

## Net Necrosis Prediction and Development in Storage<sup>1,2</sup>

P. E. Thomas<sup>3</sup>, John Shields<sup>4</sup>, Mel Martin<sup>5</sup>, and Gary McMullin<sup>6</sup>

<sup>3</sup>Agricultural Research Service, U.S. Department of Agriculture, 24106 N. Bunn Road, Prosser, WA 99350-9687, <sup>6</sup>McCain Foods, 100 Lee St., Othello, WA 99344, <sup>4</sup>Simplot, Othello, WA 99344 <sup>5</sup>Twin City Foods, Prosser, WA 99350-9687

<sup>1</sup>*Paper presented at the Annual Washington State Potato Conference and Trade Fair, Big Bend Community College, Mosses Lake, Feb. 6-8 2001.*

<sup>2</sup>*Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.*

### Introduction

Net necrosis is a dark discoloration of phloem tissue (food conducting tissues) in potato tubers. It often, but not always, develops in response to infection of the plant with potato leafroll virus (PLRV). Stem end necrosis is another type of vascular necrosis commonly observed in tubers. It is not caused by PLRV infection but by other poorly understood factors thought to be abiotic in nature. Net necrosis begins at the stem end of the tuber and may extend into the tuber to various depths and can extend the full length of the tuber. Since it markedly degrades the processing quality of tubers, netted portions of tubers must be discarded. In practice, the entire tuber may be discarded when netting extends more than an inch or so into the tuber. Thus, both the incidence and penetration depth are important factors in determining losses to net necrosis.

Previous studies (Thomas and Zielinska, 1983) demonstrated that both incidence and severity of net necrosis can increase after harvest while tubers are in storage. If processors could predict prior to harvest the subsequent development of net necrosis during storage, those lots of tubers most likely to develop net could be processed early during the harvest season to avoid net necrosis storage losses.

The studies reported here were conducted to determine the factors that affect development of net necrosis, including the effects of the strain of PLRV involved in the infection, the time and growth stage of plants at the time of infection, the storage duration and temperature, and the effect of infection with other potato viruses in combination with PLRV. We also wanted to determine the effectiveness of a rapid immuno-blot tuber test for PLRV to predict net necrosis development in storage. This report combines the results of several independent studies in an effort to provide a more complete coverage of the topic.

### General Methods

To account for both incidence and depth of net necrosis penetration in estimating losses, a disease damage index was calculated for each lot of tubers (Fig. 1). First, each tuber was scored for depth of net necrosis penetration from the stem end of the tuber. Tubers in which necrosis extended less than ½ inch were scored 0; extension to ½ inch but less 1 inch were scored 1, extension 1 inch but less than 2 inches scored 2, extension to 2 inches but 3 inches were scored 3, and extension

for more than 3 inches were scored 4. The individual scores for each tuber were totaled to determine a damage index for a lot of tubers.

To determine the actual incidence of PLRV infection in each lot of tubers, a plant was produced in a greenhouse from a melon-balled seed piece cut from the rose end of each tuber. The green tissue of each plant was assayed for PLRV and potato viruses A, M, S, X, and Y by an ELISA procedure. In the immuno-blot assay estimate PLRV infection in tubers, a small (1/4 X 1/4 inch), freshly-cut, longitudinal, cross section from the stem end of each tuber was blotted on nitrocellulose. The nitrocellulose was then developed in an ELISA-like serological procedure to detect the presence of PLRV antigen adsorbed to the nitrocellulose.

### **The Effect of Time and Growth Stage at the Time of Infection**

Two types of studies were conducted. In one, potato plots were inoculated with PLRV by placing infective green peach aphids on each plant in mid May, covering the plots with a floating screen to confine the aphids for a week, and then killing the aphids with insecticide (Fig. 2). Comparable plots were exposed naturally to the mid-summer aphid flight and became infected over approximately a 6-wk period in late July and August. In another type of experiment to measure the effect of time of inoculation, infective apterous aphids were placed on the central plants in rows of plants spaced 8-ft apart in early May. As the season progressed, the wingless aphids carried virus from the centrally inoculated plant toward the ends of the rows in both directions from the center. Plants at the ends of the rows were last to become infected (Fig 3). Tubers were harvested and rated for net necrosis.

In both experiments (Fig. 2 and 3), plants that were infected early in the season before tubers were formed or while they were still small produced relatively few net necrosis-affected tubers. Tubers from plants infected early in the season had very low damage index ratings. Typical, strong foliage symptoms were produced early in the season but the symptoms produced on new growth late in the season was much milder. In contrast, plants that were infected late in the season while tubers were bulking rapidly produced much stronger foliage growth early in the season but high incidence of net necrosis with high damage index readings (Fig. 2 and 3).

