

# Detection by Enzyme-Linked Immunosorbent Assay of *Rhizoctonia* Species on Poinsettia Stem Cuttings

D. M. BENSON, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

## ABSTRACT

Benson, D. M. 1992. Detection by enzyme-linked immunosorbent assay of *Rhizoctonia* species on poinsettia stem cuttings. *Plant Dis.* 76:578-581.

A multiwell enzyme-linked immunosorbent assay (ELISA) and rapid assay ELISA were evaluated for detection of binucleate and multinucleate isolates of *Rhizoctonia* spp. on poinsettia cuttings in propagation. Rice grain inoculum of each test isolate was positioned 2 cm on either side of the cutting. At 2, 3, and 7 days, poinsettia stem samples were rated visually for *Rhizoctonia* stem rot and then split in half for ELISA or for culture on acidified potato-dextrose agar. *Rhizoctonia* spp. were detected by ELISA and by culture within 2 days of inoculum placement for multinucleate isolates of *R. solani* and 3 days for binucleate isolates. Generally, multinucleate isolates which caused severe stem rot in 5–7 days gave absorbance readings between 1.0 and 2.0, and the same isolates gave rapid assay readings between 40 and 80. Poinsettia stems exposed to binucleate isolates generally gave much lower, but positive, test readings than multinucleate isolates. One of three binucleate isolates induced lesions of *Rhizoctonia* stem rot on poinsettia stems. A multinucleate isolate was detected in a stem lesion as small as 1 mm long (8.6 mm<sup>2</sup>) with either ELISA format. In a survey of poinsettia cuttings with symptoms of stem rot from commercial greenhouses, the multiwell kit was reliable in detection of *Rhizoctonia* spp. compared to visual assessment and culture results. The two ELISA formats proved useful for detection of *Rhizoctonia*-like fungi and *R. solani* from poinsettia.

Additional keywords: *Euphorbia pulcherrima*, pathogen detection, pest management

The rapid detection of *Rhizoctonia solani* (Kühn) causing stem rot of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) in propagation is critical to the implementation of pest management decisions for this endemic pathogen. Standard culture plate analysis of diseased tissues requires 24–48 hr to confirm a diagnosis. Agri-Diagnostics Associates (Cinnaminson, NJ) recently introduced multiwell and flow-through immunoassay formats of an enzyme-linked immunosorbent assay (ELISA) for detection of *Rhizoctonia* spp (3). The multiwell ELISA can be used to test a large

number of samples in the laboratory with standard equipment, and the flow-through or rapid assay (*Rhizoctonia* Alert kit) is designed for on-site analysis of samples. The rapid assay contains all necessary supplies and reagents in a simple-to-use format that allows sample processing in about 15 min. The use of ELISA kits to detect a specific pathogen is of value in clinical situations in which more than one pathogen causes similar symptoms.

Results with ELISA kits for detection of *Rhizoctonia* spp. in woody ornamentals have not been as encouraging as results for other pathogens (2). MacDonald et al (2) found only about a 30% agreement between test results for a culture plate assay for *Rhizoctonia* spp. and a multiwell ELISA format when a wide range of woody ornamentals with root disease symptoms were sampled. On turf, samples of asymptomatic foliage, but not thatch and root tissue, collected 30 cm from the margin of a brown patch area

on a golf green of *Agrostis palustris* Hudson tested positive with ELISA (4). Isolation of asymptomatic thatch and root tissue on water agar gave more *Rhizoctonia*-positive samples than ELISA (4). Visual assessment of disease severity for brown patch on *A. palustris* was correlated closely with meter reading for a rapid assay ELISA over the growing season (B. B. Clarke, *personal communication*).

The objective of the present work was to compare the culture plate method to both multiwell and rapid assay ELISA formats for detection of binucleate and multinucleate isolates of *Rhizoctonia* spp. on poinsettia cuttings during propagation. A preliminary report has been published (1).

