

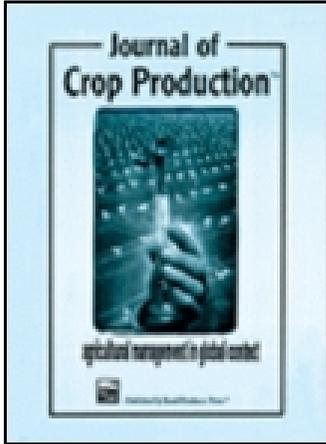
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Publisher: Taylor & Francis

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Journal of Crop Production

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/wzcp20>

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Hui-Lian Xu ^a, Xiaoju Wang ^a & Jihua Wang ^b

^a International Nature Farming Research Center, 5632 Hata, Nagano, 390-1401, Japan

^b Beijing Academy of Agriculture, Beijing, China

Published online: 20 Oct 2008.

To cite this article: Hui-Lian Xu, Xiaoju Wang & Jihua Wang (2001) Effect of a Microbial Inoculant on Stomatal Response of Maize Leaves, Journal of Crop Production, 3:1, 235-243, DOI: [10.1300/J144v03n01_19](https://doi.org/10.1300/J144v03n01_19)

To link to this article: http://dx.doi.org/10.1300/J144v03n01_19

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Effect of a Microbial Inoculant on Stomatal Response of Maize Leaves

Hui-lian Xu
Xiaoju Wang
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SUMMARY. Laboratory tests were conducted to determine the effect of a microbial inoculant on the stomatal response of maize leaves (*Zea mays* L.). The microbial inoculant investigated is known as Effective Microorganisms or EM and consists of a mixed culture of naturally occurring, beneficial microorganisms. Research has shown that EM applied to soils and plants can improve soil properties and enhance the growth, yield and quality of crops. The exact mechanisms or modes-of-action of how EM cultures elicit beneficial effects on plant growth and metabolism is not known. However, it is likely that some of these cultures can synthesize phytohormones (i.e., auxins and others) or growth regulators that stimulate plant growth. Consequently, the effects of EM and partial illumination on stomatal response of intact and excised maize leaves were evaluated. Potted plants were dried to the wilting point and rehydrated with either a 1:100 dilution of EM and water or water alone applied to the soil. Sudden illumination of plants maintained in the dark showed that the leaf stomata of the EM-treated plants opened more rapidly than water-treated control plants. When leaves were excised and subjected to dehydration, the stomata closed

Hui-lian Xu is Senior Crop Scientist and Xiaoju Wang is Research Soil Scientist, International Nature Farming Research Center, 5632 Hata, Nagano 390-1401, Japan. Jihua Wang is Crop Scientist, Beijing Academy of Agriculture, Beijing, China.

Address of correspondence to: Hui-lian Xu at the above address (E-mail. huilian@janis.or.jp).

[Haworth co-indexing entry note]: "Effect of a Microbial Inoculant on Stomatal Response of Maize Leaves." Xu, Hui-lian, Xiaoju Wang, and Jihua Wang. Co-published simultaneously in *Journal of Crop Production* (Food Products Press, an imprint of The Haworth Press, Inc.) Vol. 3, No. 1 (#5), 2000, pp. 235-243; and: *Nature Farming and Microbial Applications* (ed: Hui-lian Xu, James F. Parr, and Hiroshi Umemura) Food Products Press, an imprint of The Haworth Press, Inc., 2000, pp. 235-243. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST), E-mail address: getinfo@haworthpressinc.com].

more slowly (i.e., remained open longer) for the EM-treated plants compared with the water-treated control plants. There was no effect of EM on cuticular conductance in any of the experiments. The results of this study indicate that EM cultures contains bioactive substances that can significantly affect leaf stomatal response. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com <Website: <http://www.HaworthPress.com>>]

KEYWORDS. Cuticular conductance, effective microorganisms, EM, microbial inoculant, stomatal conductance, *Zea mays*, excised leaves

