BRIEF REPORT

Adjustment of a rapid method for quantification of *Fusarium* spp. spore suspensions in plant pathology

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Abstract The use of a Neubauer chamber is a broadly employed method when cell suspensions need to be quantified. However, this technique may take a long time and needs trained personnel. Spectrophotometry has proved to be a rapid, simple and accurate method to estimate the concentration of spore suspensions of isolates of the genus *Fusarium*. In this work we present a linear formula to relate absorbance measurements at 530 nm with the number of microconidia/ml in a suspension.

Ajuste de un método rápido para la cuantificación de suspensiones de esporas de *Fusarium* spp. en fitopatología

Resumen La cámara de Neubauer es un método ampliamente utilizado para cuantificar suspensiones de esporas. Sin embargo, esta técnica requiere personal entrenado y un tiempo considerable. La espectrofotometría demostró ser un método rápido, simple y preciso para estimar la concentración de esporas de aislados del género *Fusarium*. En este artículo breve presentamos una fórmula lineal ajustada para relacionar la absorbancia a 530 nm con el número de conidios en suspensiones utilizadas en pruebas fitopatológicas.

Cell counting is a usual task in plant pathology labs, because the need to quantify spore concentration is a basic requirement for many different assays, including resistance screening tests. It is also essential as a way of standardizing lab results allowing comparisons and repeatability.

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Although sophisticated equipment is currently available, they often represent high initial prices and expensive operation costs, especially for small laboratories. Therefore, the use of the hemocytometer or Neubauer chamber is still a widespread technique for adjusting cell suspensions. This method has many advantages, such as low cost and cell color/size independence,[6] however, it may take a long time and carry considerable mistakes in the concentration estimation.[7] Hence, the use of spectrophotometry as the measurement of absorbance of a suspension has proved to be a more rapid and simple method for quantifying conidia concentration. Some articles have mentioned the use of spectrophotometry for spore counting in *Fusarium* spp. pathogenic for humans;[4,6] however, there are no currently available publications for the use of this technique in plant pathogenic Fusaria. In the present work we describe a simple laboratory procedure for estimating the concentration of *Fusarium* spp. suspensions by spectrophotometry.

For the development of the method, we used five onion pathogenic isolates of *Fusarium* spp. The isolates belong to three different species (*F. oxysporum*, *F. proliferatum* and *F. verticillioides*) and were initially obtained from rotten onion bulbs. The conidial suspensions were prepared as follows: mycelium was collected from ten-day old colonies grown on potato-dextrose agar with a scalpel and suspended in a sterile agar-distilled water solution (500 mg agar per liter). Suspensions were then filtered through sterilized cotton filters to obtain pure conidial suspensions. From these initial suspensions, serial dilutions were prepared (9 dilutions per isolate, 45 total samples) and quantified by using a hemocytometer. At the same time, the absorbance at 530 nm[6] was measured for every sample using a spectrophotometer Spectronic 20D (Milton Roy, Rochester, NY). The presence of agar conferred stability to the suspension, avoiding conidia decantation[7] and allowing accurate quantification. Between every measurement, the absorbance data were checked and eventually blanked with sterile agar-water solution as reference blank. Data obtained with both methods were compared by regression analysis using InfoStat.[2] Figures were created using Microsoft Office Excel.

Table 1   Isolates of *Fusarium* spp. used in this study and their regression coefficients.

Isolate	Species	R^2	p-Value
LJC10002	*F. verticillioides*	0.950	7.9×10^{-8}
LJC10013	*F. proliferatum*	0.845	1.0×10^{-3}
LJC10017	*F. oxysporum*	0.775	2.4×10^{-6}
LJC10054	*F. proliferatum*	0.966	1.1×10^{-6}
LJC10081	*F. oxysporum*	0.883	4.6×10^{-6}
Model		0.934	–

Isolates belong to WDCM904 'Colección de Fitopatógenos de Cultivos Hortícolas', EEA La Consulta INTA, Mendoza, Argentina (acronym 'LJC').

Absorbance data ranged from 0 to 0.532 in accordance with the concentrations measured in the hemocytometer, which fluctuated between 45,000 and 6,125,000 conidia/ml. Only microconidia were observed during cell counting in the hemocytometer, since chlamydospores, macroconidia and hyphae were retained by the cotton filter. Absorbance values exceeding 0.400 (two samples) were not included in the statistical analysis because of their lack of linearity and due to the fact that those high conidial concentrations are rarely used in plant pathology tests. High correlation values between absorbance and spore concentration were found for each isolate and are shown in Table 1. The regression analysis of the complete data fitted to the linear model displayed in Fig. 1 (Concentration = $\beta_0 + \beta_1 \times$ Absorbance). The corresponding estimated parameters were $\beta_0 = -100,439$ and $\beta_1 = 20,080,760$, with a high total R^2 value (0.934). Individual R^2 values were calculated for every different isolate in order to check the goodness of fit for the adjusted linear model and their R^2 values were all acceptable (Table 1).

