A COMPARISON OF THREE METHODS FOR DETERMINING ROOT-KNOT NEMATODE INFECTION OF COTTON ROOTS

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ABSTRACT

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Three methods for determining the extent of infection of cotton roots (*Gossypium hirsutum* L.) by root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, were compared. Root-knot nematode eggs were added to pots containing either sterilized soil or soil amended with rye, and cotton seedlings were planted. Cotton root systems were then assessed for infection/damage after 14 and 28 days of exposure. At the 14-day sampling, the roots were stained, using the lactophenol acid fuchsin method and the juvenile nematodes counted. At the 28-day samplings, the cotton roots were given a visual rating for nematode-induced root galling (0-100), and the galls were counted. The number of stained juveniles counted in the roots at 14 days gave a poor indication of the number of galls that will ultimately develop. The visual rating and the gall counts gave similar results. In this study the visual rating proved to be faster and more useful due to difficulties encountered obtaining a representative root sample for gall counts.

Key words: Gossypium hirsutum, infection assessment, *Meloidogyne incognita*, root gall, visual rating.

RESUMEN

McBride, R. G., R. L. Mikkelsen, and K. R. Barker. 1999. Comparación de tres metodos para determinar la infección del nematodos agallador del algodón. Nematrópica 29:147-151.

Se compararon tres metodos para determinar el grado de infección en raíces de algodón (*Gossypium hirsutum* L.) por el nematodo nodular (*Meloidogyne incognita* (Kofoid and White) Chitwood). Plantulas de algodón fueron trasplantadas en macetas conteniendo suelo esterilizado. Huevecillos del citado nematodo fueron depositados en el suelo, antes del trasplante de las plantulas. Se evaluó la infección y el daño del sistema radicular de las plantas de algodón a los 14 y 28 días de exposición. Las muestras de raíces tomadas a los 14 días fueron teñidas usando el metodo del ácido lactofenol-fucsina con el propósito de contar los nematodos presentes en estado juvenil. En el muestreo de 28 días las raíces de algodón fueron evaluadas directamente en cuanto al número de agallas presentes. El número de juveniles tenidos en las raíces a los 14 días mostro una pobre indicacion de el número de agallas que a la postre se desarrollarian. La evaluación visual del grado de infestación por medio de la presencia de agallas en la raíz, y el conteo de las mismas dieron resultados similares. En este estudio la evaluación visual mostró ser la más útil y rápida, ya que resulta muy problemático obtener una muestra de raíz representativa para el conteo de agallas en la misma.

Palabras claves: evaluación visual, *Gossypium hirsutum*, infección, *Meloidogyne incognita*, número de agallas.

INTRODUCTION

Root-knot nematodes enter roots as juveniles where they remain to develop into adults and complete their life cycle. The infecting nematodes induce changes in the root cells, resulting in galls. Three methods are commonly used to assess root-knot nematode infection and damage to plants. Roots can be stained and then cleared, leaving the infecting juvenile nematodes a darker red than the surrounding root tissue (Daykin and Hussey, 1985). The stained nematodes can then be counted under a stereoscopic microscope. This staining method is successful when the roots are young. Whether or not the observed nematodes are a good indicator of subsequent root damage has received only limited study. Alternatively, galls formed by the maturing nematodes in older roots can be seen by the unaided eye and can be rated as an approximate percentage of the root galled. A less subjective method is to weigh out a portion of the root system and count the number of galls. The purpose of this study was to compare the three methods of quantifying root-knot nematode infection and damage to roots of cotton grown in root-knot nematode-infected soil in pots.

MATERIALS AND METHODS

A 1:1 mixture of steam-sterilized loamy sand and sand was used to fill 72, 15-cm clay pots. An additional 72 pots were filled with the same soil mixed with 53 g of chopped fresh rye (*Secale cereale* L.). At 12, 2-day intervals beginning immediately after adding rye, 6 pots of each substrate were infested with nematodes, planted to cotton 48 hr later, and harvested on a regular schedule to study the effect of the rye amendment at progressing levels of decomposition. In this paper we report on the relationships between our response variables, measured on 12 different occasions. For a full discussion of the rye aspect of the experiment refer to McBride *et al.* (1999).