INTRODUCTION

There is increasing evidence that mixed cultures of naturally occurring, beneficial microorganisms applied to soils and plants can improve soil quality and the growth, yield and quality of crops. One such microbial inoculant is known as Effective Microorganisms or EM and has been developed by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan (Higa and Parr, 1994). A number of theories have been proposed as to the modes-of-action of EM on plant growth and metabolism (Higa and Wididiana, 1991). However, the exact mechanisms of how beneficial effects are derived by either (a) direct effects of microorganisms on the plant or (b) indirect effects of microbially-synthesized substances (e.g., phytohormones and growth regulators) are largely unknown. In this regard, Xu et al. (1998) reported that EM significantly increased the growth and grain yield of maize by promoting root development and activity that was largely auxin-mediated. Others have also reported that a number of microbes can synthesize phytohormones and physiologically-active compounds (Arshad and Frankenberger, 1992; Kampert et al., 1975; Panosyan et al., 1963). However, these reports did not differentiate between direct effects of microorganisms on plants, or indirect effects of microbially-synthesized phytohormones. Therefore, the purpose of this study was to determine whether the liquid stock solution of EM cultures contained biologically-active substances that could affect stomatal responses of intact or excised maize leaves to partial illumination and dehydration.

MATERIALS AND METHODS

Plant Materials

Sweet corn plants (*Zea mays* L. cv. Honey Bantam) were grown in plastic pots each with a soil surface area of 0.02 m² and a height of 0.25 m. Pots were

filled with a fine textured Andosol. The N-P-K levels in the soil were 3.4, 0.025 and 0.44 g kg⁻¹, respectively, with a C:N ratio of 13. The field capacity or capillary capacity of the soil was 80% on a gravimetric basis. Chemical fertilizers used in this experiment were ammonium sulfate (5.3 g pot⁻¹), long period coated urea (2.8 g pot⁻¹), superphosphate (5 g pot⁻¹) and potassium sulfate (3.5 g pot⁻¹). One plant was grown in each pot under glasshouse conditions. The daily temperature and air humidity were not controlled and fluctuated with ambient conditions.

Pot Treatment

When the 7th leaf was fully expanded, water supply to the pots was stopped. When the leaves began to wilt, EM was diluted 1:100 with water and supplied to the pots until the plants were rehydrated (i.e., had regained maximum turgor).

Partial Illumination

Partial illumination 2000 μmol m⁻² s⁻¹ was applied to the seventh leaf using an LED light source in the leaf chamber. Other parts of the whole plant were maintained in dark. Stomatal conductance was measured using a LI-6400 instrument. The stomatal response to partial illumination (Figure 1) was analyzed using a sigmoid model as follows:

$$g_s = g_{\max} \{1/[1 + (1 - \beta t) e^{-\alpha(t-\tau)}]\} + g_{cc}(1 + \beta t),$$

where, g_{\max} and g_{cc} are the maximum stomatal conductance and cuticular conductance; β and α are the cuticular and stomatal response constants; t , time from beginning of illumination; τ , half saturation constant.

Stomatal Closure in Excised Leaves

Stomatal conductance of the 7th leaf of the fully turgid plants was measured under 2000 μmol m⁻² s⁻¹ until the maximum level was reached. The leaf was then excised from the plants and measurement continued. The stomatal response to leaf dehydration (Figure 2) was analyzed using an exponential model as follows:

$$g_s = [g'_{\max} - g_{\text{res}}(1 - \beta't)] e^{-\alpha'(t-\tau')} + g_{\text{res}}(1 - \beta't)$$

where g'_{\max} is the maximum stomatal conductance before excision; g_{res} is residual conductance after the stomata are closed; β' and α' are the cuticular and stomatal response constants; τ' is the time prior to rapid closure of stomata.

FIGURE 1. A sigmoid model for the response of stomatal conductance (g_s) to partial illumination on the leaves of sweet corn plant in the dark.

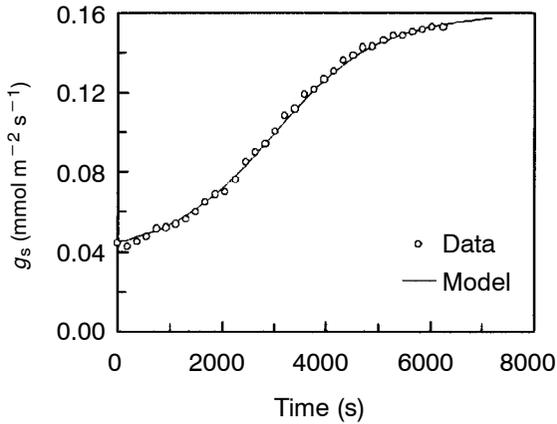
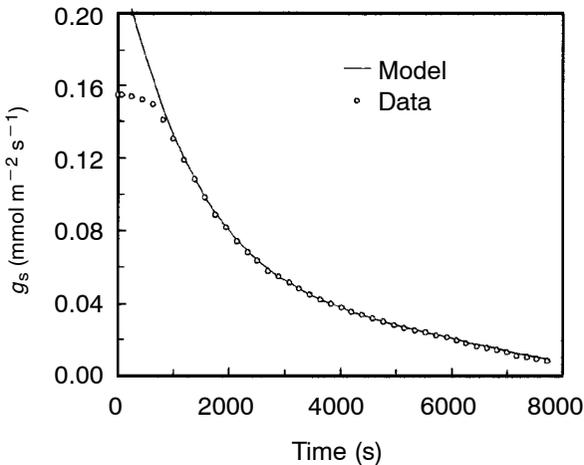


FIGURE 2. A model for the decline of stomatal conductance (g_s) in an excised sweet corn leaf blade.