## MATERIALS AND METHODS

**Inoculum and plant culture.** Isolates of *Rhizoctonia* spp. from various ornamentals, including binucleate isolates Bn 2, Bn 17, and Bn 232 from gumpo azalea, *Cotoneaster* sp., and pine bark mix, respectively, as well as multinucleate isolates Rs 3, Rs 15, and Rs 25 of *R. solani* from poinsettia, *Ilex crenata* Thunb., and *Pittosporium* sp., respectively, were cultured on rice grains (25 g of rice per 17 ml of water) for 7–10 days. An individual colonized grain for a given isolate was placed on the surface of a polyfoam rooting cube strip (Rootcubes, Smithers-Oasis USA, Kent, OH) about 2 cm on either side of the preformed hole in the strip. Poinsettia stem cuttings (Gutbier V-14 Glory) were taken from stock plants and inserted in the rooting cube strip holes. A replication consisted of a cube strip with five cuttings. There were three replications per isolate arranged in a randomized complete block design on a greenhouse mist bench. Mist was applied for 2 min twice a day. Experiments were repeated three times, with similar results.

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**Tissue samples and ELISA methods.** Beginning 2 days after placement of inoculum on the strip, a cutting from one of the three innermost cubes of each strip was selected randomly for assay. Prior to assay each stem was rated for extent of stem rot according to the following scale: 1 = no visible stem rot, 2 = lesion girdling 25% of the stem, 3 = lesion girdling 50% of the stem, 4 = lesion girdling 75% of the stem, and 5 = a completely girdled and collapsed cutting. Poinsettia stems about 4 cm long were split in half for ELISA or cultured on acidified potato-dextrose agar. Additional stems were collected 3 and 7 days after inoculum placement. Stem samples were ground on the abrasive pad provided with the ELISA kits. Samples were extracted in buffer and processed according to directions supplied by the manufacturer. Samples in the multiwell format were read at 405 nm, and samples in the rapid assay format were read with the Agrimeter II (Agri-Diagnostics). The positive-negative test threshold was set at the mean plus three times the standard deviation of the controls for the multiwell

format, and the mean plus two times the standard deviation of the control for the rapid assay format.

**ELISA sensitivity.** Unused poinsettia stems from the strips with isolate RS 3 of *R. solani* that had discrete lesions were collected. Cross sections 1, 3, or 9 mm long of diseased tissue were made and then split in half for ELISA and plate culture. An additional section of tissue from healthy stems was added to samples less than 9 mm long, so that the total length of tissue assayed by ELISA was always 9 mm.

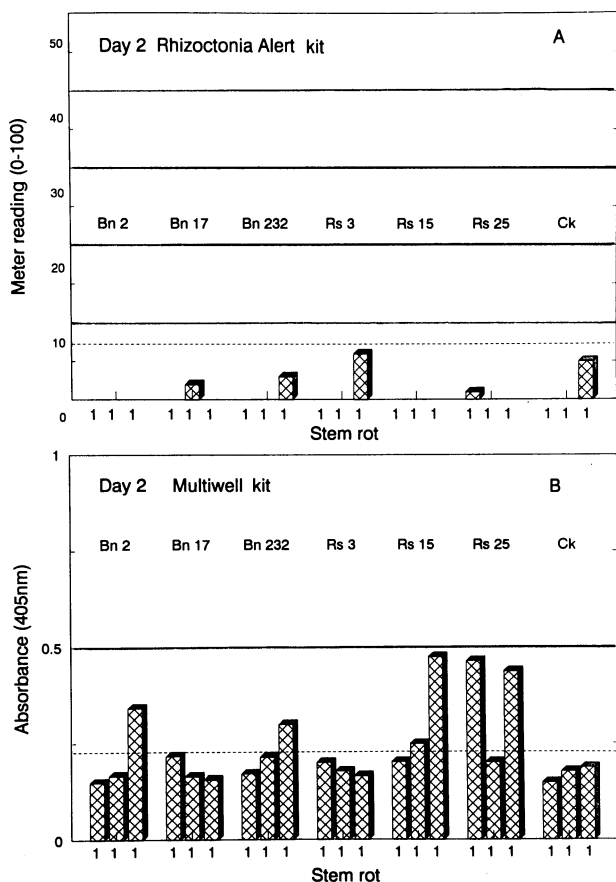
**Grower survey.** Poinsettias in propagation at three commercial greenhouses were surveyed for *Rhizoctonia* stem rot. Samples of symptomatic and asymptomatic plants were collected and returned to the laboratory for analysis by ELISA and culture plate as described above. Only the multiwell ELISA format was available for the grower survey.

