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Final Report of

# Bioreactor Evaluation for Russet Norkotah Microtuber Production

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Wisconsin Agricultural Development and Diversification Program

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# Original Intent

Wisconsin is losing millions of dollars annually, importing Russet Norkotah seed potatoes. Russet Norkotah is a major potato variety grown in Wisconsin covering more than one third of the state's potato acreage in 1992. A significant problem with the variety is that Wisconsin seed growers are losing their ability to produce Russet Norkotah seed. Wisconsin seed growers currently require five field generations to grow Russet Norkotah, and under the present heavy disease pressure from the Potato Virus Y, this is not possible. Russet Norkotah is a unique variety in that it is susceptible to PVY, yet it does not show obvious visible symptoms. This characteristic of a "symptomless carrier" of PVY, makes Russet Norkotah much more difficult to grow as seed than any other variety. Over 50% of Russet Norkotah seed now is imported from other states, and Wisconsin seed potato growers are planting less acreage of Russet Norkotah.

By using low cost, large-sized microtubers, which are test-tube potatoes, Wisconsin growers can profitably produce Russet Norkotah seed in two field generations instead of five, making certified seed potato production possible. These large-sized microtubers need to be produced in advanced bioreactors. SPI will be evaluating two advanced bioreactors in order to compare the quality and quantity of production for the Russet Norkotah variety. The most economically productive bioreactor will be used for further scale-up of the variety. The microtubers will be marketed and sold primarily in Wisconsin in order to give seed growers the ability to supply Russet Norkotah seed to commercial growers in Wisconsin. The Wisconsin certified seed potato industry has annual sales of more than $15 M and SPI plans to increase that figure by $5 M by 1998, with increased sales of Russet Norkotah certified seed.

# Accomplishments

## Job Creation

Small Potatoes during the period of the grant created one and a half new jobs producing microtubers. Those people continue to be employed with Small Potatoes. Small Potatoes plans to hire more people in the future to produce Russet Norkotah microtubers. By the year 1998 Small Potatoes projects to employ a staff of more than 20 people at its Madison location.

## Capital Investment

Small Potatoes, during the period of the grant, took in $215,000 in investment capital. This capital was used to create new equipment and jobs to make Small Potatoes more productive. The Agriculture Diversification and Development Grant helped attract investors to Small Potatoes, Inc.

## Development of New Agricultural Products

The microtuber is a new agricultural product. Although microtubers have been in existence since the 1950's, they have not yet been used in commercial applications. The principle reason has been the high cost of production and their small size. This grant has contributed significantly in developing a bioreactor that produces Russet Norkotah microtubers at a lower cost and larger size.

In addition, a new microtuber planter, developed as a joint project with Small Potatoes, Inc. and the University of Wisconsin-Madison, was used to directly seed Russet Norkotah microtubers produced under the Agriculture Development and Diversification Grant. This novel planter plants microtubers with significantly less labor input than methods used for other potato propagules. A video tape of this process is enclosed. The video shows microtubers being planted at Ron Zalewski's farm in Bryant, Wisconsin. The planter, with only one operator, can plant 2.5 acres of microtubers per hour. In order to plant minitubers or transplants, many more operators are needed and planting is much slower.

## Application of the New Technology

The microtubers created by the new bioreactors are now being used in field trials and demonstration plots in Wisconsin. Russet Norkotah microtubers are being used to create certified seed potatoes here in Wisconsin.

## Improvement of Wisconsin's Competitive Position

Wisconsin's competitive position in terms of Russet Norkotah seed potatoes, will not change overnight. Certified seed potato production takes several years to develop. The impact of microtubers on Russet Norkotah will be seen over the next three to four years.

# Evaluation of Results and Benefits

## Introduction

SPI has developed two new bioreactor prototypes, A and B, that produce large-sized microtubers. Bioreactor A is in the prototype stage and is a higher cost bioreactor, but the microtuber quality and productivity may be higher than microtubers from bioreactor B. Bioreactor B is a simple low cost design that SPI has some working experience with. Both bioreactor designs are proprietary and the details of the design will not be presented in detail in this study.

