1.305	1.999
	27	0.147	0.179	0.237
	28	0.213	0.306	0.578
	28	0.247	1.123	1.999
	30	0.110	0.225	0.278
Jurc 2 L/BF	1	0.195	0.130	0.113
	2	0.177	0.220	0.176
	3	0.194	0.143	0.190
	4	0.219	0.196	0.309
	5	0.197	0.149	0.115
	6	0.163	0.343	0.216
	7	0.121	0.194	0.153
	8	0.138	0.230	0.186
	9	0.208	0.115	0.201
	10	0.193	0.243	0.303
	11	0.234	0.581	0.496
	12	0.189	0.273	0.174
	13	0.155	0.229	0.126
	14	0.152	0.141	0.242
	15	0.116	0.106	0.263
	16	0.135	0.186	0.190
	17	0.143	0.645	0.403
	18	0.215	0.089	0.167
	19	0.218	0.291	0.303
	20	0.205	0.309	0.282
	21	0.111	0.653	0.340
	22	0.233	0.095	0.120
	23	0.237	0.237	0.113
	24	0.091	0.208	0.240
	25	0.121	0.244	0.297
	26	0.246	0.158	0.238
	27	0.205	0.277	0.308
	28	0.097	0.219	0.297
	29	0.131	0.312	0.252
	30	0.137	0.289	0.274

TABLE B-6.--Continued

Treatment (Strain, inoculation method)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
1 A 1 L/BF Continued	7	0.175	0.136	0.204
	8	0.190	0.226	0.221
	9	0.220	0.216	0.124
	10	0.143	0.281	0.127
	11	0.167	0.383	0.294
	12	0.130	0.505	0.604
	13	0.215	0.160	0.251
	14	0.235	0.156	0.326
	15	0.109	0.229	0.278
	16	0.162	0.191	0.311
	17	0.238	0.278	0.182
	18	0.108	0.147	0.292
	19	0.193	0.266	0.086
	20	0.157	0.142	0.241
	21	0.237	0.249	0.297
	22	0.280	0.147	0.205
	23	0.238	0.190	0.280
	24	0.144	0.187	0.269
	25	0.135	0.297	0.217
	26	0.208	0.223	0.217
	27	0.098	0.217	0.141
	28	0.179	0.229	0.095
	29	0.131	0.156	0.282
	30	0.191	0.243	0.335
1 A 1 B	1	0.574	1.999	1.999
	2	0.189	1.722	1.455
	3	0.276	1.505	1.972
	4	0.166	0.353	1.009
	5	0.318	0.254	0.705
	6	0.531	1.999	1.999
	7	0.119	0.392	1.602
	8	0.298	0.150	0.122
	9	0.213	0.284	0.991
	10	0.195	1.323	1.952
	11	0.422	1.999	1.999
	12	0.306	0.315	1.405
	13	0.318	0.883	1.999
	14	0.198	0.242	0.196
	15	0.262	0.178	0.222
	16	0.316	0.398	0.925
	17	0.176	0.961	0.451
	18	0.162	0.124	0.123
	19	0.254	0.661	0.370
	20	0.607	1.999	1.999

TABLE B-6.--Continued

Treatment (Strain, inoculation method)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
Jurc 2 B	1	0.273	1.999	1.999
	2	0.122	1.999	1.999
	3	0.506	1.019	1.999
	4	0.326	0.264	0.779
	5	0.194	0.226	0.172
	6	0.221	0.169	0.209
	7	0.448	0.791	1.783
	8	0.226	0.197	0.167
	9	0.286	0.197	0.167
	10	0.454	1.098	1.999
	11	0.135	1.459	1.999
	12	0.601	1.999	1.999
	13	0.381	0.750	1.999
	14	0.156	0.236	0.122
	15	0.285	0.795	1.206
	16	0.276	0.896	1.999
	17	0.185	0.722	0.874
	18	0.463	1.999	1.999
	19	0.189	0.948	1.170
	20	0.136	0.232	0.193
	21	0.572	1.999	1.999
	22	0.111	0.235	0.189
	23	0.328	0.636	1.526
	24	0.346	1.999	1.522
	25	0.124	1.770	1.999
	26	0.251	0.971	1.631
	27	0.344	1.198	1.999
	28	0.445	1.999	1.999
	29	0.219	1.999	1.999
	30	0.194	0.378	1.729
Critical ELISA Values				
$\bar{x}_{healthy}$ + 3 std. dev.		0.306	0.327	0.335
$2\bar{x}_{healthy}$		0.302	0.332	0.396

[a]Each A_{405} value represents the mean of two replicate wells per plant.

[b]Control: Plants were inoculated with extraction buffer and healthy cucumber by the leafrub/barkflap combination, respectively.

