Colorado State University-Plant Virus Investigations

Project 118 - Clark H. Livingston

Potato Virus Research

Progress during 1982 and research plans for 1983

1. Virus Eradication[1/]

   a. Ribavirin, a synthetic nucleotide has found widespread application in the control of several animal viruses having RNA as the infective agent. It was hypothesized that this agent may have eradicative potential when incorporated into the nutrient media used to culture potato meristems being grown to develop virus X and S free cultivars and seed stocks.

      Ribavirin treatments of 0, 10, 20 and 40 µg/ml were applied to doubly infected (PVX + PVS) shoot tips as a component of the nutrient culture media. Viral assays of plantlet material were made by Gomphrena local lesion tests for PVX and latex agglutination serology for PVS detection.

      Ribavirin proved to be phytotoxic at all concentrations and lethal at 40 µg/ml. Treatment delayed plantlet development by 106 and 127 days for the 10 and 20 µg/ml concentrations, respectively, when compared to the controls.

      Virus assays indicated that 93 and 83% of the plantlets were free of PVX and PVS, respectively, following treatment with 10 µg/ml. All plantlets treated with 20 µg/ml were free of both viruses. A manuscript reporting the findings in this research has been submitted to Phytopathology and accepted for publication (manuscript copy)[1/]

   b. Benomyl, a widely used systemic fungistat, has been reported to exhibit antiviral activity. Therefore, benomyl at concentrations of 25 and 50 µg/ml were tested as a medium constituent for cultured plantlets grown from meristems which had been excised from doubly infected (PVX + PVS) Russet Burbank plants. Virus assays of treated plant material were as described above. There was no evidence of phytotoxicity and both treatemtn levels increased plantlet growth rates significantly. Benomyl at a concentration of 25 µg/ml exhibited a slight eradicative effect (3-30%) on PVS, but had no effect on PVS @ 50 µg/ml. Treatment with benomyl failed to eradicate PVX. A manuscript reporting these findings has been submitted to the American Potato Journal. (manuscript copy)[1/]

[1/] Attached reprints published in 1982, covering research reported

2. Potato Leaf Roll Virus (PLRV) and Beet Western Yellows virus (BWYV) in Relation to the Leaf Roll Disease of Potatoes.

a. Foliage samples were collected from potato plants exhibiting leaf roll symptoms in two certified potato fields in the San Luis Valley. The foliage was assayed for BWYV using the ELISA serological test[2/]. Twenty seven Centennial and eight Russet Burbank plants were tested and all proved to be negative for BWYV. Four plants showing current season symptoms of leaf roll were sampled in Weld County and tested for BWYV. All proved negative. Frozen foliage samples of eight weed plants collected in 1981 in the San Luis Valley were tested serologically for BWYV with the following results:

Plant	No. positive/number of individual plants tested (278 total)
Field bindweed	0/15
Swainson's pea	0/40
Redroot pigweed	0/22, 0/18, 0/33
Lambsquarters	0/24
Black nightshade	0/28, 0/27
Black mustard	0/28
Sunflower	0/15
Lettuce	0/28

b. Indicator host trap plants were placed in yellow pans at seven locations within potato fields in the SLV. Each pan contained eight potted Physalis floridana plants which were replaced weekly. Plants which had been exposed to potential viruliferous aphid infestation for the weekly period were taken to the greenhouse for observation and development of diagnostic leaf rool symptoms. The foliage of each plant was also assayed for BWYV using the ELISA test. None of the trap plants exhibited leaf roll symptoms and of the 290 plants tested serologically, all proved negative.

This project component is the Ph.D. thesis research of R. Klein. He has been successful in preparing his own antisera to BWYV although the titre and homologous reactions have not as yet been determined. All attempts to purify PLRV from Physalis floridana have resulted in preparations having virus concentrations too low to elicit adequate antibody formation in rabbits. Future attempts at PLRV isolation and purification will be made using virus free Russet Burbank foliage rather than Physalis foliage. Bob has completed his course work this semester so he will be registered for research credits and devote his full time to PLRV and BWYV purification and serology. The deep freeze is full of collected plant materials to be tested as soon as the appropriate sera are available. Additional sampling of potato fields in both the San Luis Valley and Northern Colorado will be made during the 1983 growing season for BWYV and PLRV tests.

2/ BWYV antisera supplied by Dr. J. E. Duffus.

c. Cultivar WC-230-14 was planted into pots in the greenhouse. Half were grafted to PLRV infected Russet Burbanks while the other half were infested with viruliferous aphids. Non-inoculated control plants and leafroll Russet Burbanks served as controls. The progeny tubers have been harvested and will be planted in the field in 1983 for evaluation of the WC-230-14 reaction to PLRV.

3. Chipping variety trials are established each year at five locations in the U.S. under the sponsorship of the Potato Chip Snack Food Association. I am responsible for the trials at Aberdeen, Idaho in cooperation with Drs. Pavek and Corcini and the Colorado trial at Fort Morgan. Seed of five varieties was supplied by Dr. Okeefe and planted at Wiggins, Colorado. Early blight reaction and yield data were obtained and sent to Dr. Okeefe for computer input. Field samples of tubers were also shipped for sugar analysis and storage studies.