These results suggest that net necrosis is a shock reaction of the potato plant to virus infection typical of the initial shock reactions produced by many viruses on specific hosts. The initial symptoms following infection with a plant virus are often severe and often involve veinal necrosis of infected tissues. Plants typically show at least some degree of recovery following the initial symptoms, and in many instances new growth may be completely asymptomatic. So it is with PLRV infection of potato. Current season symptoms, those caused by new infections in the current season, are always much more severe than chronic symptoms, those produced when plants are grown from PLRV-infected seed pieces. The fact that net necrosis is associated with current season infections and rarely, if ever, with chronic infections is widely recognized among potato growers. Microscopic examination of foliage tissues associated with the initial current season symptoms reveals the same necrosis of veinal tissues as occurs in tubers that express net necrosis. The current season symptoms on plants infected very early in the season often recover to a point where they resemble those of the milder chronic symptoms of PLRV on new growth near the end of the growing season.

## **The effect of virus isolate on net necrosis**

Some isolates of PLRV appear to be more virulent than others. Our results (Fig. 4) suggest that the strain of virus has a strong influence on incidence and severity of net necrosis development. The strain of virus that may invade a growers field is not a variable that can be controlled. However, differences in the mix of strains of PLRV in different regions may play a role in determining net necrosis losses.

### A Prediction Assay for Net Necrosis Storage Losses

In cooperation with three major processors, a total of 79 circles of potatoes from the Columbia Basin of Washington were surveyed. There were some slight variations in the exact procedures used with each of the processors. In general, however, three tubers were harvested from each of 60 randomly selected plants in each field. One tuber was placed in each of three bags so that each bag contained one each of 60 sets of sister tubers from each of 60 plants in the circle. At harvest, each tuber from one of the three bags harvested from each of the 79 circles was scored for net necrosis, assayed for PLRV using the stem-end blot, grown-out in a greenhouse and assayed for PLRV and potato viruses A, M, S, X, and Y using an ELISA procedure. The other two bags were divided, and half of each bag was stored at 40 or 48 degrees F for 3 or 7 months. Each bag was scored for net necrosis at the end of the specified storage period.

Overall, 24% of the tubers were infected with PLRV, 3.8% were infected with PVY, and less than 0.1% were infected with PVA, PVM, and PVX. As expected, PVS infected 100% of the tubers in most fields, but a few circles were nearly free of the virus. Infection of tubers with PVS or PVY in addition to PLRV did not influence net necrosis.

The tuber blot assay underestimated PLRV infection by 6%. Although the tuber blot assay selected circles of potatoes most likely to develop necrosis in storage with a fair degree of accuracy, the assay was time consuming and cumbersome. Furthermore, it was more likely to miss the late season infections that are most likely to cause net necrosis. Rather than use it, we think that careful field inspection coupled with limited ELISA assays of foliage samples to confirm visual assessments of infection would provide a more reliable prediction of net necrosis in storage. The visual inspection provides an easy distinction between early and late season infection.

The greenhouse grow-out coupled with ELISA revealed that most tubers that received a net necrosis score of 1 were not infected with PLRV, and nearly all that scored higher than 1 were infected with PLRV. Therefore, the tubers with a score of 1 were excluded from our calculation of effects of storage temperature and duration on net necrosis development (Fig. 5).

With score category 1 excluded, the damage index for net necrosis increased approximately 2.5 fold during the first 3 months at 48 degrees F. The increase in net necrosis was 1.9 fold at 40 F, slightly lower at 40 than at 48 F. Only slight increases in net necrosis occurred after the first three months of storage.

The results from these studies show clearly that there is a very large increase in incidence and severity of net necrosis during the first three months of storage and the lots of tubers most likely to develop net necrosis in storage can be selected at harvest time.

Literature Cited

Thomas, P.E. and Zielinska, L. 1983. Use of IKI leafroll test to reduce net necrosis storage losses of potatoes. Am. Potato J. 60:309-320.

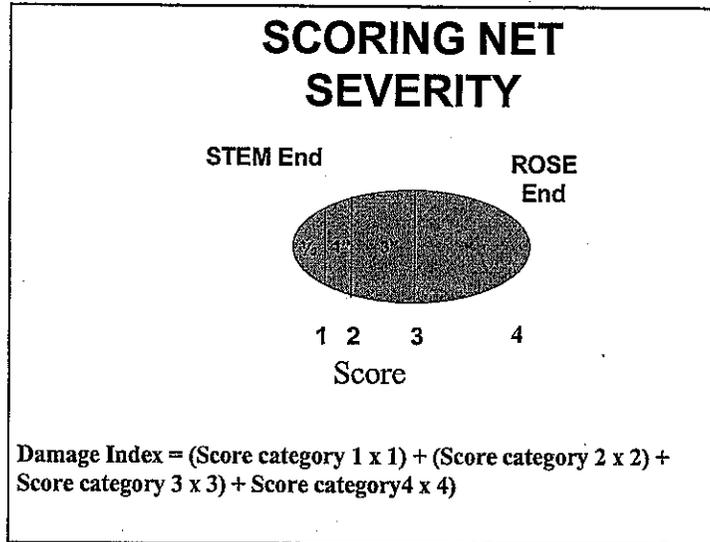


Figure 1.