TABLE B-7.--Mean A_{405nm} values[a] for each plant that was ELISA tested on three dates in inoculation Experiment VII. Plants were inoculated with PNRSV strain 1 A 1 of Jurczack 2 (Jurc 2) by the bud method on June 30, 1984

Treatment (Strain)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
Control[b]	1	0.131	0.209	0.284
	2	0.233	0.160	0.259
	3	0.107	0.079	0.109
	4	0.089	0.223	0.127
	5	0.099	0.126	0.188
	6	0.118	0.151	0.164
	7	0.169	0.237	0.281
	8	0.142	0.230	0.225
	9	0.208	0.190	0.193
	10	0.170	0.207	0.163
	11	0.123	0.121	0.198
	12	0.132	0.082	0.191
	13	0.224	0.156	0.228
	14	0.219	0.115	0.184
	15	0.109	0.116	0.221
	16	0.248	0.150	0.199
	17	0.123	0.150	0.220
	18	0.206	0.131	0.196
	19	0.155	0.099	0.203
	20	0.104	0.216	0.153
	21	0.143	0.223	0.216
	22	0.147	0.171	0.167
	23	0.153	0.121	0.231
	24	0.276	0.094	0.235
	25	0.127	0.081	0.255
	26	0.137	0.091	0.227
	27	0.107	0.189	0.231
	28	0.199	0.106	0.222
	29	0.120	0.140	0.116
	30	0.097	0.110	0.178
Jurc 2	1	1.999	1.822	1.999
	2	1.999	1.641	1.999
	3	0.714	1.999	1.999
	4	0.537	1.999	1.999
	5	1.999	1.999	1.999
	6	0.791	1.449	1.240
	7	0.442	±.999	1.999
	8	0.806	1.309	1.279
	9	1.126	1.999	1.732

TABLE B-7.--Continued

Treatment (Strain)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
Jurc 2	10	0.978	1.999	1.999
	11	0.475	1.417	1.999
	12	0.688	1.999	1.999
	13	0.099	0.123	0.199
	14	1.999	1.858	1.999
	15	0.837	1.999	1.611
	16	1.312	1.630	1.999
	17	0.730	0.582	1.484
	18	0.353	0.620	1.176
	19	0.607	1.424	1.999
	20	0.653	0.616	1.999
	21	0.372	0.519	1.486
	22	0.435	1.627	1.999
	23	1.136	1.858	1.422
	24	0.435	1.215	1.999
	25	1.999	1.999	1.999
	26	1.999	1.999	1.999
	27	1.730	1.999	1.999
	28	0.913	0.750	1.005
	29	0.239	0.563	0.905
	30	1.075	1.226	1.999
1 A 1	1	0.825	1.999	1.999
	2	1.133	1.416	1.857
	3	0.625	1.999	1.999
	4	0.604	1.796	1.233
	5	1.143	1.911	1.469
	6	0.289	0.359	0.839
	7	0.117	0.199	0.126
	8	0.204	0.311	0.432
	9	0.237	0.370	0.383
	10	0.533	1.865	1.749
	11	0.670	1.999	1.999
	12	0.537	1.999	1.669
	13	0.289	0.331	0.622
	14	0.208	0.168	0.112
	15	1.999	1.999	1.999
	16	1.999	1.502	1.999
	17	0.422	0.616	1.175
	18	0.165	0.147	0.172
	19	0.327	0.790	1.246
	20	0.193	0.235	0.517
	21	0.315	0.216	0.531
	22	0.142	0.092	0.197

TABLE B-7.--Continued

Treatment (Strain)	Plant	1984 Sample Dates of ELISA Tests		
		8/13	9/3	10/24
1 A 1 Continued	23	0.457	1.740	1.999
	24	0.195	0.124	0.148
	25	0.415	0.618	0.535
	26	0.575	1.604	1.464
	27	0.925	1.999	1.999
	28	0.232	0.313	1.324
	29	0.840	0.627	1.728
	30	1.650	1.999	1.999
Critical Values				
$\bar{x}_{healthy}$ + 3 std. dev.		0.305	0.298	0.334
2 $\bar{x}_{healthy}$		0.308	0.298	0.405

[a]For each plant sampled, two replicate wells were used in the ELISA tests and A_{405} values were averages.

[b]Control plants were inoculated with extraction buffer and healthy cucumber by the leafrub/barkflap combination, respectively.

Literature Cited

Barnett, O. W. and R. W. Fulton. 1969. Some chmeical properties of prunus necrotic ringspot and tulare apple mosaic viuruses. Virology 39:556.

