Ann Sci For (1997) 54, 463-472

Original article

Use of stem diameter variations for detecting the effects of pathogens on plant water status

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(Received 14 June 1996; accepted 18 September 1996)

Summary – Linear variable displacement transducer (LVDT) sensors were used on *Cistus* plants, non-inoculated or inoculated with the pathogens *Botryosphaeria stevensii* and *Hypoxylon mediterraneum*, to determine the suitability of this method to detect the effects of the infection process. Control and *H mediterraneum* infected plants did not differ in either the maximum daily shrinkage or the daily evolution of stem diameter. However, the pathogenic effects of *B stevensii* were clearly detected. Daily increase in stem growth stopped 19 days after the plants were inoculated and before any noticeable external symptom. Maximum daily shrinkage occurred 5 weeks after inoculation when foliar chlorosis and stem cankers were visible and preceded the plant mortality. Stem contractions were related to the amount of daily solar radiation when stress caused by pathogens did not exist. This methodology can be useful in the study of disease development, especially for those pathogens that affect the plant–water relations.

***Botryosphaeria stevensii* / *Hypoxylon mediterraneum* / *Cistus* / pathogenic effect / stem variation / LVDT sensor**

Résumé – Utilisation des variations de diamètre de tige pour la détection d'effets de pathogènes sur l'état hydrique de la plante. Le suivi des variations micromorphométriques du diamètre de tige à l'aide de capteurs de déplacements linéaire (LVDT) a été réalisé sur des plantes de *Cistus* sains et inoculés par deux agents pathogènes différents, *Botryosphaeria stevensii* et *Hypoxylon mediterraneum*, dans le but de déterminer les effets éventuellement liés à l'infection pathogénique. Les amplitudes de contractions maximales et la croissance journalière du diamètre de tige des plantes témoins et inoculés par *H mediterraneum* ont été similaires. L'effet du pathogène *B stevensii* a, en revanche, clairement été détecté. Une absence de croissance a été enregistrée 19 j après l'inoculation

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Abbreviations: DE: daily evolution; LVDT: linear variable displacement transducer; MDS: maximum daily shrinkage.

des plantes avant toutes observations de symptômes externes. Les amplitudes maximales des contractions journalières ont augmenté 5 semaines après l'inoculation, accompagnées d'une chlorose foliaire et du développement d'un chancre au niveau de la tige précédant la mort des plantes. Une relation linéaire entre les amplitudes de contractions journalières de tige et le rayonnement global a été trouvée uniquement pour les plantes non affectées. Il apparaît ainsi que cette méthode peut être utilisée pour étudier l'évolution de l'infection pathogénique, en particulier dans le cas où le fonctionnement hydrique de la plante est affecté.

***Botryosphaeria stevensii* / *Hypoxylon mediterraneum* / *Cistus* / effet pathogène / micromorphométrie / capteur LVDT**

INTRODUCTION

Among all the proposed methods for measuring plant water status, such as leaf water potential, relative water content or stem tracers, the micromorphometric method based on stem shrinkage seems to be the most efficient one (Simonneau et al, 1993).

Daily variations in stem and other growth parameters have been observed in plants for many years. A plant loses water through the transpiration process with an increase in solar radiation. As a result, an internal water deficit occurs, which induces a stem contraction. In the afternoon, the plant begins to rehydrate and absorption exceeds transpiration, causing an increase in stem diameter. It has been proved that plant stem contraction depends on soil water content and atmospheric demand (Huguet et al, 1992). The variation in plant stem width due to cyclical shrinkage and swelling processes is a very sensitive indicator of the plant response to water availability or shortage (Ameglio and Cruiziat, 1992), and of the plant water status, both in trees (Garnier and Berger, 1986; Cohen et al, 1992; Huguet et al, 1992) and in herbaceous plants (Schoch et al, 1989; Brun and Tournier, 1992; Cohen et al, 1996).