## RESULTS

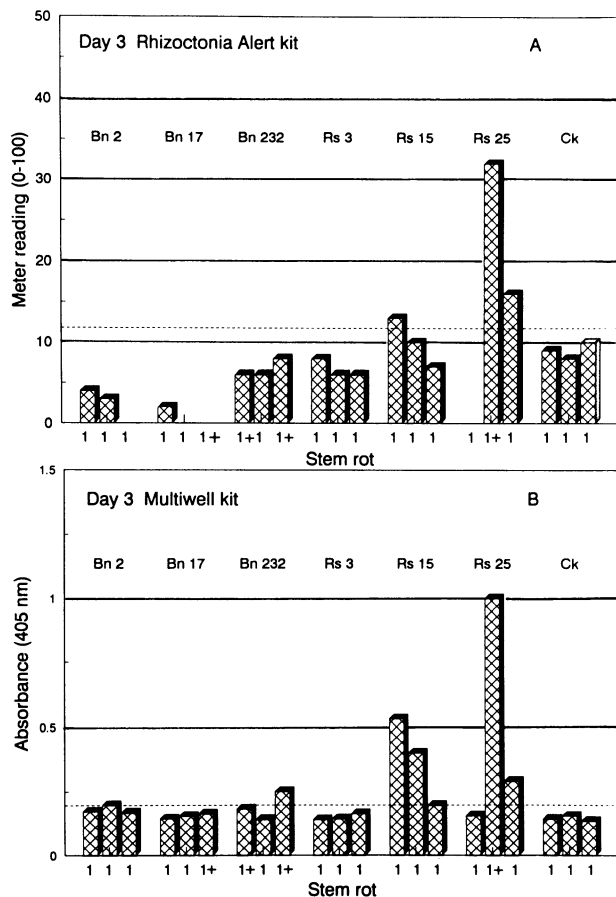
**Comparison of ELISA with the culture plate method.** Two days after inoculum placement, two of three poin-

settia cuttings inoculated with multinucleate isolate Rs 25 were judged positive with the multiwell ELISA (Fig. 1B). Absorbance value also was above the positive-negative test threshold for two of three cuttings inoculated with multinucleate isolate Rs 15. Positive test results were also obtained with one of three cuttings inoculated with Bn 2 and Bn 232 (binucleate isolates) at day 2. No cuttings were positive by the rapid assay kit or by the culture plate method at day 2 (Fig. 1A).

After 3 days, two of three cuttings inoculated with isolate Rs 25 gave readings above the positive-negative test threshold in the rapid assay test (Fig. 2A), and isolates Rs 25 and Rs 15 had absorbances well above the test threshold from cuttings tested with the multiwell ELISA (Fig. 2B). *Rhizoctonia* spp. were recovered by the culture plate method at 3 days from one of three cuttings inoculated with Rs 25 and Bn 17 and from two of three cuttings inoculated with Bn 232. *R. solani* was not isolated from any of the cuttings inoculated with isolate Rs 15 at day 3. No symptoms of



**Fig. 1.** Detection of *Rhizoctonia* spp. with (A) rapid assay ELISA kit (Rhizoctonia Alert kit) and (B) multiwell ELISA 2 days after poinsettia cuttings were inoculated with rice grains colonized by either binucleate or multinucleate isolates of *Rhizoctonia* spp. The *Rhizoctonia* stem rot rating for each cutting was based on disease severity, with 1 = healthy cutting and 5 = completely girdled and collapsed cutting. There were three cuttings per isolate. The positive-negative test threshold is indicated by the horizontal dashed line in each figure.