Berkley, G. H. and R. S. Willison. 1948. Yellows and necrotic ringspot of sour cherries in Ontario: inoculation experiments. Phytopathol. 38:509-518.

Boxus, P. and M. Quoirin. 1974. The culture of apical meristems of several species of Prunus. Bulletin de la Societe Royal de Botanique de Beligique. Tome 107, Fasicule 1.

Boyle, J. S., J. D. Moore and G. W. Keitt. 1954. Cucumber as a plant host in stone fruit virus research. Phytopathol. 44:303-312.

Cochran, L. C. and L. M. Hutchins. 1941. A severe ringspot virosis of peach. Phytopathol. 31:860. (Abstr.)

Cole, A, G. I. Mink, and S. Regev. 1982. Location of prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathol. 72:1542-1544.

Constantine, D. R. and A. J. Abbott. 1978. Microvegetative propagation and modification of Prunus spp. Rep. Long Ashton Res. Stat. for 1977:63-64.

Cropley, R. 1964. Further studies on European rasp-leaf and leaf-roll diseases of cherry trees. Ann. Appl. Biol. 53:333-341.

Davidson, R. T. and J. A. George. 1964. Spread of necrotic ringspot and sour cherry yellows virus in Niagara peninsula orchards. Can. J. Bot. 44:471-484.

Dunstan, D. I. 1981. Transplantation and post-transplantation of micropropagated tree fruit rootstocks. In: Proc. Int. Plant Prop. Society. 31:39-44.

Feucht, W. and B. Dausend. 1976. Root induction of easy-to-root Prunus psuedocerasus and difficult-to-root Prunus avium Scien. Hortic. 4:49-54.

Fridlund. P. R. 1970. Intrageneric graft compatibility in relation to graft transmission of Prunus viruses. Sucr. Stiint. Inst. Agron. 'Nicolae Balcescu' Agron. 23:5-10.

Fridlund, P. R. 1970. Temperature effects on virus disease symptoms in some Prunus, Malus, and Pyrus cultivars. Washington Agricultural Experiment Station, Bulletin No. 726. 6 pp.

_____. 1968. Effects of high temperature on graft transmission of prusus ringspot virus. Phytopathol. 58:1192-1193.

_____. 1967. The relation of inoculum-receptor contact period to the rate of graft transmission on 12 Prunus viruses. Phytopathol. 57:1296-1299.

_____. 1967. The effect of time and temperature on the rate of graft transmission of prunus ringspot virus. Phytopathol. 57:230-231.

_____. 1966. Transmission or lack of viruses through Prunus seed. Plant. Dis. Reptr. 50:902-904.

Fulton, R. W. 1970. Prune dwarf virus. Descriptions of Plant Viruses. No. 19. Commonwealth Mycological Institute/Association of Applied Biologists. Kew, Surrey. Unpaged.

_____. 1970. Prunus necrotic ringspot virus. Descriptions of Plant Viruses. No. 5. Commonwealth Mycological Institute/ Association of Applied Biologists. Kew, Surrey. Unpaged.

_____. 1958. Identity of and relationships among certain sour cherry viruses mechanically transmitted to Prunus species. Virology 6:499-511.

George, J. A. and T. R. Davidson. 1964. Further evidence of pollen transmission of necrotic ringspot ans sour cherry yellows in sour cherry. Can. J. of Plant Sci. 44:383-384.

Gilmer, R. M. and R. D. Way. 1960. Pollen transmission of necrotic ringspot and prune dwarf viruses in sour cherry. Phytopathol. 50:624-625.

Halk, E. L. and R. W. Fulton. 1978. Stabilization and particle morphology of prune dwarf virus. Virology 91:434-443.

Hammerschlag, F. 1980. Peach micropropagation. In: Proc. conf. on nursery production of fruit plants through tissue culture-applications and feasibility, pp. 40-52. U.S.D.A. S.E.A., ARR-NE-11.

Hartman, H. T. and D. E. Kester. Techniques of propagation by cuttings. pp. 272-274. In: Plant Propagation, Prentice Hall Inc. Englewood Cliffs, New Jersey. 662 pp.

Helton, A. W. and B, Bowyn. 1964. Comparative value of bud-shield and bark patches in transfer of prunus ringspot virus from 'Italian Prune' to 'Shirofugen'. Phytopathol. 54:1339-1341.

Hildebrand, E. M., G. H. Berkeley and D. Clayton. 1942. Handbook of virus disease of stone fruits in north america. Michigan Agricultural Experiment Station Misc. Publ., 76 pp.

Hildebrand, E. M. et al. 1942. Prune Dwarf. Phytopathol. 32:741-751.