On the other hand, preliminary results (Luque, unpublished) demonstrate the susceptibility of *Cistus salviifolius* L, a frequent understorey species of cork oak (*Quercus suber* L) forests in Spain, to the fungi *Botryosphaeria stevensii* Shoem and *Hypoxylon mediterraneum* (de Not) Ces and de Note. *Botryosphaeria stevensii* has been reported to be pathogenic to *Q suber* (Luque and Girbal, 1989) and to species of the following genera: *Acacia, Fraxinus, Juniperus, Lycopersicon, Malus, Pinus, Piper, Populus, Prunus, Pyrus, Quercus, Spilanthes, Solanum, Syringa, Thuja, Ulmus* and *Vitis* (Shoemaker, 1964; Sutton, 1980; Vajna, 1986; Tisserat et al, 1988). The fungus can induce dieback and trunk canker formation depending on the host species and the infection site. *Hypoxylon mediterraneum* is a well known pathogen of *Q suber* (Malençon and Marion, 1952; Natividade, 1990) and other species of *Quercus*: *Q cerris* L (Capretti and Mugnai, 1987), *Q ilex* L (Torres Juan, 1975), *Q pubescens* Willd and *Q trojana* Webb (Luisi et al, 1993). It has been reported that water-stressed cork oak seedlings infected with *H mediterraneum* develop more acute symptoms than non-stressed plants (Jacobs et al, 1993).

The main objective of this study was to evaluate whether linear variable displacement transducer (LVDT) sensors, which continuously record stem diameter variations, can detect the effect of the presence of pathogens in plants. Two pathogens, *B stevensii* and *H mediterraneum*, which differ in symptom expression and mode of action, were used. The experimental plants used were *C salviifolius* owing to the evident visual symptoms developed when inoculated with either one of the two pathogens.

MATERIALS AND METHODS

Plant material

One month before the setting up of the experiment (April 1995), eight plants of *C salviifolius* ranging 50–80 cm in height and 8–11 mm in diameter, measured at 30 cm height, were transplanted from the field into 5 L containers filled with a mixture of sterilized peat/vermiculite (1:1; v/v). The plants were maintained in a glasshouse and watered as needed. The substrate was maintained humid throughout the experimental period.

Fungal strains

Two fungal strains isolated from *Q suber* were used: *B stevensii* (isol 24 January 1992; Vallgorguina, Barcelona, Spain; UTM 31TDG6008) and *H mediterraneum* (isol 24 September 1992; La Bisbal, Girona, Spain; UTM 31TEG0640). Strains were maintained in potato dextrose agar (PDA) plugs in tubes with sterile distilled water at 4 °C until use. Four days before the inoculations the strains were recovered by placing a small piece of the mycelium in a PDA petri dish and incubating it at 25 °C.

Inoculations

The plants were inoculated on 16 May 1995. A superficial wound of 1 cm long was made on the stem of every plant with a sterile blade at 15 cm above the ground level. A mycelial plug (5 mm in diameter) from an actively growing colony was placed on the wound with the mycelium touching the stem tissues. The wounds were sealed with Parafilm® (American National Can, Greenwich, CT, USA). Two groups of three plants were inoculated with each fungus. Controls consisted of two additional plants treated in a similar way but with plugs of PDA only. At the end of the experiment isolations were made from all the plants to confirm the presence of the pathogens in the inoculated plants. Reisolations were made from the point of inoculation and 3 cm above it. Wood pieces were disinfected with 70° ethanol, plated in PDA and incubated at 25 °C for fungal identification.

Monitoring

The microvariations of stem diameter were measured with a set of LVDT (model DF ± 2.5 mm, accuracy ± 10 µm, from Solartron [Solartron Metrology, Bogno Regis, UK]), attached to the plant stem about 15 cm above the inoculation points, and with a special holder made of Invar and aluminium material. The LVDT outputs were recorded into a datalogger (model CR10 with AM416 multiplexer from Campbell [Campbell Scientific Ltd, Logan, UT, USA]) every 30 s and averaged every 30 min. The system was powered by a standard car battery (12 V) and an automatic charger. The assay was performed from 16 May to 18 July. The stored data were downloaded to a personal computer twice a week. Any visually appreciable changes in the plants were annotated. Main meteorological data during the monitoring period were recorded from an automatic weather station, located 200 m from the glasshouse.