Table 1. NORTHERN COLORADO CHIPPING CULTIVAR TRIAL - 1982

Cultivar	Manona	Norchip	Belchip	Croatan	Crystal	Wis-726
Early Blight % Leafsurface	7	16	6	6	3	3
Covered by lesions 9/16/82	B1/	A	BC	BC	D	D
Total Yield LBS/25 ft of row		67.4	56.3	90	91.3	51.1
Mean of 7 reps.		B1/	B	A	A	B

1/ Mean Values having a common letter do not differ significantly according to Duncan's multible range test

The results of this trial as well as those conducted at Idaho, North Dakota, Maine, California and Louisiana will be composited, analyzed and made available as a report by the by the Potato Chip Snack Food Association. Anyone wishing to receive a copy of this report please contact me.

Budget Request 1982-83

Area #3 Potato Administrative Committee - Greeley

Travel	$ 300.00
Equipment	900.00
Supplies	500.00
	$1700.00

Area #2 Potato Administrative Committee - San Luis Valley

Travel	$ 1250.00
Equipment	700.00
Supplies	1750.00
	$ 3700.00

ERADICATION OF POTATO VIRUS X FROM POTATO BY RIBAVIRIN TREATMENT OF CULTURED POTATO SHOOT TIPS[1]

Robert E. Klein and Clark H. Livingston[2]

Abstract

Ribavirin treatment of cultured potato shoot tips was tested as a means of eradicating potato virus X (PVX) from two potato cultivars. Shoot tips were cultured on liquid medium containing 0, 1, 10 or 100 μg/ml ribavirin. Cultures were evaluated periodically for viability, and scored for vigor on a relative growth scale. Developed plantlets were assayed for PVX by transmission tests to *Gomphrena globosa*.

Ribavirin treatment was phytotoxic at all tested concentrations, and lethal to all cultivars treated at 100 μg/ml. Treatment also delayed plantlet development by up to 2 months at 1 and 10 μg/ml as compared with non-treated controls. PVX assays indicated that 80 and 83% of the plantlets were free of PVX following treatment with 10 μg/ml for cultivars Russet Burbank and Red McClure, respectively. Five and 6% of the plantlets developed from the 1 μg/ml treatment were PVX-free, whereas 0 and 2% of the controls were PVX-free for the same cultivars. Six to 8 months were required to develop plants from shoot-tip cultures treated with 10 μg/ml ribavirin.

Resumen

Tratamiento con Ribavirin de ápices de brotes de papa "in vitro" fue probado como un medio de erradicar al virus X de la papa (PVX) de dos cultivares. Apices de brotes fueron cultivados en medio líquido conteniendo 0, 1, 10 ó 100 μg/ml de Ribavirin. Los cultivos fueron evaluados periodicamente para determinar su viabilidad y calificados con relación a su vigor en una escala relativa de crecimiento. Las plántulas que desarrollaron fueron analizadas para PVX por pruebas de transmisión a *Gomphrena globosa*.

El tratamiento con Ribavirin fue fitotóxico en todas las concentraciones probadas y letales a todos los cultivares a 100 μg/ml. El tratamiento también redujo el desarrollo de las plántulas hasta en 2 meses a 1 y 10 μg/ml en comparación con los testigos no tratados. Las pruebas para PVX indicaron que 80 y 83% de las plántulas estuvieron libres de PVX con el tratamiento de 10 μg/ml para los cultivares Russet Burbank y Red McClure, respectivamente. Cinco y 6% de las plántulas que desarrollaron del tratamiento con 1 μg/ml estuvieron libres de PVX, mientras que 0 y 2% de los testigos estuvieron libres para los mismo cultivares. Se necesitaron 6 a 8 meses para desarrollar plantas a partir de ápices de brotes tratados con 10 μg/ml de Ribavirin.

[1]Supported by the Colorado State University Experiment Station and published as Scientific Series Paper No. 2677.

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KEY WORDS: Ribavirin, PVX, shoot-tip culture, chemotherapy.

Introduction

Potato (*Solanum tuberosum*) serves as a host for a number of viruses. One of the most commonly encountered is potato virus X (PVX). Field studies have shown yield depressions of 7-22% when PVX-infected stocks are compared with PVX-free stocks (1, 4, 18). Thus, the eradication of PVX from seed potato stocks is of potential economic importance to potato growers.

Stace-Smith and Mellor (16) successfully combined thermotherapy of mother stock plants with axillary bud culture to obtain PVX-free clones. This method has become widely adopted for obtaining PVX-free potato plants. Although it is possible to eradicate PVX by shoot-tip culture alone (5, 11), reported results are variable, probably due to different PVX strains and potato cultivars as well as simultaneous infection with other viruses. This method is normally more difficult and time consuming than the thermotherapy-bud culture method (8) due to the minute amount of shoot tip that must be excised to achieve virus eradication. Consequently, shoot-tip culture alone has not received widespread adoption as a means of eradicating PVX.

