Disease Control and Plant Growth Promotion of *Miscanthus* × *giganteus* with *Trichoderma* Bio-Inoculants

Diwakar R. W. Kandula
Lincoln University, New Zealand

Janaki Kandula
Lincoln University, New Zealand

Harmanjeet Kaur
Lincoln University, New Zealand

Hossein Alizadeh
Lincoln University, New Zealand

John G. Hampton
Lincoln University, New Zealand

The XXIII International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

Paper ID: 900
Theme: 2. Grassland Production and Utilization
Sub-Theme: 2.2 Integration of plant protection for optimizing production

Disease control and plant growth promotion of *Miscanthus* × *giganteus* with *Trichoderma* bio-inoculants

D.R.W. Kandula*, Janaki Kandula, Harmanjeet Kaur, Hossein Alizadeh, John G. Hampton
*Corresponding author e-mail: Diwakar.Kandula@lincoln.ac.nz
Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand

Keywords: Biological control, *Miscanthus* × *giganteus*, *Rhizoctonia solani*

Introduction

The second-generation bioenergy crop Miscanthus (*Miscanthus* × *giganteus*) is being assessed in New Zealand for its potential to provide shelter on irrigated dairy farms. Miscanthus is a perennial sterile hybrid vegetatively propagated rhizomatous C_4 grass and the young rhizomes and roots are prone to infection by soil-borne fungal pathogens (Glynn *et al*., 2015) which can cause deleterious effects on plant establishment and growth. In Europe, several species of *Fusarium* such as *F. avenaceum*, *F. culmorum*, *F. moniliforme* and *F. oxysporum* have been implicated as causal agents of root and rhizome rot (Thinggaard, 1997; Covarelli *et al*., 2012) leading to poor field establishment of *in-vitro* propagated Miscanthus plants. When tested for their ability to cause disease of Miscanthus, *Rhizoctonia solani* (Kuhn) was reported as the most aggressive species among nineteen fungal pathogens of cereal crops (Glynn *et al*. 2015). In New Zealand, *R. solani* reduces seedling emergence and plant establishment of several herbage species and the problem may be alleviated through biocontrol using *Trichoderma* fungi (Kandula *et al*., 2015). In a glass-house study, the effect of four *T. atroviride* isolates on growth of tissue culture propagated Miscanthus plants in a soil naturally infested with *R. solani* was investigated.

Materials and Methods

Soil from Lincoln University field known to contain *R. solani* (Kandula *et al*., 2015) was collected, passed through a 3 mm sieve and mixed with pumice in a 3:1 v/v soil: pumice ratio. Four New Zealand *T. atroviride* isolates were grown in sterile wheat bran and peat (3:1 w/w ratio). Four grams of colonised wheat bran (containing approximately 2×10^8 cfu/g) was added to 800 g of soil and thoroughly mixed. Nursery grown plant plugs (Plate 1) were transplanted into the soil in 1l pots. Uninoculated soil was the control. Pots (5 replicates of each treatment) were arranged in a completely randomised block design in a glass-house at Lincoln University maintained at an average temperature of 20°C for the duration of the experiment. Pots were watered using overhead irrigation as required. After 5 months (December 2013-April 2014), rhizosphere soil was sampled and enumerated for *Trichoderma* colonisation using soil-dilution plating on a *Trichoderma* selective medium (TSM). Plants were then transferred to larger pots (8.5l) and filled with 5kg of infected soil and allowed to grow for a further 12 months (May 2014- April 2015). After 17 months growth, shoot number was counted and shoots were cut at the base of the plant and bagged. Roots were carefully removed, rhizosphere soil was sampled for final enumeration of *Trichoderma* colonies and then washed to remove the soil. 100 fine root pieces (2-3mm) from each pot were surface sterilised and plated on TSM to obtain *Trichoderma* root colonisation data. Roots were separated from rhizomes (Plate 1) and bagged separately. Shoots, rhizomes and roots were oven dried at 60°C for 48 hours and dry weights were recorded. All data were subjected to analysis of variance with a probability level of $P< 0.05$ used for mean separation using Fisher's unprotected least significant difference (LSD) test.

Plate 1: Photograph showing nursery grown plant plug of *Miscanthus* × *giganteus*; root system (selected areas indicate rhizome growth) of control and *Trichoderma atroviride* isolate 1 treatments after 17 months growth in 8.5l pots in a glasshouse.

Results and Discussion

Miscanthus mean shoot number per plant did not differ among the four isolates, but only isolate (I)3 had significantly ($P<0.05$) more shoots (+114%) than the untreated control (Table 1). Shoot dry weight per plant was increased ($P<0.05$) by I1 and I3, but not by I2 and I4. All four isolates significantly increased rhizome dry weight per plant, with the increases ranging from 57% (I2) to 108% (I3), and all isolates except for I4 increased root dry weight per plant by between 67 to 140% (Table 1).