Aberkane *et al.*[1] state that the spectrophotometric method needs to be adjusted for every different fungal species, due to variability in spore color and size. In our study we used isolates from three different species of the genus *Fusarium*, which had been previously characterized (data not shown). It was found that the difference in size of
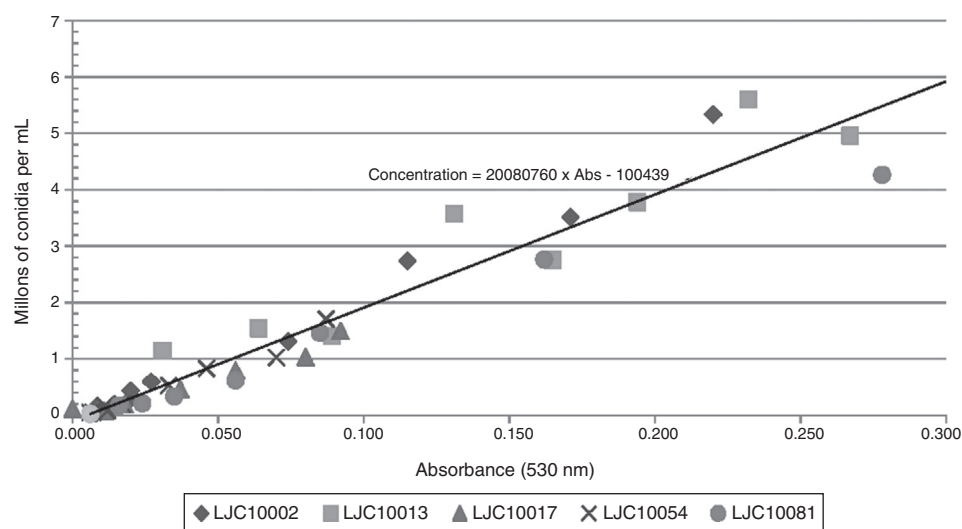

Figure 1   Adjustment of linear regression model to experimental data

their microconidia was not significant, therefore, not influencing the spectrophotometric measurements, which was confirmed by the uniform distribution of the points in Fig. 1 and the high individual R^2 values in Table 1. Although every isolate conferred different colors to the suspensions, these colors did not affect the adjustment of individual data to the model, in accordance with Lage *et al.*[5]

Spectrophotometry has been reported as a quick, simple and familiar method to most lab personnel.[3] The results allow to conclude that the use of this technique is also an accurate and rapid method for quantification of *Fusarium* conidial suspensions.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Aberkane A, Cuenca Estrella M, Gomez Lopez A, Petrikkou E, Mellado E, Monzón A, Rodriguez Tudela JL, Network E. Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi. J Antimicrob Chemother. 2002;50: 719–22.
2. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat Software. Córdoba, Argentina: Universidad Nacional de Córdoba.
3. Espinel-Ingroff A, Kerkering TM. Spectrophotometric method of inoculum preparation for the susceptibility testing of filamentous fungi. J Clin Microbiol. 1991;29:393–4.
4. Gehrt A, Peter J, Pizzo PA, Walsh TJ. Effect of increasing inoculum sizes of pathogenic filamentous fungi on MICs of antifungal agents by broth microdilution method. J Clin Microbiol. 1995;33: 1302–7.
5. Lage L, Panizo MM, Ferrara G, Reviakina V. Validación del inóculo por densitometría para las pruebas de susceptibilidad a los antifúngicos en especies del género *Fusarium*. Rev Soc Ven Microbiol. 2013;33:46–52.
6. Petrikkou E, Rodriguez Tudela JL, Cuenca Estrella M, Gomez A, Molleja A, Mellado E. Inoculum standardization for antifungal susceptibility testing of filamentous fungi pathogenic for humans. J Clin Microbiol. 2001;39:1345–7.
7. Valdez JG, Piccolo RJ. Use of spectrophotometry as a tool to quantify the sporulation of *Penicillium alli* in garlic lesions. Fitopatol Bras. 2006;31:595–7.