Chemotherapy generally has not been recognized as an effective method for eradicating PVX from infected potato plants (8, 9, 10, 17). However, a synthetic riboside, ribavirin (Virazole[3]), recently has been reported to have antiviral activity against a wide range of plant viruses (2, 3, 6, 12, 14), including PVX (7, 13, 15). The objective of these studies was to determine the efficacy of ribavirin treatment of cultured potato shoot tips as a means of eradicating PVX.

Materials and Methods

Potato plants used in this study were grown in the greenhouse from cv. Russet Burbank and Red McClure seed tubers previously ascertained to be PVX-infected. When the plants were approximately 30 cm tall, terminal buds were removed to promote axillary shoot formation. Lateral shoots having several axillary buds were removed from the parental potato plants and cut into segments, each bearing one axillary bud. The shoot segments were surface disinfested by sequential immersion in 70% ethanol for 30 seconds, 0.5% sodium hypochlorite for 2-3 minutes, and several changes of sterile distilled water. The most terminal tissue of each axillary bud, approx-

[3]ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio.

imately 0.2 mm long, was excised with a razor blade fragment and transferred to the surface of a filter-paper wick in a culture tube containing culture medium.

Liquid nutrient culture medium for shoot-tip culture was prepared from stock solutions as described by Mellor and Stace-Smith (8). Ribavirin was added to the medium at concentrations of 1, 10 and 100 μg/ml just before adjustment to pH 5.7; a control medium without ribavirin was included. The culture medium was sterilized by filtration through a 0.01 μm Seitz filter to avoid heat degradation of ribavirin. Aliquots of 3.5 ml of the sterile medium were pipetted into pre-sterilized 16 x 10 mm culture tubes containing hooped filter-paper wicks and capped with sterile culture tube closures. This volume was sufficient to immerse all but the top surface of the wick. Culture medium was used within 1 week of preparation. Sufficient shoot tips were excised from each of five parental plants to provide 15 subsample cultures for each ribavirin concentration and the control of each parental plant. Shoot-tip cultures were maintained in a growth chamber in a completely randomized design under a 15 h photoperiod of 30,000 lx and a temperature of 25 C. At approximately monthly intervals, the cultured shoot tips were transferred to culture tubes containing freshly prepared medium. When both shoot and roots had been initiated, the plantlet was transplanted into a 10 cm-diam plastic pot containing potting mix for continued growth under greenhouse conditions.

Individual shoot tips were rated after 4, 10, 17, 21, 26, 32, 43, and 52 weeks of culture on a 0 to 5 relative growth scale as follows: (0) = the culture appeared to be inviable; (1) = the culture showed no readily observable growth and development differentiation from the initially excised tissue; (2) = one or two leaves were evident; (3) = more than two leaves were evident; (4) = the culture showed evidence of shoot formation and development; and (5) both shoot and roots had been initiated. Dead and microbially-contaminated cultures were discarded.

Values determined by relative growth ratings (omitting zero scores) were combined by treatment across replications for each cultivar and analyzed with a one-way analysis of variance (AOV). The least significant difference (LSD) test at P = 0.05 was applied to compare the ribavirin treatment means to the control mean when justified by a significant F value in the AOV.

The phytotoxicity of ribavirin to cultured shoot tips was measured by determining the percentage of inviable shoot-tips receiving each ribavirin treatment and the nontreated control. The percentages of inviable cultures were transformed to arcsin values for statistical analysis. Each cultivar was analyzed as a single experiment with five replications. A one-way AOV was performed on each of the periodic observations and LSD tests at P = 0.05 were applied to compare the ribavirin treatment means to the control mean when justified by a significant F value in the AOV.

When each plantlet was approximately 15 cm tall, several leaflets were removed and triturated in a small amount of 0.05 M phosphate buffer, pH 7.2. The triturate was used to mechanically inoculate vigorously-growing *Gomphrena globosa* L. plants, which served as a local lesion assay host. Potato plants which initially assayed PVX-free were periodically assayed for several months.

The percentage of plantlets of each cultivar that assayed as PVX-free was determined for each of the ribavirin treatments and for the nontreated controls.

Results

The mean relative growth ratings for each ribavirin treatment and the corresponding culture period are summarized for each cultivar in Table 1. Ribavirin treatment initially reduced growth in both cultivars, but shoot-tip cultures treated with 1 or 10 μg/ml ribavirin eventually equalled the growth levels attained by the nontreated control cultures. Cultures treated with 100 μg/ml ribavirin failed to develop into plantlets.

TABLE 1. — *Average relative growth over time of shoot-tip cultures from two potato cultivars exposed to each of three ribavirin levels*[1].