_____. 1942. Rapid transmission techniques for stone fruit viruses. Science. 95:52.

Jones, O. P. and M. E. Hopgood. 2979. The successful propagation in vitro of two rootstocks of Prunus: the plum rootstock 'Pixy' (P. insititia) and the cherry rootstock f12/1 (P. avium). Jour. of Hort. Science. 54(1):63-66.

Keitt, G. W. and C. N. Clayton. 1939. A destructive bud transmissable disease of sour cherries in Wisconsin. Phytopathol. 29:821-822.

Keitt, G. W. et al. 1951. Sour cherry yellows. pp. 152-158 In: Virus Diseases and Other Disorders with Virus-like Symptoms of Stone Fruits in North America. U.S. Dept. Agr. Handb. 10.

Keitt, G. W. and C. N. Clayton. 1943. A destructive virus disease of sour cherry. Phytopath. 33:449-468.

Keitt, G. W. and J. D. Moore. 1943. Masking of symptoms of sour cherry yellows by temperature effects. Phytopathol. 33:1213-1215.

Klos, E. J. and K. G. Parker. 1960. Yields of sour cherry affected by ringspot and yellows virus. Phytopathol. 50:412-415.

Lewis, F. H. 1951. The effect of ringspot and yellows on yield of 'Montmorency' cherry. Phytopathol. 33:1213-1215. (Abstr.).

Lindner, R. C., H. C. Kirkpatrick and T. E. Weeks. 1959. The airbrush as a tool in virus inoculations. Phytopathol. 49:507-509.

Linsmaier, E. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18:100-127.

Loesch, L. S. and R. W. Fulton. 1975. Prunus necrotic ringspot virus as a multicomponent system. Virology 68:71-78.

Milbrath, J. A. 1959. Mechanical transmission of the virus responsible for sour cherry yellows, prune dwarf and peach stunt. Phytopathol. 49:545. (Abstr.).

Mills, W. D. 1946. Temperature effects on the expression of the yellows virus in sour cherry. Phytopathol. 36:353-358.

Moore, J. D., J. S. Boyle and G. W. Keitt. 1948. Mechanical transmission of a virus disease to cucumber form sour cherry. Science 108:623-624.

Moore, J. D. and G. W. Keitt. 1946. Relation of temperature to expression of symptoms of sour cherry yellows and necrotic ringspot virus. Phytopathol. 36:406-407.

Moore, J. D. 1945, The etiology of cherry yellows and necrotic ringspot. Ph. D. Thesis. University of Wisconsin. Madison, WI.

Moore, J. D. and G. W. Keitt. 1944. Host range studies of necrotic ringspot and yellows of sour cherry. Phytopathol. 34:1009.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nyland, G. 1960. Heat inactivation of stone fruit ringspot virus. Phytopathol. 50:380-382.

Popov, Y. G., V. A. Vysotiskii and V. G. Trushechkin. 1976. Cultivation of isolated stem apices of sour cherry. Pisiologiya Rastenii 23(3):513-518.

Posnette, A. F., et al. 1968. The incidence of virus diseases in English sweet cherry orchards and their effects on yield. Ann. Appl. Biol. 61:351.

Rosati, P., G. Marino and C. Swierczewski. 1980. In vitro propagation of Japenese plum (Prunus salicina Lindl. cv. 'Calita'). Proc. J. Amer. Soc. Hort. Sci. 105(1):126-129.

Snir, I. 1982. In vitro micorpropagation of sweet cherry cultivars. Proc. 5th Cong. Plant Tissue and Cell Culture. Plant Tissue Culture. 735-736.

Swenson and Milbrath. 1964. Insect and mite transmission tests prunus ringspot virus. Phytopathol. 54:399-404.

Thomas, H. E. 1940. Graft transmission persistence and migration of some viruses in fruit trees. Phytopathol. 30:790 (Abstr.).

Thomas, H. E. and G. H. Hildebrand. 1936. A virus disease of prune. Phytopathol. 26:1145-1148.

Vertesy, J. and J. Nyeki. 1974. Effect of different ringspot viruses on the flowering period and fruit set of 'Montmorency' and 'Pandy' sour cherries. I. Acta Phytopathologica Academiae Scientiarum Hungaricae 9:19-22. (see also Acta Horticultrae)

Waterworth, H. E. and R. W. Fulton. 1964. Variation among isolates of necrotic ringspot virus and prune dwarf virus in sour cherry. Phytopathol. 34:1037-1049.

Way, R. D. and R. M. Gilmer. 1963. Reductions in fruit sets on cherry trees pollinated with pollen from trees with sour cherry yellows. Phytopathol. 53:399-401.

Willison, R. S. 1944. Strains of prune dwarf. Phytopathol. 34:1037-1049.