**Fig. 2.** Detection of *Rhizoctonia* spp. with (A) rapid assay ELISA kit (Rhizoctonia Alert kit) and (B) multiwell ELISA 3 days after poinsettia cuttings were inoculated with rice grains colonized by either binucleate or multinucleate isolates of *Rhizoctonia* spp. The *Rhizoctonia* stem rot rating for each cutting was based on disease severity, with 1 = healthy cutting and 5 = completely girdled and collapsed cutting. There were three cuttings per isolate. The positive-negative test threshold is indicated by the horizontal dashed line in each figure. Plus sign by stem rot rating indicates recovery of *Rhizoctonia* spp. by culture plate.

stem rot were seen on any cutting at day 2 or day 3.

Absorbance values for cuttings exposed to the binucleate isolates were judged positive at day 7, but relative absorbance values were much lower than values for cuttings exposed to multinucleate isolates. Only Bn 17 caused stem rot among the binucleate isolates tested (Fig. 3). Very high meter readings were recorded for multinucleate isolates, particularly isolates Rs 3 and Rs 15, and low but positive readings for binucleate isolates with the rapid assay test (Fig. 3A). Severe stem rot was evident on all cuttings inoculated with multinucleate isolates, and multiwell ELISA absorbance values were off-scale (Fig. 3B). *Rhizoctonia* spp. were isolated by culture plate from all cuttings at day 7, except the uninoculated control.

**Sensitivity of the ELISA tests.** Both the multiwell and rapid assay test detected isolate Rs 3 of *R. solani* from stem lesions 1–9 mm long on poinsettia. High absorbance values and meter readings well above the test threshold were obtained from lesion tissue samples as small as 1 mm long (8.6 mm<sup>2</sup>), about

the practical limit for tissue sectioning and handling (Fig. 4).

***Rhizoctonia* spp. from poinsettias in commercial greenhouses.** Most cuttings rated visually for stem rot were judged positive by multiwell ELISA (Fig. 5). In general, cuttings with a symptom rating of 2 (25% of stem girdled) had lower absorbances (but were still judged positive by ELISA) than those with ratings of 3 or higher. One cutting with a symptom rating of 3 did not test positive by ELISA or by culture plate (grower 3, Fig. 5). Healthy-appearing plants collected as a control for ELISA had low absorbances and negative culture plate results.

## DISCUSSION

Both the multiwell and rapid assay ELISA formats were useful for detection of binucleate and multinucleate isolates of *Rhizoctonia* spp. from poinsettia cuttings in propagation. It is interesting to note that isolate Rs 25 (detected within 2 days of inoculum placement) was originally isolated from *Pittosporium* sp., and isolate Rs 15 (detected on day 3) was originally isolated from *I. crenata*.

Neither isolate was detected by culture plate at day 2. Rs 3, an isolate of *R. solani* originally from poinsettia, was not detected by ELISA or culture plate until day 7. In a preliminary experiment, Rs 3 was not detected at day 3, but it was detected on day 5.

In general, multinucleate isolates caused more stem rot and gave higher absorbance values and meter readings than binucleate isolates. Only Bn 17 caused symptoms of stem rot on poinsettia, yet isolates Bn 2 and Bn 232 were judged positive by ELISA and were recovered by culture plate at day 7. Apparently Bn 2 and Bn 232 grew ectotrophically on stem tissue without causing stem rot symptoms.