Cultivar	Ribavirin concentration	Culture period (weeks)							
		4	10	17	21	26	32	43	52
Russet Burbank	Control	1.9	2.2	2.8	4.1	4.8	4.9	4.9	5.0
	1 μg/ml	1.9	2.2	3.0	3.7*	4.0*	4.4*	4.9	5.0
	10 μg/ml	1.9	1.9*	2.2*	2.9*	4.3*	4.5*	4.9	5.0
	100 μg/ml	1.1*[2]	1.2*	1.1*	1.5*	2.3*	3.6*	3.0*	[3]
Red McClure	Control	1.9	2.5	3.4	4.0	4.9	5.0	5.0	5.0
	1 μg/ml	1.9	1.9*	2.7*	3.4*	4.3*	4.6*	4.9	5.0
	10 μg/ml	1.8	2.0*	2.1*	2.9*	4.1*	4.4*	4.7*	5.0
	100 μg/ml	1.2*	1.4*	1.5*	2.2*	1.7*	3.5*	3.2*	[3]

[1]Growth scale as follows: 0 = inviable; 1 = viable but no growth; 2 = one or two leaves; 3 = more than two leaves; 4 = shoot formation; 5 = shoot and root formation.

[2]Asterisks designate a significant difference between the control and the asterisked value at P = 0.05. Non-asterisked values do not differ significantly from the control.

[3]All cultures treated with 100 μg/ml ribavirin were inviable at this point and, consequently, could not be analyzed.

Comparisons of growth ratings for the ribavirin-treated shoot-tip cultures and the nontreated control indicated that 1 μg/ml ribavirin delayed plantlet development by approximately 1 month, whereas 10 μg/ml ribavirin delayed plantlet development approximately 1 to 22 months. Cultures of the cultivar Red McClure appeared to be slightly more sensitive to ribavirin treatment than cultures of the cultivar Russet Burbank.

The percentage of inviable shoot-tip cultures of each ribavirin treat-

in Table 2. Treatment of shoot-tip cultures with ribavirin for 21 weeks or more, regardless of the concentration, significantly increased numbers of inviable shoot tips compared with the nontreated control. Treatment of shoot-tip cultures with 100 μg/ml ribavirin resulted in the death of all cultures. Generally, the higher ribavirin concentrations resulted in greater shoot-tip mortality and phytotoxicity was also detectable earlier in cultures treated with the higher ribavirin concentrations. Shoot-tip cultures of the cultivar Red McClure appeared to be more sensitive to ribavirin treatment than those of Russet Burbank.

TABLE 2. — *The percentages over time of inviable shoot-tip cultures of two potato cultivars for each of three ribavirin levels.*

Cultivar	Ribavirin concentration	Culture period (weeks)							
		4	10	17	21	26	32	43	52
		%							
Russet Burbank	Control	0	0	2.0	4.0	11.1	12.6	12.6	12.6
	1 μg/ml	1.3	1.3	3.6	16.9*	30.3*	42.7*	54.7*	54.7*
	10 μg/ml	0	1.3	6.7*	14.7*	19.0*	25.3*	34.2*	34.2*
	100 μg/ml	5.9*[1]	9.7*	24.0*	25.5*	86.1*	89.0*	89.0*	100.0*
Red McClure	Control	0	0	0	0	25.6	25.6	25.6	25.6
	1 μg/ml	1.3	2.8	10.6*	20.1*	39.0*	53.0*	53.0*	59.8*
	10 μg/ml	1.3	4.0	6.5*	13.5*	48.3*	64.1*	70.4*	70.4*
	100 μg/ml	5.9*	24.3*	41.2*	41.2*	91.0*	91.0*	91.0*	100.0*

[1]Asterisks designated a significant difference between the control and the asterisked value at $P = 0.05$. Non-asterisked values do not differ significantly from the control.

Results of PVX assays of plantlets developed from excised shoot tips treated with ribavirin and the nontreated control are shown in Table 3. When the data were analyzed across cultivars by linear regression, a significant linear relationship ($R^2 = 0.76^*$) was found between the ribavirin concentration and the degree of PVX-eradication.

TABLE 3. — *Percentage of developed plantlets of two potato cultivars that assayed PVX-free following treatment of shoot-tip cultures with ribavirin at two concentrations.*

Cultivar treated	Ribavirin concentration μg/ml	Number of developed plantlets	% plantlets PVX-free
Russet Burbank	0	52	0
	1	21	5
	10	35	80
Red McClure	0	40	2
	1	18	6
	10	12	83

Discussion

Phytotoxic responses due to ribavirin treatment were evident at concentrations lower than those reported to cause phytotoxicity in other plant host-virus combinations (6, 13, 15). The reported concentrations ranged from 1000 μg/ml (6) to 10 μg/ml (15). In these studies, phytotoxicity as evidenced by growth inhibition and shoot-tip culture mortality was detected in concentrations as low as 1 μg/ml. Schuster (13) reported that tissue damage associated with ribavirin treatment was more severe in virus-infected plants. Thus, the phytotoxicity associated with ribavirin treatment may well be related to the interaction of the host plant, ribavirin, and the virus.

It is conceivable that treatment with ribavirin at concentrations that are known to be slightly phytotoxic might be regarded as desirable. In several instances (6, 13, 15), virus titer was reduced only by treatment with ribavirin concentrations that also resulted in tissue damage. Some level of phytotoxicity may have to be tolerated to achieve the eradication of PVX from potato shoot-tip cultures.