Data analyses

The raw data were corrected to the same initial 0 (zero) value. Three parameters were studied: i) the total increase in the stem diameter, ii) the maximum daily shrinkage (MDS) of stem diameter (difference between the maximum and minimum of each daily curve) and iii) the daily evolution (DE), increasing or decreasing of the stem diameter (difference between the maximum values of 2 consecutive days). Data were analyzed using the SYSTAT statistical package (SYSTAT, 1992). If necessary, data were transformed to obtain a normal distribution with homogeneous variances. After that, analysis of variance (ANOVA) was performed, and means compared by either Tukey (within treatments) or Dunnett (between treatments and control) tests. Additionally, correlation and regression coefficients were calculated between the variables and the global solar radiation recorded during the experiment.

RESULTS

At the end of the experiment, the pathogens were successfully reisolated from the inoculated plants. No fungus was isolated from the control plants. Plants inoculated with *H mediterraneum* looked and behaved similarly to non-inoculated plants in growth, production of leaves and flowering capacity throughout the experiment. However, plants

inoculated with *B stevensii* began to develop stem cankers in the third week after inoculation (31 May–6 June). An early symptom of canker formation was the darkening of the bark. In the fourth week (6–13 June) chlorosis of the leaves appeared, and the plants began to decline after the sixth and seventh weeks (20 June–4 July). The cankers were characterized by darkening and depression of the stem, owing to the necrosis of the bark and vascular tissues. They averaged 10 cm in length at the end of the experiment.

The stem diameter evolution of plants for the different treatments during the experiment is shown in figure 1. Mean overall growth was 1.57 mm for control plants, 1.44 mm for *H mediterraneum* inoculated plants and –0.32 mm for those inoculated with *B stevensii*.

The growth rate of the stem diameter of the non-inoculated and *H mediterraneum* inoculated plants was approximately 25 µm per day. The difference between two maximum values of stem diameter, within a 24 h cycle, can be divided into three chronological phases (Cohen, 1992): 1) a rapid decrease in the stem diameter, starting soon after sunset (the stem reservoir loses water during the rapid increase of transpiration); 2) a rapid increase in the stem diameter corresponding to rehydration, starting soon after the afternoon decrease in transpiration; and 3) a second slight increase in stem diameter, which reaches a maximum value at around 3 to 6 am. This maximum is higher than the previous maximum value, 1 day before. This is interpreted as a growth phase. There is no clear limit between phases 2 and 3. Both groups of plants grew but with decreasing growth rates throughout the experiment.

The plants inoculated with *B stevensii* underwent four chronologically distinct growth phases: increase during the first 18 days, then cessation, and a sharp decrease