Although the tuberization processes conducted at SPI has produced microtubers for all varieties attempted thus far, Bioreactor B had previously not been used for tuberization of Russet Norkotah. Every potato variety responds differently to physical and chemical stimuli, and each variety must be "fined tuned" to the appropriate bioreactor. The primary objective of this study is to evaluate the two bioreactors for their ability to produce high quality Russet Norkotah at a low cost.

## Materials and Methods

SPI purchased a line selection of Russet Norkotah from the Wisconsin State Seed Program. The individual shoots were multiplied on Murashige and Skoog tissue culture medium under 80 *u*Einsteins of flourescent light at 22$^{o}$C. Single node explants of the microshoots were transferred to fresh containers every 21 to 28 days. The multiplication process was conducted for four months in order to generate a sufficient number of plants to conduct the study.

The Russet Norkotah microshoots were randomized and then transferred into both Bioreactors A and B. The Bioreactors were randomized to insure equal exposure to light and temperature in the growth room. Bioreactors A and B received the same quantities of medium per explant in order to minimize differences between the treatments. All of the microtubers were harvested eight weeks after the tuberization process was started. At the harvest a sample of microtubers from the Bioreactors were sized, graded and weighed.

## Results

The microtuber productivity of each bioreactor is not statistically different (Figure 1). The fresh weights of the microtubers produced in

each bioreactor are not significantly different from each other. In addition the productivity of the bioreactors A and B did not differ significantly from each other.

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Figure 1. Microtuber fresh weights (FW) and number of microtubers per size catagory harvested from each bioreactor type. The microtubers harvested represent averages from eight replicates. Each treatment was arranged in a Randomized Complete Block.

| | FW | Microtuber number/BR greater than ... (inches) | | | |
|---|---|---|---|---|---|
| | (grams) | 0.375 | 0.3125 | 0.25 | 0.1875 |
| BR A (agar) | 23.03a | 4.88b | 16.25c | 36.00d | 45.50e |
| BR A (liquid) | 34.29a | 5.88b | 18.88c | 48.00d | 49.88e |
| BR B | 28.7a | 4.25b | 15.25c | 39.38d | 45.25e |

Identical letters within a column indicate no significant difference at the 0.05 significance level using the LSD test.

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Figure 2. Bioreactor cost per microtuber. The cost per use of Bioreactor A is $0.70 while the cost per use of Bioreactor B is $0.265. These calculations assume that handling costs are equal.

| | BR Cost/ use (US$) | MT Cost/ FW (US$) | Total MT/ BR | Cost/ MT (US$) |
|---|---|---|---|---|
| BR A (agar) | 0.700 | 0.0300 | 102.63 | 0.0068 |
| BR A (liquid) | 0.700 | 0.0200 | 122.64 | 0.0057 |
| BR B | 0.265 | 0.0092 | 104.13 | 0.0025 |

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Bioreactor B was able to produce more microtubers at a lower cost than Bioreactor A. The cost of a microtuber on a fresh weight basis and on a per microtuber basis were lower for Bioreactor B (Figure 2.). The overall size and quality of microtubers from Bioreactor B and Bioreactor A were not significantly different. Using Bioreactor A with liquid medium did produce slightly larger microtubers, but not significantly so. It is possible that with slightly different parameters Bioreactor A could produce larger size microtubers which would justify its use. SPI is in the process of investigating those parameters.

The cost per microtuber of Bioreactor A at $0.007 is still well below the actual sales price of the microtubers at $0.25. However, as microtuber prices continue to fall, the cost of the bioreactor becomes more significant. Microtuber prices may be less than $0.10 by the year 2000. It was the hypothesis of this investigation that Bioreactor A would produce significantly larger microtubers which would justify its larger per microtuber cost. However, it appears that the number and size of microtubers harvested from Bioreactor A are not significantly different from Bioreactor B. It is difficult to justify the extra expense of Bioreactor A.

At the beginning of this study the contamination rate of Bioreactor B was considerably higher than that of Bioreactor A. Further work on Bioreactor B has considerably reduced the contamination problem.

### Benefits

During the month of May, Small Potatoes planted Russet Norkotah microtubers in an experimental plot at the Langlade County Airport and in a demonstration plot with a selected grower. The experimental plot will compare the growth and yield of Russet Norkotah microtubers, transplants and minitubers. The progress on a demonstration plot with a selected grower is being videotaped through the 1994 growing season.