RESULTS AND DISCUSSION

Stomatal Response to Illumination

Preliminary experiments have shown the response of stomatal conductance (g_s) to partial illumination of sweet corn leaves that had equilibrated in

the dark. The rapid increase in g_s upon illumination and the close agreement between the sigmoid model and experimental data are shown in Figure 1. The decline in stomatal conductance (g_s) that occurs when a leaf is excised from a corn plant is shown in Figure 2, which indicates close agreement between the model and experimental data.

When the leaf was partially illuminated in the dark, the stomata started to open slowly. In this case, the stomata in the EM-treated leaves opened faster than those of the control plants (Figure 3). The parameters analyzed from the model are presented in Table 1. The variable g_{\max} shows the maximum g_s .

FIGURE 3. Comparison of the sigmoid curves for the response of stomatal conductance (g_s) to partial illumination on the leaf blade of sweet corn plants treated with EM liquid and water in the dark.

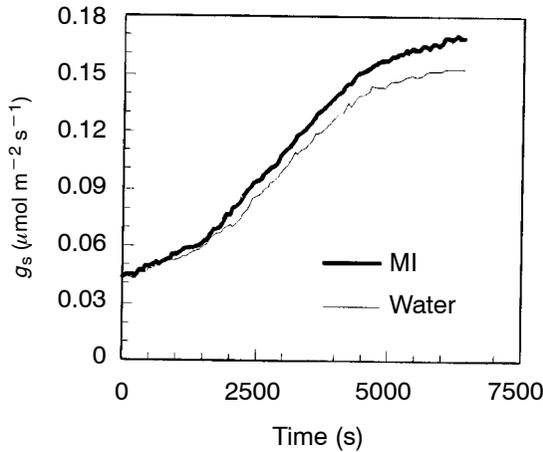


TABLE 1. Parameters of the sigmoid curve of leaf stomatal response to illumination in the dark for intact leaves of sweet corn plants treated with a microbial inoculant (EM) compared with water-treated and untreated control plants.

Treatment	g_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_{cc} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	α (10^{-3}s^{-1})	β (10^{-5}s^{-1})	τ (s)
EM-absorbed	0.118 a	0.0398 a	-1.10 a	4.01 a	2795 a
Water-absorbed	0.110 a	0.0401 a	-1.03 a	2.10 b	2985 b
Control (untreated)	0.113 a	0.0396 a	-1.05 a	2.19 b	2953 b

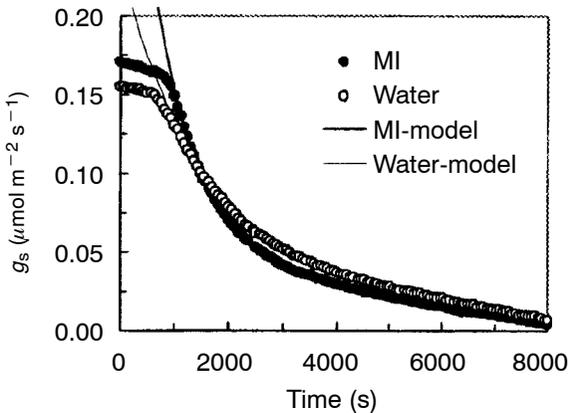
g_s , stomatal conductance; g_{\max} , the maximum g_s ; g_{cc} , cuticular or residual conductance in dark, α , stomatal response constant; β , residual conductance response constant; τ , time at which stomatal conductance reaches half of the maximum.

