Monitoring Current Season Potato Leafroll Virus Movement With an Immunosorbent Direct Tissue Blotting Assay

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ABSTRACT

A direct tissue blotting assay (DTBA) was used to track the movement of potato leafroll virus (PLRV) from newly infected foliage to the tubers. Plant and tuber characteristics were recorded to assess plant growth stage at inoculation and PLRV effect on yield. Russet Burbank potatoes were planted at different times in 1991 and 1992 to provide plants of different maturities which were then inoculated using PLRV carrying aphids. Aphids were allowed to feed two to three days after which an insecticide was applied. Stems and tubers were tested periodically for PLRV with DTBA after inoculation. Indexed tubers were grown out and ELISA tested in the greenhouse the following winter to confirm results of summer serological tests. Plant age affected percentages more consistently than did inoculation date. When plants approximately 43 days from planting were inoculated at different dates, early inoculation produced a higher percentage of infected plants. Conversely, when plants approximately 62 days from planting were inoculated at different dates, late inoculation resulted in a higher percentage of infected plants. However, early inoculation of young plants resulted in the highest infection percentages. Tuber size and yield were negatively affected by higher percentages of leafroll regardless of the stage of growth at inoculation. DTBA is best used for detecting PLRV in foliage of plants grown from infected tubers (i.e. secondary PLRV). DTBA is less accurate for detecting primary PLRV.

RESUMEN

Una técnica de absorción directa de tejidos (direct tissue blotting assay - DTBA) fue usada para rastrear el movimiento del Virus del Enrollamiento de la Hoja de la Papa (PLRV) del follaje recién infectado hasta los tubérculos. Se registraron las características tanto de las plantas como de los tubérculos, a fin de calcular la etapa del crecimiento de las plantas al inocularse, y los efectos del PLRV sobre el rendimiento. Las papas Russet Burbank fueron sembradas en diferentes épocas en 1991 y 1992 para proveer plantas de diferentes maduraciones, las cuales fueron entonces inoculadas con áfidos portadores de PLRV. A los áfidos se les permitió alimentarse durante dos o tres días y después se les aplicaba un insecticida. Los tallos y tubérculos fueron examinados periódicamente a fin de detectar la presencia de PLRV usando DTBA después de inocularse. El invierno siguiente los tubérculos indexados fueron cultivados y sometidos a la prueba de ELISA en el invernadero para confirmar los resultados de las pruebas serológicas del verano. La edad de las plantas afectó los porcentajes más consistentemente que la fecha de su inoculación. Cuando las plantas a 43 días de ser sembradas fueron inoculadas en diferentes fechas, la inoculación temprana produjo un porcentaje más alto de plantas infectadas. Al contrario, cuando las plantas fueron inoculadas aproximadamente 62 días de ser sembradas en diferentes fechas, la inoculación tardía produjo un porcentaje más alto de plantas infectadas. Sin embargo, la inoculación temprana de plantas juveniles dio como resultado los porcentajes de infección más altos. El tamaño de los tubérculos y su rendimiento fueron afectados negativamente por porcentajes mayores del Enrollamiento de la Hoja de Papa cualquiera que fuera la etapa de crecimiento al inocularse. El DTBA trae mejores resultados cuando se usa para detectar PLRV en el follaje de plantas cultivadas de tubérculos infectados (i.e. PLRV secundario). El DTBA es menos preciso en el caso de la detección del PLRV primario.

INTRODUCTION

Symptom expression of current season (primary) PLRV infection in potato plants varies and may be absent when
infection occurs late in the season (Knutson and Bishop, 1964; Woodruff and Barker, 1986). One purpose of winter grow-out tests performed by certification agencies is to determine the amount of virus in seed lots that results from current season spread. Seed growers apply more insecticide than commercial potato growers in order to limit current season spread. These and other methods help to limit the spread of viruses.

Knowledge of relative plant susceptibility and aphid population dynamics in a growing region would allow a grower to better utilize control measures to reduce PLRV. If aphids are present when plants are young and more susceptible to infection, preventive insecticide sprays applied early and consistently compared to later in the season when plant susceptibility is low should result in better control of PLRV. A better understanding of how the age of the plant affects the time required for PLRV to move from infection sites to tubers is needed (Storch and Manzer, 1985). This may allow time for vine killing before tubers become infected.