## Description of Educational Materials

### Extension Report

On February 17th, 1994, Peter Joyce gave a presentation to the Wisconsin seed potato growers on the microtuber field trials conducted in 1993. The yield results from the trial were presented and were discussed with an economic perspective. A copy of the presentation, including graphs, was published in the "University of Wisconsin-

Extension (UWEX) Proceedings of Wisconsin's Annual Potato Meetings 1993". A copy of the talk was also included with monthly grant reports.

### Field Days

A field trial with Russet Norkotah microtubers, generated by this grant, was planted at the Langlade County Experimental Farm. The trial compares the canopy growth and yields from minitubers, microtubers and microshoots of Russet Norkotah. The mid-season results of which will be discussed with the growers at Antigo Field Days, on July 20th, 1994.

In addition the results from the trial will be presented to the growers at the extension meetings in February of 1995. These results will include an economic discussion of using microtubers. The talk will be published in the "UWEX Proceedings of Wisconsin's Annual Potato Meetings 1995."

## Future Projections Resulting From Grant

### Improvements in Bioreactor Use

This study has allowed Small Potatoes, Inc. to make significant improvements in its bioreactor design that has increased productivity for the variety Russet Norkotah. The bioreactor capital costs on a per microtuber basis is less than $0.01.

### Lower Cost Production of Russet Norkotah

The lowering of production costs with this bioreactor allows microtubers to be produced at a lower price. A more effecient use of bioreactors lowers the costs of microtuber production.

### Increased Sales of Russet Norkotah Microtubers in Wisconsin

Small Potatoes, Inc. has recently offered to all Wisconsin seed potato growers the opportunity to purchase Russet Norkotah microtubers for the spring of 1995 for $0.25 each. Microtubers, at $0.25 each, can outproduce minitubers of a larger size, on a cost per tuber basis. Minitubers which retail from $0.50 to $1.00 are more expensive than microtubers and have equal yield in terms of tuber weight and number.

## Lower Incidence of PVY in Russet Norkotah

Microtubers used in a two year flush through program will lower the incidence of PVY in Russet Norkotahs grown in Wisconsin. Seed potatoes grown in the field for only 2 years have less exposure to PVY than seed potatoes grown for more than 2 years. Russet Norkotah seed potatoes grown in Wisconsin typically are exposed to PVY for 5 to 6 years.

## More Sales of Wisconsin Produced Foundation and Certified Russet Norkotah Seed Potatoes

In order to increase sales of Foundation and Certified Russet Norkotah seed potatoes, Wisconsin growers need to accomplish several tasks. One task would be to reduce the incidence of PVY in Russet Norkotah seed potatoes. One method to reduce the incidence of PVY in Russet Norkotah, is to have a two year flush through program. This program is only possible with high yielding, low cost propagules for the first generation of seed potatoes.

It is possible to sell more Wisconsin produced Russet Norkotah seed potatoes in the next two to three years, but it is more realistic that increases in Russet Norkotah seed production will be seen in the four to six year time frame.

## Field and Grower Trials with Russet Norkotah

As a result of the Wisconsin Agriculture Diversification and Development Grant, microtuber field trials and grower demonstration plots were set up in Langlade County. The University of Wisconsin, in coordination with Langlade County Extension, conduct field trials with potatoes at the Langlade County Airport. The field trials are first reviewed by the growers at Field Days in mid-July and then the information is presented more formally during the February winter meetings. Small Potatoes set up a field trial at the Langlade County Airport with collaboration from the extension agent, Dr. Steven Zimmerman. At this field trial, growers will be able to compare the performance of Russet Norkotah microtubers, minitubers and transplants. Growers will evaluate the three types of propagules in terms of tuber weight and tuber numbers. The yields will also be evaluated on the basis of their starting seed costs.

In addition, Small Potatoes is conducting a grower trial with Ron Zalewski. Ron is the first grower in Wisconsin to try Small Potatoes' new microtuber planter. The planter is a modified corn air planter, adjusted for microtubers. This microtuber trial is visible from the road for other growers and visitors to see. Both of these trials are directly a result of the Wisconsin Agriculture Diversification and Development Grant.