At five months *Trichoderma* rhizosphere colonisation (cfu/g rhizosphere soil) was higher than that of the control (Table 1) and among the isolates was greatest for I3. However by 17 months only I1 and I3 had more cfu/g rhizosphere soil than the control, and I3 was still significantly higher than the other isolates. Percentage *Trichoderma* root colonisation was only just over 3% in the control, significantly lower than that for all the isolates (Table 1). Among the isolates I1 had the highest root colonisation percentage.

Table 1: Effect of four *Trichoderma atroviride* isolates on *Miscanthus* × *giganteus* shoot number, dry weight of plant components, rhizosphere colonisation [colony forming units (cfu)/g soil] and root colonisation (%) of potted plants when grown in soil with a natural infestation of *Rhizoctonia solani* for 17 months.

		Dry weight (g/plant)				*Trichoderma* cfu/g soil	
Treatments	Shoot number	Shoot	Rhizome	Root	*Trichoderma* root colonisation	5 months	17 months
Control	2.8 b	53.3 b	37.5 b	28.8 c	3.2 e	3.1×10^{2} d	5.4×10 c
Isolate 1	5.4 ab	87.5 a	68.2 a	68.2 a	75.6 a	2.9×10^{5} b	1×10^{4} b
Isolate 2	4.2 ab	68.6 b	59.0 a	48.3 b	44.0 c	1.4×10^{5} c	3.4×10^{3} bc
Isolate 3	6.0 a	85.2 a	78.1 a	58.6 ab	59.6 b	4.6×10^{5} a	1.9×10^{4} a
Isolate 4	4.8 ab	64.3 b	71.6 a	43.2 bc	30.8 d	1.7×10^{5} bc	1.7×10^{3} c
LSD (*P*<0.05)	**2.87**	**15.32**	**19.97**	**17.06**	**13.06**	**1.2×10^{5}**	**7.9×10^{3}**

Mean values followed by a different letter in the columns indicate significant differences according to Fisher's unprotected LSD test.

In the presence of *R. solani*, all four *Trichoderma* isolates increased rhizome dry weight, three increased root dry weight, and two increased shoot dry weight per plant. While it is possible that these responses were due to growth promotion by the *Trichoderma* isolates, it is unlikely, as I1 and I3 do not have growth promotion properties and are known to be active against *R. solani* (Kandula *et al*., 2015). Isolates 1 and 3 were strong rhizosphere soil and root colonisers and significantly increased the dry weight of all three plant growth parameters recorded. This result is consistent with those recorded for temperate pasture grasses (Kandula *et al*., 2015: Kandula unpublished data). Beneficial microorganisms such as *Trichoderma* and plant pathogens compete to colonise the rhizosphere (Raaijmakers *et al*., 2009), and the introduction of *Trichoderma* fungi can reduce the negative impact of soilborne pathogens on plant establishment and vegetative growth (Kandula *et al*., 2015). Plant propagation and establishment problems have been identified as factors limiting the success of Miscanthus as a commercial crop (Xue *et al*., 2015). Biocontrol of soil-borne pathogens by *Trichoderma* may offer an option for disease management in this species.

Conclusion

Trichoderma isolates persisted in the rhizosphere soil for 17 months and actively colonised Miscanthus roots. In the presence of *R. solani*, two isolates increased shoot, rhizome and root dry weight per plant by between 60 to 140% over that of the untreated control. Verification of these results in field experiments is now required.

References

Covarelli, L., Beccari, G and L. Tosi. 2012. Miscanthus rhizome rot: A potential threat for the establishment and the development of biomass cultivations. *Biomass and bioenergy* 46: 263-269.

Glynn, E., Brennan, J.M., Walsh, E., Feechan, A and K.P. McDonnell. 2015. The potential of *Miscanthus* to harbour known cereal pathogens. *European journal of plant pathology* 141: 35-44.

Kandula, D. R. W., E. E. Jones, A. Stewart, K. L. McLean and J.G. Hampton. 2015. *Trichoderma* species for biocontrol of soil-borne plant pathogens of pasture species. *Biocontrol science and technology* 25: 1052-1069.

Raaijmakers, J. M., T. C. Paulitz, C. Steinberg, C. Alabouvette and Y. M. Loccoz. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant soil 321: 341-361.

Thinggaard, K. 1997. Study of the role of Fusarium in the field establishment problem of Miscanthus. *Acta Agriculturae ScandinavicaB. Soil & Plant science* 47: 238-241.

Xue, S., O. Kalinina and I. Lewandowski. 2015. Present and future options for *Miscanthus* propagation and establishment. *Renewable and sustainable energy reviews* 49: 1233-1246.

Acknowledgement
We thank Mr Chris P. Littlejohn for providing the plant material.