A quick, reliable serological test for both current season and tuber-borne PLRV would be useful for research as well as seed certification purposes. The enzyme-linked immunosorbent assay (ELISA) detects current season (primary) PLRV less accurately than tuber-borne (secondary) PLRV (Flanders et al., 1990). The recently developed direct tissue blotting assay (DTBA) is a relatively simple, but accurate serological test (Bravo-Almonacid et al., 1992). It is as accurate as ELISA for detecting secondary PLRV in cvs. Russet Burbank and Russet Norkotah plants (Whitworth et al., 1993). DTBA and ELISA utilize the same enzyme-linked antibody tag detection system; however, intact tissue is blotted directly onto a nitrocellulose membrane with DTBA, while tissues are homogenized and diluted in a buffer for ELISA. DTBA has been reported to be relatively more sensitive than ELISA for detecting potato viruses X and Y (Bravo-Almonacid et al., 1992), but direct comparison between the two methods is difficult because of the different tissue preparations required. Since undiluted plant sap is used to apply viruses to nitrocellulose in DTBA, it is potentially more sensitive than ELISA for detecting current season leafroll virus infection.

This study was done to determine 1) how quickly PLRV moves to the tubers when plants are infected at different ages and times during the season, 2) how accurately DTBA can be used to detect PLRV in primary infected vines and tubers and 3) PLRV effect on yield.

**MATERIALS AND METHODS**

**Field Plot Layout and Seed Preparation**

Russet Burbank seed potatoes were planted in a randomized complete block design at the Lewis-Brown Horticultural Farm near Corvallis, Oregon. This site in the Willamette Valley was chosen, despite high aphid populations, because of the relative absence of seed and commercial potatoes and PLRV inoculum. Plots were replicated six times in 1991 and three times in 1992.

Certified, generation three (fourth field year) seed pieces were planted both years. Certification readings for PLRV and mosaic viruses in the field and in greenhouse winter grow-out tests were zero for both seed lots. Seed tubers were cut on May 6, 1991 but planting was delayed until May 24 because of heavy rains. Seed tubers were cut on May 6, 1991 but planting was delayed until May 24 because of heavy rains. Seed for 1992 trials was cut three days before planting on May 22 and June 8. Seed was planted on the two dates to provide plants of different ages for inoculation. Cut seed pieces were held in 10 C storage until planting in 1991 and at room temperature in 1992. Seed piece size was 43 to 57 grams both years.

**Crop Production Practices**

Fertilizer (15-15-15) was broadcast and incorporated at 560 kg/ha preplant and banded at 481 kg/ha on both sides of the seed piece at planting. Each plot row was planted with 30 seed pieces spaced 23 cm apart using a hand-feed assist planter. Rows were 86 cm apart. Each plot consisted of two planted rows, two blank rows, and two border rows, planted completely around each plot. Border rows were sidedressed with aldicarb insecticide at 10.9 kg/ha before plant emergence. This arrangement provided bare ground and insecticide-treated potato barriers around each plot to hinder movement of apterous (non-winged) aphids. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. This arrangement provided bare ground and insecticide-treated potato barriers around each plot to hinder movement of apterous (non-winged) aphids. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. This arrangement provided bare ground and insecticide-treated potato barriers around each plot to hinder movement of apterous (non-winged) aphids. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. Pesticide sprays of chlorothalonil (Bravo) were alternated with aldicarb insecticide at 10.9 kg/ha before plant emergence. Pesticide sprays of chlorothalonil (Bravo) were alternated with aldicarb insecticide at 10.9 kg/ha before plant emergence. This arrangement provided bare ground and insecticide-treated potato barriers around each plot to hinder movement of apterous (non-winged) aphids. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. Pesticide sprays of chlorothalonil (Bravo) were alternated with aldicarb insecticide at 10.9 kg/ha before plant emergence. Pesticide sprays of chlorothalonil (Bravo) were alternated with aldicarb insecticide at 10.9 kg/ha before plant emergence. This arrangement provided bare ground and insecticide-treated potato barriers around each plot to hinder movement of apterous (non-winged) aphids. Weeds were controlled by one application of metribuzin (0.6 kg/ha) at early plant emergence. Pesticide sprays of chlorothalonil (Bravo) were alternated with aldicarb insecticide at 10.9 kg/ha before plant emergence.

**Inoculation of Field-grown Plants with Viruliferous Aphids**

Apterous *Myzus persicae* were supplied by Dr. Harold Toba and Mr. Lee Fox of the USDA-ARS Entomology Research Laboratory in Yakima, Washington. The original