PVX could not be detected in over 80% of the plantlets developed from shoot-tip cultures treated with 10 μg/ml ribavirin. Although plantlets in this study were assayed for 3 to 4 months, plants developed in additional experiments have been maintained for up to 9 months without developing detectable PVX infections. Thus, it appears that PVX was eradicated and undetectable PVX titers were not evident during the duration of the assays.

The time required to develop PVX-free plantlets by treatments of shoot tips with 10 μg/ml ribavirin was 6 to 8 months. This time period is substantially longer than that reported by Stace-Smith and Mellor (16) for eradication by thermotherapy shoot tip cultures. Obviously, additional comparisons of the two methods are needed before the relative merits of each can be determined. The major advantage of ribavirin treatment is the elimination of thermotherapy. Potential disadvantages may include culture transfers to fresh media, and increased culture periods. The effects of thermotherapy on other common potato viruses are known while the effect of ribavirin treatment is not.

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Eradication of Potato Viruses X and S from Potato Shoot-tip Cultures with Ribavirin

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ABSTRACT

Klein, R. E., and Livingston, C. H. 1982. Eradication of Potato Viruses X and S from Potato Shoot-tip Cultures with Ribavirin. Phytopathology _____.

Ribavirin treatment of cultured potato shoot-tips was tested as a means of eradicating PVX and PVS. Doubly-infected shoot tips were cultured on a liquid medium containing 10, 20, or 40 µg/ml ribavirin and a control medium without ribavirin. Cultures were evaluated periodically for relative growth rate, inviability, and the time required for plantlet regeneration. Developed plantlets were assayed for PVX by transmission tests to Gomphrena globosa, and serologically for PVS by the latex agglutination test. Ribavirin proved to be phytotoxic at all concentrations tested, and resulted in the inviability of all cultures treated with 40 µg/ml. Treatment delayed plantlet development by 106 and 127 days for the 10 and 20 µg/ml treatments, respectively. Virus assays indicated that 93 and 87% of the plantlets were free of PVX and PVS,

respectively, after treatment with 10 µg/ml. All plantlets developed from the 20 µg/ml treatment were free of both viruses, whereas 10 and 0% of the controls were free of PVX and PVS, respectively.

Additional key words: chemotherapy

Potato viruses X (PVX) and S (PVS) are two of the most commonly encountered viruses infecting potatoes (Solanum tuberosum L.). Potato yield depressions associated with infection by each of these viruses often are difficult to detect (8,18), although yield depressions of up to 20% (16) and 17% (18) have been reported due to PVS and PVX infections, respectively. Yield depressions of up to 38% can be realized due to the dual infection with these viruses (17).

The most common method of eradicating PVX and PVS from infected seed potato stocks has been heat treatment of rooted cuttings followed by axillary shoot-tip culture. This method has been particularly effective against PVX (15), but eradication of PVS by this method has proven to be more difficult and yields variable results (7,9,10).

Recently, a synthetic riboside, ribavirin (Virazole)[1/], has been reported to have antiviral activity against a wide range of plant viruses (2,3,5,6,11,12,13,14). The eradication of PVX from infected shoot-tip cultures by ribavirin treatment has been reported (4). There are no reports of PVS eradication studies involving chemotherapy. Therefore, this study was undertaken to determine the efficacy of ribavirin treatments of cultured potato shoot tips as a means of eradicating PVX and PVS from doubly-infected potatoes.

[1/] ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio

MATERIALS AND METHODS

Liquid nutrient culture medium containing 10, 20, and 40 µg/ml ribavirin as well as a control medium lacking ribavirin was prepared as described by Mellor and Stace-Smith (10). The medium was sterilized by filtration. Aliquots of 3.5 ml of the sterile medium were pipetted into presterilized 16 x 100 mm culture tubes containing hooped filter paper wicks; tubes were capped to ensure sterility. This volume was sufficient to immerse all but the top surface of the wick.

Potato plants (cv Russet Burbank) previously ascertained to be doubly infected with PVX and PVS served as a source of shoot tips. After surface disinfestation with 70% ethanol and 0.5% sodium hypochlorite, the most terminal tissue of each axillary bud, 0.2 mm to 0.5 mm in length, was excised and transferred to the domed surface of a filter paper wick. Shoot-tip cultures were maintained in a controlled environment chamber under a 15 hr photoperiod and a temperature of 25 C. At approximately monthly intervals, the shoot tips were transferred to culture tubes containing freshly prepared liquid medium and a filter paper wick. Regenerated plantlets were transplanted into 10-cm-diam plastic pots containing potting mix for continued growth under greenhouse conditions.

When each plant was approximately 20 cm tall, several leaflets were removed and assayed for both PVX and PVS. PVX assays were performed by mechanical inoculation of the local lesion indicator host, Gomphrena globosa L. Several leaflets were triturated in a small aount of 0.1 M phosphate buffer, pH 7.2. The triturate was rubbed onto G. globosa leaves which had been dusted with 600 mesh carborundum. Local lesions commonly developed within 10 days of inoculation. PVS assays were

performed serologically using the latex agglutination test (1). Plants that tested positive for both viruses were discarded. Those testing negative were periodically assayed over a period of several months.