Eggs of the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood were collected from the root systems of infected tomato plants (*Lycopersicon esculentum* Mill.) by NaOCl extraction (Barker, 1985). At each of the 12 time intervals, the egg/water suspension was adjusted to 333 eggs/ml and pot inoculation was accomplished by removing the top 2 cm of soil, adding 30 ml egg suspension, and then replacing the soil.

Forty-eight hours after the addition of the nematode eggs, eight germinated cotton seeds (var. Delta Pine 50) were planted into each pot. The cotton was watered daily and fertilized weekly with N, P_2O_5, and K_2O in the irrigation water. The transplanted cotton plants were carefully watered to avoid leaching the soil and to maintain aerobic soil conditions. Fourteen days after planting, three of the cotton plants were removed with the root system intact. This was accomplished by loosening the soil around the root system with a small spatula and gently shaking the roots free. At this time the remaining plants were thinned to three per pot. Twenty-eight days after planting, the remaining three plants were removed. The roots were recovered by submerging in water and gently rinsing away the soil.

The roots of the cotton plants that were collected after 14 days of growth were stained using the lactophenol acid fuchsin method (Daykin and Hussey, 1985). The stained roots were mounted by pressing between two microscope slides and the number of juveniles counted. The roots of the 28-day-old cotton plants were rated visually. The visual rating estimated the percent of the root system damaged, but

did not consider the severity of the infection as may be required in a field study (Zeck, 1971). From these roots, the root-knot nematode-induced galls were counted in a 5 g sub-sample.

For each time interval, the average responses (infection rate, root gall index, and number of galls per gram root) were determined for the 6 pots of each soil treatment.

RESULTS AND DISCUSSION

The root stain method was a poor predictor of the eventual root damage caused by nematodes. The numbers of stained juvenile nematodes per plant varied widely, even among plants from the same pot. This variation can be interpreted as either a problem with the method, the inherent difficulty obtaining a representative root sample, that only some of the juveniles entering the root matured to form observable galls, or that individual galls contained variable numbers of nematodes. The root stain method did not correlate well with the visual gall rating or the gall counts despite the fact that the root-stain data were obtained from plants growing in the same pots as those used for the visual ratings and gall counts (Fig. 1).

There were some discrepancies between the visual rating, where the entire root system was rated, and the gall count where only a 5 g subsample of the root system was counted for a given pot. It was observed during root recovery that the major nematode infections of the cotton root systems were concentrated near the soil surface. This response is likely a result of the conservative way in which the cotton plants were watered and the initial placement of the eggs. The eggs were added approximately 2 cm under the soil surface and were not leached deeply into the pot during the experimental period. This lack of uniform infection of the entire root system made it difficult to get a representative sample of each root system. The lack of uniform infection may explain the fairly low correlation between the root-gall rating and the gall count systems ($r = 0.71$). Nevertheless, both the visual gall rating and the gall-count methods were more accurate than the root staining method for assessing nematode damage to cotton roots.

CONCLUSIONS

Although staining roots and counting the number of juvenile nematodes present is an effective means to measure root infection rates, the root-stain method gave a poor indication of the number of galls that ultimately developed, and is therefore less useful as a predictive tool. The visual gall rating, while somewhat subjective, gave results that were fairly consistent with gall counts ($r = 0.71$). The visual rating proved to be the most practical method and was much faster than the other two methods used. To insure that the visual ratings are consistent throughout an experiment, the same person should rate all root systems. Problems were encountered acquiring a representative sample to conduct gall counts. Some roots systems had an even distribution of galls, whereas others had higher concentrations near the surface. For evaluating larger numbers of plants such as screening for root-knot resistance, visual ratings, based on a standard gall index, have proven to be most useful (Kinloch, 1990). A range of subjective rating schemes for root-knot development with and without associated root necrosis has been used (Barker, 1985; Barker and Davis, 1996). When assessing root damage caused by root-knot nematodes, investigators should consider employing the visual rating method, gall count method, or a combination of both methods. Molecular tools