Both the multiwell and rapid assay formats detected *R. solani* in stem lesions as small as 1 mm long (8.6 mm<sup>2</sup>), making the assays very sensitive for detection of *R. solani* in small samples. Detection of *Rhizoctonia* spp. with either ELISA format was also positive from tissue without lesions, as evidenced by the positive readings for nonpathogenic isolates Bn 2 and Bn 232 on day 7. Although absorbance and meter readings were positive for nonpathogenic, binucleate isolates,

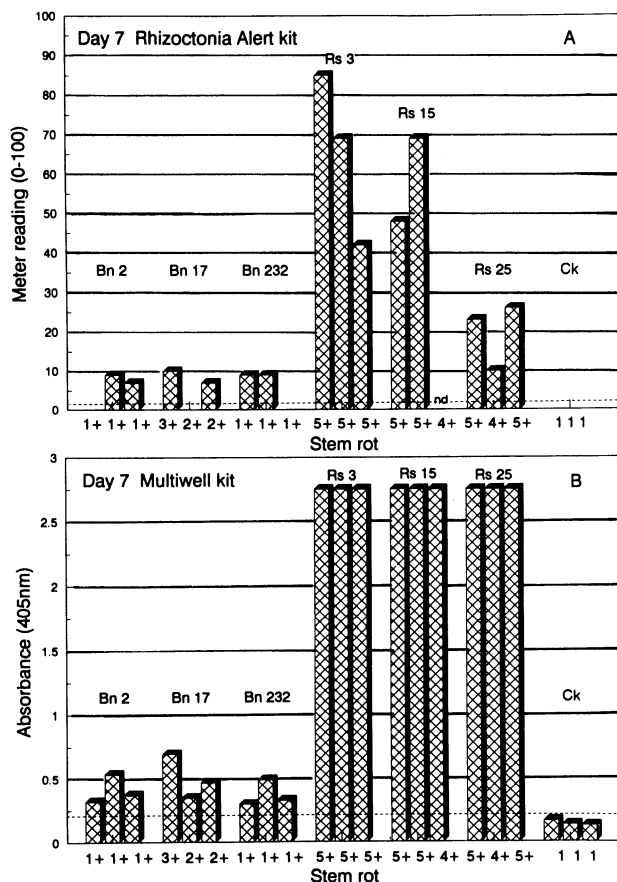


Fig. 3. Detection of *Rhizoctonia* spp. with (A) rapid assay ELISA kit (Rhizoctonia Alert kit) and (B) multiwell ELISA 7 days after poinsettia cuttings were inoculated with rice grains colonized by either binucleate or multinucleate isolates of *Rhizoctonia* spp. The *Rhizoctonia* stem rot rating for each cutting was based on disease severity, with 1 = healthy cutting and 5 = completely girdled and collapsed cutting. There were three cuttings per isolate. The positive-negative test threshold is indicated by the horizontal dashed line in each figure. Plus sign by stem rot rating indicates recovery of *Rhizoctonia* spp. by culture plate.