Sufficient shoot tips were excised to provide three replications of 12 subsample cultures for each experimental ribavirin concentration and the nontreated control. Cultured shoot-tips were maintained in the controlled environment chamber according to a completely random design. Individual shoot tips were evaluated after each month of culture on a 0 to 5 relative growth scale (4).

Values determined by relative growth ratings (omitting zero scores) were averaged for each replication and analyzed by a one-way analysis-of-variance (AOV). When justified by a significant F value, treatment means were compared to the control mean with the least significant difference (LSD) test of $P \leq 0.05$. Shoot-tip cultures were examined frequently to determine the time required for plantlet regeneration. Regeneration times were averaged across each replication and analyzed with a one-way AOV. Treatment means were compared to the control mean with the LSD test at $P \leq 0.05$.

RESULTS

A comparison of mean relative growth scores presented in Table 1 indicates that all three ribavirin treatments inhibited shoot-tip growth after one month of culture. The extent of inhibition is directly related to the ribavirin concentration.

Comparison of mean times required for plantlet regeneration (Table 2) show that ribavirin at 10 and 20 µg/ml significantly delayed

regeneration; a concentration of 40 µg/ml was lethal. Although the ranges of regeneration times overlap, treatment of shoot-tip cultures with 10 µg/ml ribavirin delayed plantlet regeneration by approximately 106 days, whereas treatment with 20 µg/ml ribavirin delayed regeneration by approximately 127 days when compared with the nontreated control.

Ribavirin treatment resulted in a significant increase in shoot-tip inviability, which increased with increasing ribavirin concentration (Table 3). Even at the relatively low concentration of 10 µg/ml, ribavirin treatment resulted in a six-fold increase in culture inviability as compared with the nontreated control. Treatment with 40 µg/ml ribavirin resulted in the inviability of all cultures.

Ribavirin was effective as an eradicant for both PVX and PVS at treatment levels of 10 and 20 µg/ml (Table 4). Control plantlets exhibited 10% PVX eradication and 0% PVS eradication, whereas plantlets regenerated from cultures treated with 10 µg/ml ribavirin exhibited 93% and 87% eradication of PVX and PVS, respectively. All plantlets regenerated from cultures treated with 20 µg/ml ribavirin were free of both PVX and PVS.

DISCUSSION

Treatment of cultured shoot tips with ribavirin resulted in growth inhibition, culture inviability, and delayed plantlet regeneration. The extent of growth inhibition and culture inviability was similar to that reported earlier (4) for a ribavirin treatment level of 10 µg/ml. The delay in plantlet regeneration was greater than that reported in the earlier experiments. However, the percentage of plantlets that developed from cultures treated with 10 µg/ml ribavirin and tested negative for

PVX was greater than previously reported. The difference is most likely due to experimental variation, but may also be indicative of variance in PVX strains to treatment.

Ribavirin at 10 and 20 µg/ml was also effective as a PVS eradicant. Treatment with ribavirin at 10 µg/ml resulted in PVS eradication from 87% of the plantlets and 20 µg/ml resulted in 100% eradication. PVS is considered to be one of the potato viruses most difficult to eradicate (10). The results of these studies suggest that PVS may be amenable to chemotherapy using ribavirin.

TABLE 1. Average relative growth scores[a] of shoot-tip cultures of cv Russet Burbank exposed to each of three ribavirin treatment levels and the corresponding treatment period.

Ribavirin concentration	Culture period (months)									
	1	2	3	4	5	6	7	8	9	11
Control	1.9	2.5	4.1	4.6	4.8	4.9	4.9	5.0	5.0	5.0
10 µg/ml	1.6	2.2	3.2*	3.5*	3.7*	4.0*	4.2*	4.5	4.5	5.0
20 µg/ml	1.4*[b]	2.0*	2.8*	3.2*	3.3*	3.9*	3.8*	3.6*	4.3*	5.0
40 µg/ml	1.3*	1.6*	2.5*	2.4*	2.8*	2.8*	2.5*	---[c]	---	---

[a] Growth scale as follows: (0) - inviable; (1) - no evident change from originally excised tissues; (2) - one or two leaflets visible; (3) - three or more leaflets visible; (4) - stem present; (5) - stem and roots present and plantlet ready for transplanting.

[b] Asterisks within each column designate a significant difference at $P \leq 0.05$ from the control.

[c] All cultures treated with 40 µg/ml ribavirin were inviable at this point and, consequently, could not be analyzed.

TABLE 2. Mean regeneration times (in days) and range of regeneration times for plantlets developed from shoot-tip cultures exposed to each of three ribavirin treatment levels.

Ribavirin concentration	Regeneration times (days)	
	Mean	Range
Control	123	79 - 213
10 µg/ml	229*[a]	178 - 313
20 µg/ml	250*	201 - 313
40 µg/ml	---[b]	---

[a] Asterisks designate a significant difference from the control (at $P \leq 0.05$).