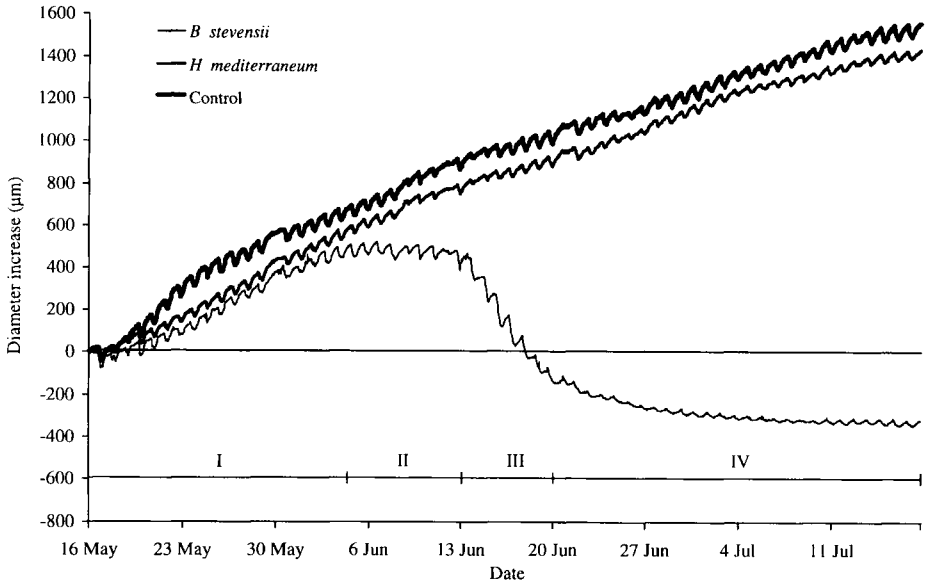

Fig 1. Mean stem diameter evolution of *Cistus salviifolius* plants inoculated with *Botryosphaeria stevensii* and *Hypoxylon mediterraneum*. The four analysis periods are indicated above the X axis.

Table I. Time subdivisions of the experiment determined by the evolution of the plants inoculated with *Botryosphaeria stevensii*.

Period	*Date*	*No of days*	*Plant response*
I	16 May–3 June	18	Growing period in all plants
II	4 June–12 June	9	End of growth; chlorotic leaves; canker formation
III	13 June–20 June	8	Decreasing stem diameters
IV	21 June–18 July	27	Death of plants

followed by a progressive decline (fig 1, table I).

Mean MDS values for control and *H mediterraneum* inoculated plants ranged from 0 to 65 µm. Minimum values were recorded on cloudy and rainy days (30 May, 12 June and 21 June) when transpiration rates were low (fig 2). The plants inoculated with *B stevensii* exhibited the greatest MDS just before their death, in the fifth week, and thereafter the MDS values were lower than those of any other treatment (fig 2).

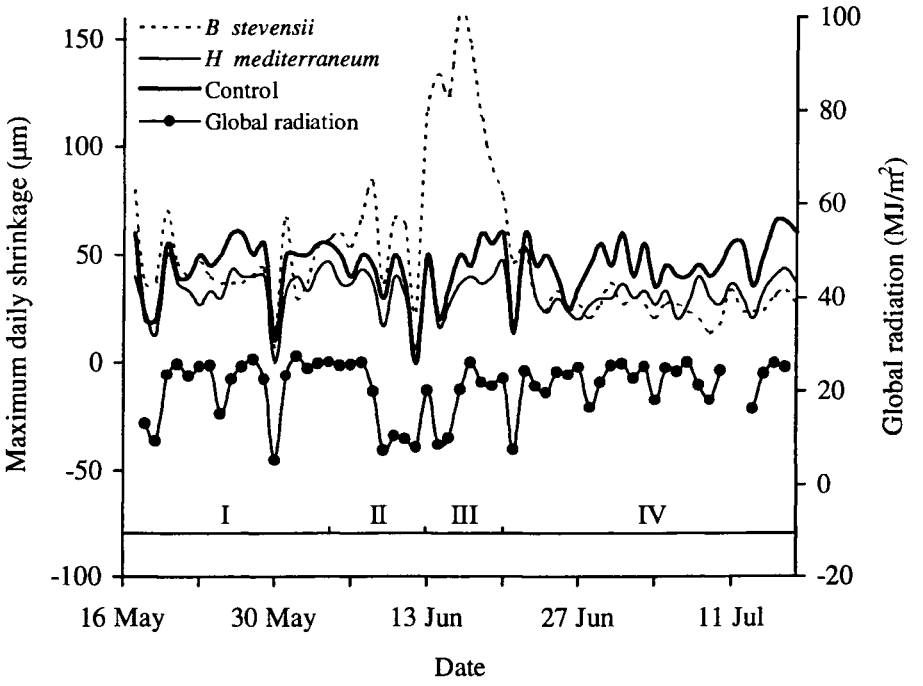

Fig 2. Mean maximum diameter shrinkage measured daily for *Cistus salviifolius* plants inoculated with *Botryosphaeria stevensii* and *Hypoxylon mediterraneum*. The four analysis periods are indicated above the X-axis.