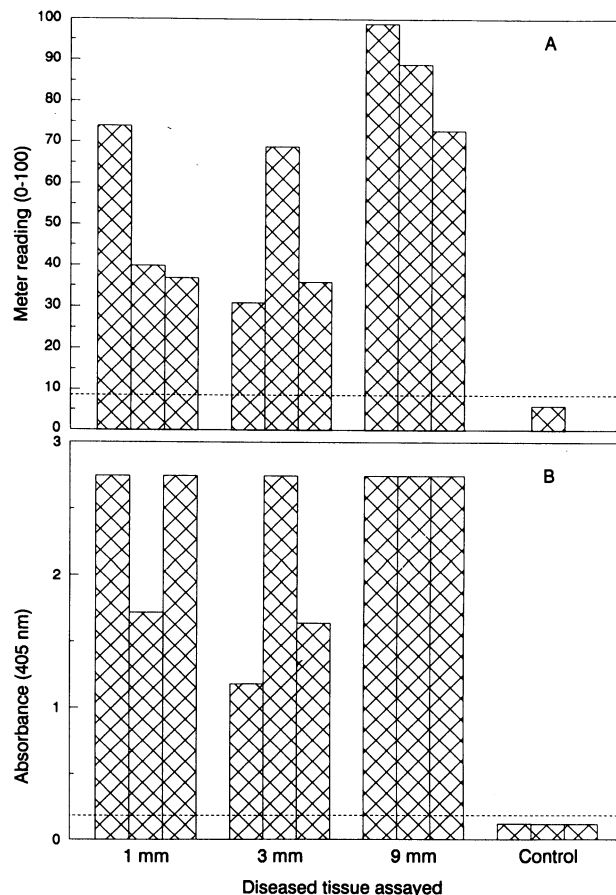


Fig. 4. Sensitivity of ELISA formats. Cross sections of lesioned tissue, either 1, 3, or 9 mm long, from poinsettia stems inoculated with isolate Rs 3 of *Rhizoctonia solani* were assayed by either (A) the rapid assay ELISA kit (Rhizoctonia Alert kit) or (B) the multiwell ELISA format. There were three cuttings per lesion size class. The positive-negative test threshold is indicated by the horizontal dashed line in each figure. The pathogen was isolated in culture from all lesioned tissue.

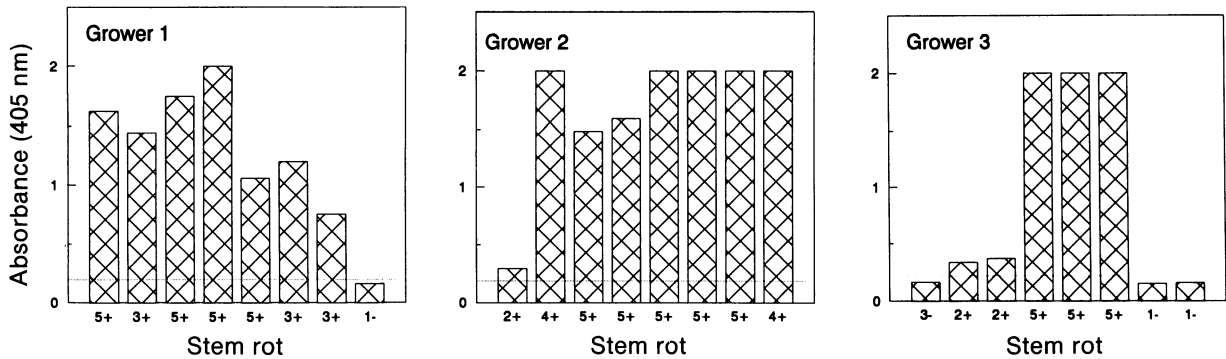


Fig. 5. Detection of *Rhizoctonia* spp. from poinsettia cuttings with symptoms of *Rhizoctonia* stem rot from three commercial greenhouse growers. The *Rhizoctonia* stem rot rating for each cutting was based on disease severity, with 1 = healthy cutting and 5 = completely girdled and collapsed cutting. The positive-negative test threshold is indicated by the horizontal dashed line in each figure. Plus sign by stem rot rating indicates recovery of *Rhizoctonia* spp. by culture plate.

absolute values of the readings were low compared to values for multinucleate isolates. Thus in a clinical setting, positive ELISA results could be misleading when nonpathogenic, binucleate isolates were present, unless visible symptoms of stem rot were seen. However, in the case of poinsettia, the natural occurrence of binucleate isolates of *Rhizoctonia* spp. is unknown.

Most poinsettia cuttings with stem rot symptoms from three commercial greenhouses tested positive for *Rhizoctonia* spp. In one sample, however, the lesion girdled 50% of the stem, but the sample tested negative by ELISA and plate

culture. This illustrates the benefit of ELISA detection of pathogens when visual symptoms from more than one causal agent, such as *Pythium* spp., *Phytophthora* spp., or bacterial stem canker pathogens could be confused.

#### ACKNOWLEDGMENTS

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