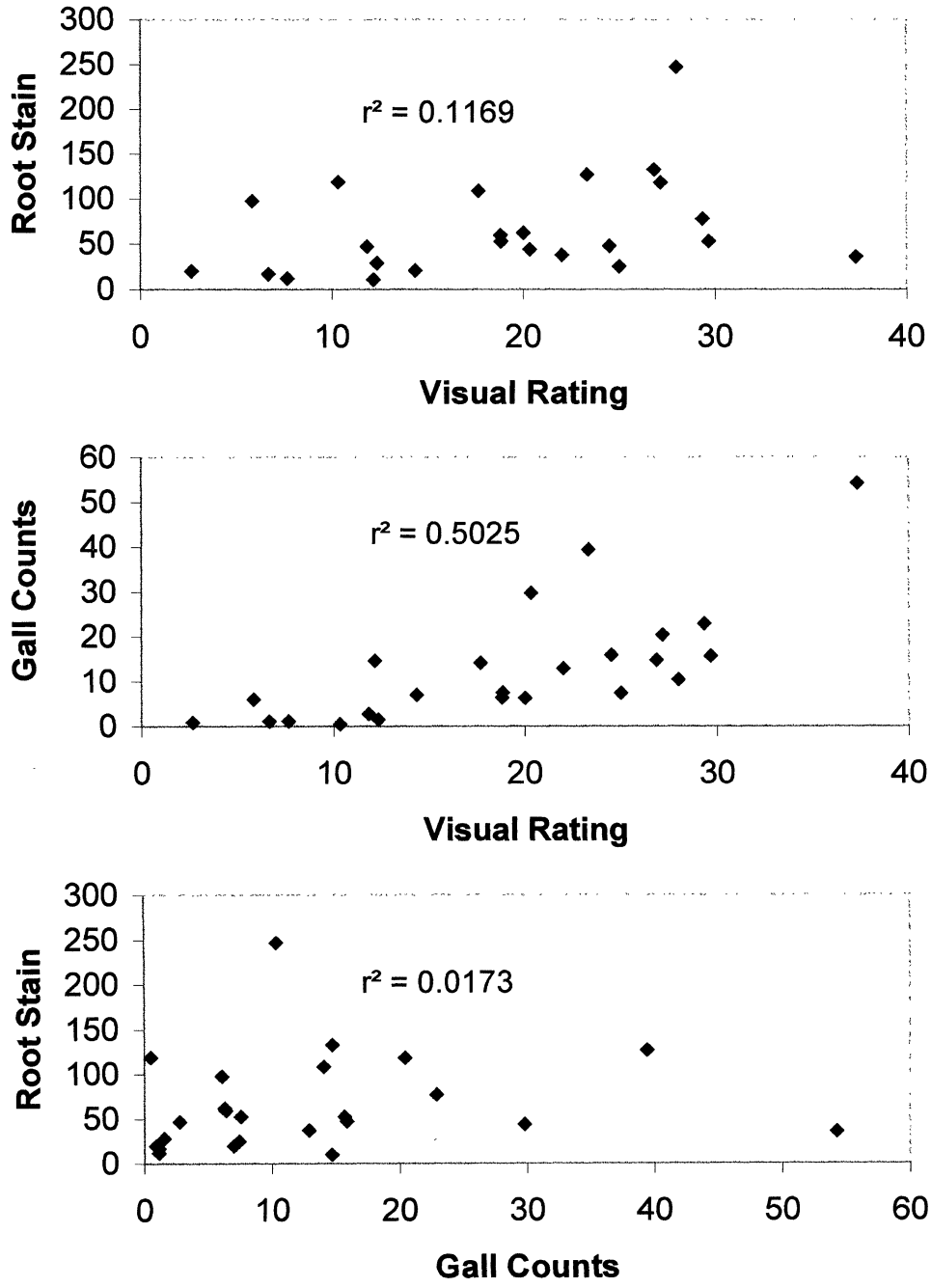

Fig. 1 Relationships between three methods of estimating root-knot nematode infection and damage to plant roots. Root stain represents the number of nematodes per gram of 14-day-old cotton roots, measured by a root staining method. Visually rated root galling (percent of root system infected) and gall counts (root galls per gram) were taken from 28-day-old cotton roots from the same pots. Cotton plants were planted two days after the addition of nematode eggs. Data points represent an average infection of 6 pots, 3 plants each.

also may eventually be refined for the identification and quantification of nematodes in roots as well as soils (Curtis *et al.*, 1997).

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