To statistically analyze the MDS and DE data, four arbitrary periods were defined, characterized by the differential trend of the *B stevensii* group (table I). Prior to statistical analysis, MDS data were log-transformed to obtain normal distributions with homogeneous variances. No transformation was needed for the DE data. ANOVA tests of both variables showed a significant ($P < 0.05$) interaction between the inoculation treatment and the period of the assay. Because of the interaction, two additional one-way ANOVA tests were made for each variable, selecting either the inoculation treatment or the date interval as factors.

The results from the MDS data analyses are given in table II. The greatest MDS average value for all periods was detected in the *B stevensii* group (62 μm), but with high variability among the different periods. MDS means for control and *H mediterraneum* inoculated plants were 44.5 μm and 33.2 μm, respectively. The mean MDS of the plants inoculated with *B stevensii* were significantly different from the control in the last two periods, when plants gradually reduced their diameter and died (especially in the period IV). In period II, although the differences were not significant, MDS was greater in infected plants than in the control plants. In the plants inoculated with *H mediterraneum* MDS values were always smaller than the control ones but only in the last period were they significantly different.

For the control plants the means of the MDS values were similar and did not differ significantly. Mean values ranged from 39.4–46.9 μm. Likewise, the MDS values of the *H mediterraneum* inoculated plants were not significantly different. They ranged from 30.5–36.3 μm. The plants inoculated with *B stevensii* exhibited non-significantly different MDS values between the first two chronological periods (table II). A progressive increase in the mean MDS was observed, reaching the maximum in period III (120.4 μm), when the plants began to die. In the last period, MDS values were consequently the smallest (fig 2).

The results from the DE data analyses are given in table III. Control and *H mediterraneum* inoculated plants behaved similarly; there was a progressive decrease during the experimental period. For the control plants the means varied from 37.4 (period I) to 18.6 μm per day (period IV). DE values for the *H mediterraneum* inoculated plants were 30.2 and 18.1 μm per day in the same time period. MDS and DE values for *H mediterraneum* inoculated plants were always smaller than for the control plants but not significantly different. The *B stevensii* DE data differed significantly from the controls from period II and thereafter. During the first time interval, the growth rate was lower

Table II. Maximum daily shrinkage (μm) means of *Cistus salviifolius* plants inoculated with *Botryosphaeria stevensii* and *Hypoxylon mediterraneum*.

Treatment	*Period*			
	I	*II*	*III*	*IV*
Control	$45.6^{a\,A}$	$39.4^{a\,A}$	$46.9^{a\,A}$	$46.1^{a\,A}$
B stevensii	$43.5^{a\,B}$	$56.7^{a\,B}$	$120.4^{b\,A}$	$27.5^{b\,C}$
H mediterraneum	$33.3^{a\,A}$	$32.6^{a\,A}$	$36.3^{a\,A}$	$30.5^{b\,A}$

Values with different lowercase letters within the same column indicate significant differences from the control mean according to the Dunnett test ($P < 0.05$). Values with different capital letters within the same row indicate significant differences among means according to the Tukey test ($P < 0.05$). Original data were log-transformed.

Table III. Daily evolution (μm/day) means of *Cistus salviifolius* plants inoculated with *Botryosphaeria stevensii* and *Hypoxylon mediterraneum*.