Method of scoring net necrosis severity and of calculating the damage index

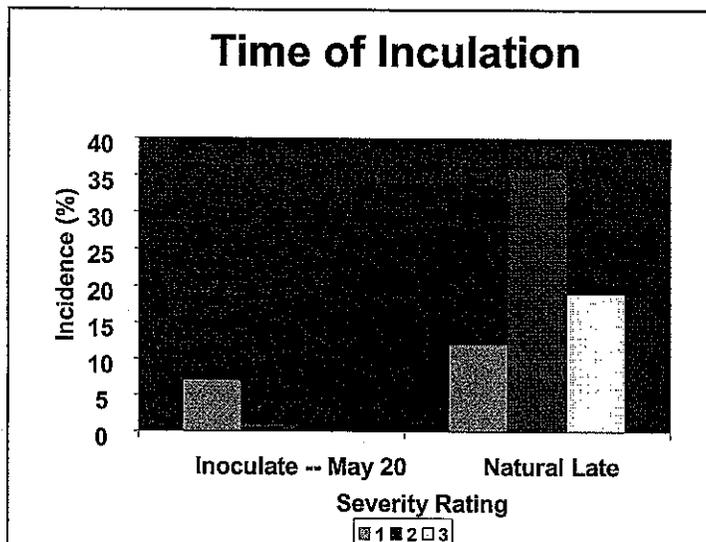


Figure 2.

Effect of time of inoculation on incidence and severity of net necrosis

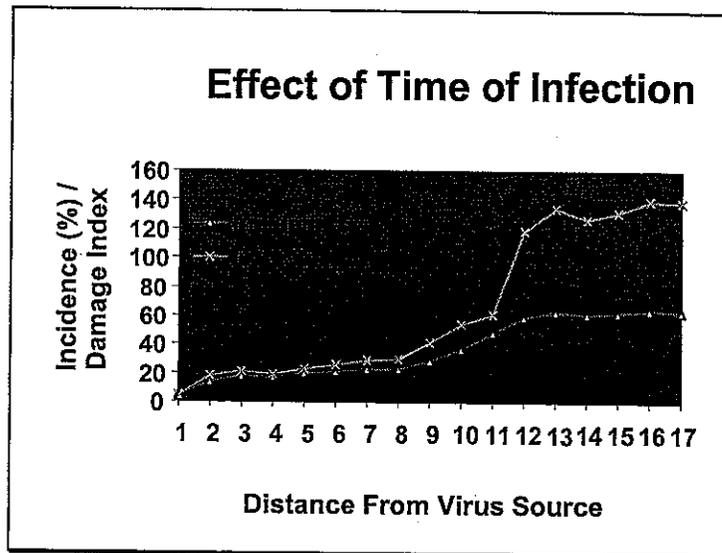


Figure 3.

Effect of time of inoculation on incidence and severity of net necrosis

### Net Necrosis

#### Effect of Virus Isolate

Isolate	Incidence	Severity Score			Mean
		1	2	3	
LR-1	27	5	10	12	2.3
LR-6	14	7	7	0	1.5
LR-7	34	8	10	16	2.2
LR-21	12	4	6	2	2
LR-43	16	8	9	4	1.8

Figure 4.

Effect of virus isolate on incidence and severity of net necrosis

Effect of Storage Time and Temperature						
		% per Score Category				Damage
		1	2	3	4	Index
At Harvest	Inc 1	9.9	3.3	1.1	0.9	23.2
	Exc 1		6.6	3.3	3.4	13.3
3 Mo. - 47°F	Inc 1	9.5	5.3	3.0	3.2	41.9
	Exc 1		10.6	9.0	12.8	32.4
3Mo. - 40°F	Inc 1	9.7	3.8	2.1	2.9	35.0
	Exc 1		7.6	6.3	11.6	25.5
7 Mo. - 47°F	Inc 1	9.5	5.8	2.8	3.5	43.5
	Exc 1		11.6	8.4	14.0	34.0
7Mo. - 40°F	Inc 1	9.8	3.8	2.2	3.1	36.2
	Exc 1		7.6	6.6	12.4	26.6

**Figure 5**  
**Increase in net necrosis during storage. Effects of storage temperature and duration**