[b] Plantlets could not be regenerated from shoot tips treated with 40 µg/ml ribavirin.

TABLE 3. Mean inviability of shoot-tip cultures exposed to each of three ribavirin treatment levels after 11 months of culture.

Ribavirin concentration	Mean inviability (%)
Control	6.9
10 g/ml	41.6*[a]
20 g/ml	68.4*
40 g/ml	100.0*

[a] Asterisks designate a significant difference from the control (at $P \leq 0.05$).

TABLE 4. Numbers of plantlets which assayed PVX-free or PVS-free following shoot-tip culture treatment with each of two ribavirin concentrations.

Ribavirin concentration	PVX-free	PVS-free
Control	2/20[a/]	0/19
10	14/15	13/15
20	5/5	7/7

[a/] Denominator indicates the number of plants assayed; the numerator indicates the number that assayed virus-free.

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Effect of Benomyl on Shoot-Tip Cultures from PVX- and PVS-infected Potatoes[1/]

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Abstract

Benomyl (50% WP) at 25 and 50 µg ai/ml was tested as a constituent of potato shoot-tip culture medium. Both concentrations increased shoot-tip growth rate when compared to a nontreated control. The 25 µg/ml benomyl treatment significantly reduced the amount of time required for plantlet regeneration. Benomyl had no eradicative effect on potato virus X, but it appeared to exhibit a weak eradicative effect on potato virus S.

Introduction

Benomyl, a widely used systemic fungistat, has been reported to exhibit antiviral activity. Benomyl root drenches suppressed symptom expression of tobacco mosaic virus and beet western yellows virus infections in Nicotiana tabacum (3,4,5,11,12) and lettuce (12,13),

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respectively. However, benomyl had no effect on tomato spotted wilt virus in petunia leaf strips (2), and benomyl treatment increased the virus content of cucumber mosaic virus-infected cucumber cotyledon disks and seedlings (3).

Benomyl also has exhibited cytokinin-like activity (increased cell division, delayed senescence, promotion of organ formation, and lateral bud development) in several studies (3,6,8,9,11), and asparagus explants cultured on a medium containing benomyl exhibited an increase in shoot development (14).

Benomyl was tested as a constituent of potato shoot-tip culture medium to determine if benomyl treatment increased shoot-tip growth, and if potato viruses X (PVX) and S (PVS) could be eradicated.

Materials and Methods

Greenhouse-grown potato (cv Russet Burbank) plants previously ascertained to be doubly infected with PVX and PVS were used as a source of shoot tips. Shoot tips approximately 0.3 to 0.5 mm long were excised and maintained as previously reported (6). Culture medium was prepared as described by Mellow and Stace-Smith (7) except that Benlate 50 WP[3/] was added to the medium as a benomyl source before pH adjustment. Two benomyl concentrations, 37.5 and 75 µg/ml active ingredient (ai) were used. Approximately one-third of the ai is destroyed by autoclaving (C. Delp, personal communication); consequently, the effective concentrations of benomyl were calculated to be

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25 and 50 µg ai/ml, respectively.

Three replications of 12 shoot-tip subsamples were excised and maintained in a controlled environment chamber in a completely random design for each of the two benomyl treatments and a nontreated control. At monthly intervals, cultures were examined and scored on a 0 - 5 relative growth scale as previously reported (6). Scores (omitting zero scores) were averaged across subsamples for each replication and analyzed by a one-way analysis-of-variance (AOV). When justified by a calculated significant F value, benomyl treatment means were compared to the control mean with a least significant difference (LSD) test of $P \leq 0.05$.

Cultures were examined frequently to determine the number of days from excision to plantlet regeneration. The distribution of regeneration times for each of the two benomyl treatments was compared with the control by means of the Kolmogorov-Smirnov two-sample test (10).

Plantlets regenerated from excised shoot-tips were transplanted, maintained in the greenhouse, and assayed for PVX and PVS when approximately 20 cm tall. Several leaflets were removed and triturated in a small amount of 0.1M phosphate buffer, pH 7.2. The triturate was used to mechanically inoculate Gomphrena globosa L. plants which served as local lesion indicator hosts. PVS assays were performed serologically by the latex agglutination test (1). Plants that tested positive for the presence of both viruses were discarded. Remaining plants were assayed periodically for several months, or until both viruses had been detected. This experiment also was repeated with two replications of eight shoot tips solely for virus assays.

These experiments also were repeated with four replications of twelve shoot-tip subsamples which had been excised from plants solely infected with PVS. Data were collected in the same manner as in the initial experiment.

Results

Mean relative growth scores presented in Table 1 for the doubly-infected and PVS-infected shoot-tip cultures show that 25 and 50 µg ai/ml benomyl treatments tended to increase shoot-tip growth. However, after four months of culture, the growth of shoot tips subjected to benomyl treatments no longer differed significantly from the nontreated control.