Treatment	*Period*			
	I	*II*	*III*	*IV*
Control	37.4 $^{a\,A}$	26.1 $^{a\,AB}$	19.4 $^{a\,B}$	18.6 $^{a\,B}$
B stevensii	28.2 $^{a\,A}$	1.1 $^{b\,B}$	−71.7 $^{b\,C}$	−8.1 $^{b\,B}$
H mediterraneum	30.2 $^{a\,A}$	25.9 $^{a\,AB}$	19.2 $^{a\,B}$	18.1 $^{a\,B}$

Values with different lowercase letters within the same column indicate significant differences from the control mean according to the Dunnett test ($P < 0.05$). Values with different capital letters within the same row indicate significant differences among means according to the Tukey test ($P < 0.05$).

than for the control plants, 28.2 μm per day, but not significantly different. After that, the growth stopped (1.1 μm per day in the second period) and finally, negative values were observed, thus indicating a decrease in stem diameter, which corresponds to the extension of the cankers and finally the death of the plants.

The relations between the global incident radiation and both MDS and DE parameters are given in figure 3. Global radiation data correlated positively with MDS for the control and *H mediterraneum* treatments ($r = 0.702$ and 0.655, respectively). On the contrary, the correlation coefficient for the *B stevensii* group was negative ($r = -0.082$), owing to the great variability observed in MDS data throughout the experiment. In the same way, only regressions corresponding to MDS for controls and plants infected with *H mediterraneum* were significant ($P < 0.05$), indicating a possible relation between the amount of daily solar radiation and the stem contractions. Daily evolution was poorly correlated with solar radiation in all cases and no significant regressions were obtained.

DISCUSSION

The progressive reduction of plant growth rate in both control and *H mediterraneum* infected *Cistus* (fig 1, table III) observed during the experiment could be explained by the growth pattern of Mediterranean-climate plants as summer approaches (Mooney, 1983).

As expected, since the experimental conditions included ready availability of water to the plants, the results indicated subtle pathogenic effects due to *H mediterraneum*. No differences were detected in DE between the control and *H mediterraneum* inoculated plants, although the control plants had slightly higher values at the beginning of the experiment. This represented a delay in the growth of infected plants, but similar trends were evident between both treatments. There was also little difference in MDS values between control and *H mediterraneum* inoculated plants; only the values of the last period were significantly different.

However, the pathogenic effect of *B stevensii* was clearly reflected by the LVDT sensors. In all cases the fungus produced stem cankers, characterized by the necrosis of the bark and the underlying tissues. The damage of the xylem vessels precluded a regular water flux, thus inducing a plant response similar to drought stress. *Cistus* plants reacted similarly to other woody species such as apricots, cherries, citrus, peaches, plums and walnuts (Huguet, 1985; Garnier and Berger, 1986; Li et al, 1989a; Huguet et al, 1992; Cohen, 1994), and some