The value of g_{\max} was higher for EM treatment than for water-treated or untreated plants because EM increased the stomatal opening. The variable g_{cc} shows the cuticular or residual conductance in the dark. There was no significant difference between treatments in this parameter as related to cuticular characteristics. The coefficient α shows the slope of the fast opening phase of the stomatal response curve, and β shows the initial slope of the conductance response curve. The value of β was higher in the EM-treated plants than in water-treated or untreated control plants. This means that the EM treatment increased stomatal opening at the beginning of illumination. Moreover, there was also a difference in α between treatments. The value of α showed the stomatal response property and was larger for the EM-treated plants than for the control plants. The coefficient τ shows the time at which stomatal conductance reaches half of the maximum. The value of τ was higher in the EM-treated plants than in the water-treated or untreated control plants. This suggests that stomatal conductance was maximized earlier in EM-treated plants than in the water-treated or untreated control plants.

Decline of Stomatal Conductance in the Excised Leaf Blade

When the leaf was excised from the plant, stomata started to close soon in response to leaf dehydration. In this case, stomata in the EM-treated leaves remained open longer than those in the control plants (Figure 4). However, when stomatal closure reached a fast phase, stomata in the EM-treated leaves closed faster than those in leaves of control plant.

FIGURE 4. Comparison of the modeling curves for the decline of stomatal conductance (g_s) in excised leaf blades of sweet corn plants treated with EM liquid and water.



The parameters analyzed from the model are presented in Table 2. The variable g'_{\max} shows the initial total g_s before the leaf is excised. As mentioned earlier, EM treatment increased stomatal opening and consequently g'_{\max} was higher in EM-treated plants than in water treated or untreated plants. The variable g_{res} shows the cuticular or residual conductance when stomata are roughly closed. There was no statistically significant difference in g_{res} found between these treatments.

The coefficient α' shows the stomatal response constant. The value of α' was higher for the EM-treated plants than in water-treated or untreated control plants. The variable g_{res} shows the cuticular or residual conductance. This indicated that the stomata in leaves of the EM-treated plants closed more rapidly in response to water loss from the excised leaves compared with the controls. There was no difference in the residual conductance response constant (β') between treatments. Obviously, the short-term treatment with a microbial inoculant would not change the morphological structure of the leaf cuticle. The coefficient τ' indicates the time required for rapid stomatal closure to begin. The value of τ' was higher for the EM-treated plants than for water-treated or untreated control plants. This suggests that the leaf stomata in the EM-treated plants could remain open longer under leaf water-deficit conditions than those of the control plants.

Throughout these experiments, there was no significant effect of EM on cuticular conductance. Overall, the results indicate that EM contains substances that can affect stomatal response. Although we do not have direct evidence to support this conclusion, the various species that comprise EM used in the present study have been studied extensively for decades. A number of them are known to synthesize phytohormones, growth regulators and other biologically-active substances (Arshad and Frankenberger, 1992). Barea et al. (1976) found that among 50 bacteria isolated from the rhizosphere

TABLE 2. Parameters of the stomatal closure curve for excised leaves of sweet corn plants treated with a microbial inoculant (EM) compared with water-treated and untreated control plants.

Treatment	g'_{\max} (mmol m ⁻² s ⁻¹)	g_{res}	α' (10 ⁻³ s ⁻¹)	β' (10 ⁻⁵ s ⁻¹)	τ' (s)
EM-absorbed	0.169 a	0.0505 a	-1.24 a	7.4 a	873 a
Water-absorbed	0.158 b	0.0575 a	-1.13 b	7.9 a	546 b
Control (Untreated)	0.153 b	0.0593 a	-1.16 b	7.1 a	596 b

g'_{\max} , the initial total g_s before the leaf is excised; g_{res} , cuticular or residual conductance when stomata are roughly closed. α' , stomatal response constant; β' , residual conductance response constant; τ' , time at which stomatal conductance get into sharp closing course.

of various plants, 86, 58, and 90% produce auxins, gibberellins, and kinetin-like substances, respectively. Kampert et al. (1975) reported that 55% of bacteria and 86% of fungi isolated from the rhizosphere of *Pinus silvestris* could produce gibberellins and their derivatives. *Actinomyces* and *Streptomyces* produce auxins and similar substances (Purushothaman et al., 1974; Mahmoud et al., 1984), gibberellins (Arshad and Frankenberger, 1992), and cytokinins (Bermudez de Castro et al., 1977; Henson and Wheeler, 1977). Some fungi like *Aspergillus niger* also produce gibberellins (El-Bahrawy, 1983). The promotion of stomatal responses by intact and excised leaves of EM-treated plants is likely due to the effects of plant growth regulators existing in the liquid phase of the cultures. Further studies are needed to determine the exact mechanisms and modes-of-action whereby these bioactive compounds can affect leaf stomatal responses, growth and metabolism of plants.

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