The 25 µg/ml benomyl treatment resulted in a significantly earlier regeneration time than both the nontreated control and the 50 µg/ml treatment (Table 2). This occurred regardless of whether the shoot tips were doubly-infected or solely PVS-infected. The regeneration times of the 50 µg/ml treatment proved to be more variable. When the excised shoot tips were doubly-infected, treatment with 50 µg/ml benomyl increased, but not significantly, the time required for plantlet regeneration compared with the nontreated control. However, when the shoot tips were solely PVS-infected, treatment reduced regeneration time.

Virus eradication studies (Table 3) indicate that benomyl does not have an eradicative effect on PVX, but PVS eradication varied between approximately 3 and 30%, depending on the particular experiment.

Discussion and Conclusions

Benomyl proved to be effective as a growth promoting agent at both concentrations tested, and it appears that the 25 µg ai/ml treatment was more effective than the 50 µg ai/ml treatment. Cultures treated with either benomyl concentration usually were chlorotic before transfer to freshly prepared culture medium, but eventually they became nomal following the transfer. The observed chlorosis probably was due to medium exhaustion, and was not regarded as a phytotoxic response to benomyl treatment. Phytotoxic responses were not evident in the cultures treated with 25 µg/ml benomyl, but some of the cultures treated with 50 µg/ml benomyl exhibited phytotoxic-like symptoms, such as a persistent chlorosis and thickened shoots. The symptoms were similar to those reported by Yang (14) for asparagus explants treated with benomyl.

Treatment of shoot-tip cultures with 50 µg ai/ml benomyl did not consistently reduce the time required for plantlet regeneration. However, treatment with 25 µg ai/ml benomyl did consistently and significantly reduce the regeneration time when compared with the control. The reduction in regeneration time was from 10 to 28% of the time required for the nontreated controls. Thus, it appears that benomyl has potential as a growth promoting agent in the culture of potato shoot tips. The optimum concentration was approximately 25 µg ai/ml. Similar results have been reported with asparagus explants (14), except that the optimum treatment concentration was 50-100 µg ai/ml, and phytotoxicity was evident at benomyl concentrations exceeding 100 µg ai/ml.

Assays indicated that benomyl was not effective as a PVX

eradicant. The occurrence of PVX-free plantlets in both the control and the 25 g/ml benomyl treatment was probably fortuitous.

PVS assays seemed to indicate that benomyl was effective as a PVS eradicant only when the treated plant tissue also was infected with PVX. It is most likely that the great variability was due to the sample size, and that benomyl has only a weak PVS-eradicative effect.

The weak anti-PVS effect of benomyl precludes its use as a sole means of PVS eradication. It may, however, be appropriate to include benomyl in culture medium of shoot tips following thermotherapy, because it could increase the number of PVS-free plantlets, promote shoot-tip growth, and decrease regeneration time.

Table 1. Average relative growth scores[1/] of potato shoot-tip cultures exposed to each of two benomyl treatment levels and the corresponding treatment period in two separate experiments.

Parent material	Benomyl concentration	Treatment period (months) 1	2	3	4	5	6	7	8
PVX + PVS-infected	Control	1.9	2.5	4.1	4.6	4.8	4.9	4.9	5.0
	25 g/ml	2.0	3.3*	4.6*	4.7	4.7	5.0	5.0	5.0
	50 g/ml	2.2	2.9*	3.9	4.3	4.4	5.0	5.0	5.0
PVS-infected	Control	2.0	2.4	3.0	4.1	5.0			
	25 g/ml	2.7*[2/]	4.0*	4.5*	4.9	5.0			
	50 g/ml	2.2	3.6*	4.2*	4.8	5.0			

[1/] Growth scale was as follows: (0) - inviable; (1) - no evident change from originally excised tissues; (2) - one or two leaflets visible; (3) - three or more leaflets visible; (4) - stem present; (5) - stem and roots present and plantlet ready for transplanting.

[2/] Asterisks designate a significant difference at $P \leq -0.05$ between the control and the asterisked value.

Table 2. Mean number of days required for plantlet regeneration of potato shoot-tip cultures exposed to two benomyl treatment levels in two experiments.

Benomyl concentration	PVX + PVS infected parent plant	PVS-infected parent plant
Control	115 a[1/]	101 a
25 µg/ml	103 b	73 b
50 µg/ml	132 a	87 c

[1/] Values followed by the same letter are not different at $P \leq 0.025$ in the Kolmogorov-Smirnov test.

Table 3. Numbers of potato plantlets that assayed PVX-free or PVS-free following shoot-tip culture treatment with two concentrations of benomyl and the nontreated control in three separate experiments.

Parent Plant	Benomyl concentration	PVX-free	PVS-free
PVX- and PVS-infected	Control	1/20[1/]	0/19
	25 µg/ml	2/21	6/20
	50 µg/ml	0/6	0/6
PVS-infected	Control	---	1/21
	25 µg/ml	---	1/38
	50 µg/ml	---	0/38
PVX- and PVS-infected	Control	0/13	0/13
	25 µg/ml	0/6	1/7
	50 µg/ml	0/16	0/16

[1/] Numerator indicates the number of plantlets that assayed either PVX- or PVS-free; denominator indicates the number of plantlets assayed.

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