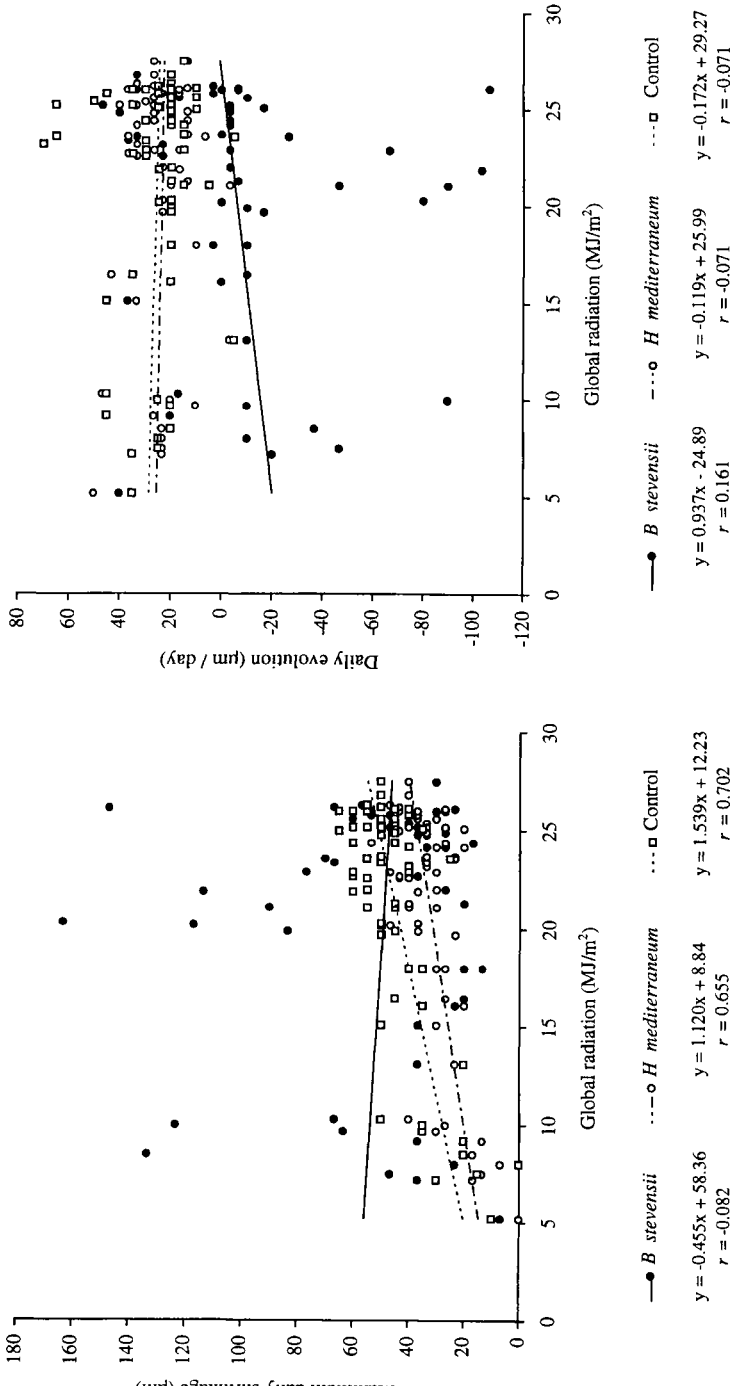

Fig 3. Relation between maximum daily shrinkage and daily evolution parameters with the global incident radiation during the assay.

herbaceous plants such as corn, eggplant, pepper and tomato (Schoch et al, 1989, 1990; Cohen et al, 1996). This reaction to water stress is characterized by a gradual release of water from internal reserves as water stress increases, which is later reflected in greater MDS values (Huguet et al, 1992). *B stevensii* affected both parameters, MDS and DE. The first indication of pathogenic stress was the cessation and decrease of DE, as it was reported in many other studies in plants submitted to water stress (Huguet, 1985; Li et al, 1989b; Schoch et al, 1989; Katerji et al, 1990). As the infection became more severe, DE was reduced and MDS increased considerably, indicating a strong internal dehydration.

The visual symptoms of *B stevensii* infection, characterized by stem canker formation, were detected in the third week after inoculation. The yellowing, drying and later falling of the leaves, were observed in the fourth week. Despite non-significant differences in the first period, changes in MDS and DE were early detected by the LVDT sensors.

Stem contractions seem to be related to the amount of daily solar radiation rather than to the stem daily evolution when stresses caused by pathogens do not exist. Simonneau et al (1993) demonstrated that changes in stem diameter are closely related to changes in the transpirational demand, depending on the solar radiation among other factors. This shows the high sensitivity of LVDT sensors in detecting the real water status of the plant.

The micrometric measurement of shrinkage and swelling of stem diameter seems to be a sensitive indicator of plant response to environmental conditions and could be used as a reliable method for logging changes in plant water status. The use of this methodology permits the detection of stresses caused by the presence of pathogens that affect plant physiology, especially when related to hydric aspects. This technique is very sensitive and could be useful when studying disease development or applied to detect infections when external symptoms are still undetectable.

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