ROOT AND BASAL STEM ROT DISEASE OF SUGARCANE IN LAMPUNG, INDONESIA

By

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Abstract

THE ROOT and basal stem rot disease is new to Indonesia. It was detected in 1993 on a commercial variety grown in Gunung Madu sugarcane plantation, Lampung. Infected plants have been more frequently found thereafter on newer varieties and the disease is becoming more important to the plantation. Based on field observations, the infection in contaminated fields may reach 11% stalk infection. Symptoms appear on mature plant crops (9 month and older), and on younger plants in ratoon crops (5 month and older), as yellowing or drying of cane leaves. Under very severe infection, the symptoms could be visible much earlier and may be followed by patches of dying stools across the field. Because of the rotten roots and basal stem, the infected plant is easily pulled out of the ground. The key indicator of the infection is the emergence of multiple branched stroma that protrude from the ground around the cane stool, and from the dead stubbles. In general, the disease characteristics are very similar to the root and basal stem rot disease reported from Taiwan. The CABI Identification Services, England, upon examinations on the anamorphic and teleomorphic states of the causal pathogen, has almost certainly confirmed that the causal pathogen in Indonesia and Taiwan is similar, namely *Xylaria* cf. *warburgii*. Yield loss assessment conducted on artificially inoculated plant crops in the field revealed that at 25–26% infection severity the particular disease could potentially reduce the cane and sugar yield by 12.3% and 15.4%, respectively.

Introduction

The root and basal stem rot, a relatively new and rare disease of sugarcane, was first reported from Taiwan. Fang *et al.* (1986) reported that the disease was caused by *Xylaria* cf. *warburgii*. A disease with similar characteristics was detected in 1993 in Indonesia, on a commercial variety grown in PT Gunung Madu Plantations (GMP), a sugar plantation in Lampung province (approximately 4°42'S/105°12'E).

The characteristics of the disease are similar to that reported from Taiwan. Quite often disease occurrence is recognised very late, where patches of drying and dying stools across the field have become very pronounced.

Infected plants have been more frequently found on newer varieties thereafter, and the disease is becoming more important in GMP. Based on field observations in 2002–2007, the infection in contaminated fields may reach 11% stalk infection (unpublished data). Fang *et al.* (1994) in Taiwan estimated that *X. warburgii* caused a yield loss of 5% in plant crops and 30% or higher in ratoons.

Despite the similarity, it was not clear whether the causal pathogen is the same as in Taiwan. The present paper reports the findings of studies related to the disease in GMP. The studies have been conducted since 2002, aiming to characterise disease infection in the field; to understand fungus morphology for necessary identification and pathogen confirmation; and to assess yield loss

caused by the disease. The outputs are expected to complement current knowledge on this rare disease of sugarcane.

Material and methods

Characterisation of disease infection in the field

This is to characterise typical appearance of disease infection in the field, to help plantation staff to be alerted to any symptoms of disease. Spots with premature yellowing or drying leaves in mature plant crops were inspected. The stalks were pulled out and split longitudinally. Typical appearance of both above- and underground parts was then characterised upon confirmation of disease infection. Similar approaches but on younger canes were done in ratoons.

Morphologicaldescription

Materials from infected plants, including protruding stromata associated with the infection in the fields, were collected and brought to the laboratory for detailed morphological examination under the microscope. The morphological description was used for pathogen identification.

Confirmation of pathogen

The basal stems of the infected plants were thoroughly cleaned, split up, and chopped to take a small piece (ca. 1 cm^3) of stem tissues covering the transitional zone of the advancing pathogen and healthy tissue in front of it. This material was rinsed and dipped in an alcohol solution for 5 min. and the rind was removed aseptically. The inner portion was finely sliced and transferred to a PDA medium containing terramycine 0.02%. The developing culture was purified and the pure isolates were further propagated on the same media to be used on second step propagation on coarse media.

Mango twigs media were used for second propagation, following Fang *et al.* (1986) and Fang *et al.* (1994). In the present study, the same isolates were also propagated on fine slices of sugarcane tissues. The mango twigs and sugarcane slices were dipped separately in water for 2 hrs and transferred into 500 mL flasks. About 300 g of each medium was put in a flask. The flasks and these coarse media were autoclaved for 1 h (121^{o}C, 15 psi), and this step was repeated one more time the next day. One tube of pure isolates as already prepared (of 1 month age) was used to inoculate each flask of coarse medium, followed by incubation for 2 months under 26–28^{o}C. The colonies produced were used to inoculate the plants in subsequent pot/poly-bag experiment.

The use of colonies on coarse media, rather than on standard agar media, was preferred for an inoculation under soil environment in a pot experiment. A previous experiment suggested that standard inoculum failed to enhance infection of fungus on target plants under such environment, likely because the inoculated fungus could not persist for a longer time due to limited nutrient reserves in the standard agar medium.

The fungi colony would find the mango twigs and sugarcane slices as better nutrient resources in the soil before any infection on target tissues could take place.

Sugarcane and maize were used as test-plants. They were grown on poly-bags filled with 10 kg pre-sterilised soils. Maize was selected for having a shorter life cycle which should facilitate the trial, while it was also reported as susceptible and responsive to root and basal stem rot disease infection in Taiwan (Fang *et al.* 1994).

Each poly-bag with two plants of either sugarcane or maize was inoculated by burying (5–10 cm deep) the previously prepared colonies (inoculated mango twigs and sugarcane tissues). Sterilised, non-inoculated mango twigs and sugarcane materials were buried instead in control treatments. Each treatment was replicated 5 times. Regular watering was done to maintain plant growth but no fertiliser was applied.

Any wilting, yellowing, or drying plant was pulled out of the poly-bag. The basal stem was split and observed for any disease symptom. The diseased tissues were again isolated to confirm the causal pathogen.

Yield loss assessment

A field experiment was carried out in plant cane in 2002–2003. Plot size was 9 rows x 10 m. Two local varieties (GM19 and PSGM88-5052) with three inoculum dosages (0/control, 250 g, and 500 g for each metre of row) were used. A complete randomised block design with 3 replications was applied.

Inoculum was prepared by shredding the roots and basal stems of diseased plants. The materials were air dried for 2–3 weeks under ambient temperature. At planting, these inoculum materials were broadcast on the bottom of planting furrows, and then the cane setts were manually planted and covered.

The observations were done at harvest age. Three rows in each plot were dug out and the number of millable stalks per metre was counted. The length of discoloration (representing infection symptom) and stalk diameter were measured, then the ratio of length of symptom to stalk diameter was calculated. The ratio figures were specifically used in the assessment of severity of infection, to eliminate any bias on disease impacts that may have resulted from variable stalk diameter within the stalk population. Disease infection severity was then calculated by the following formula,

$$I = \frac{\sum (n \,.\, v)}{N \,.\, V} \text{ x } 100\%$$

where
I = disease infection severity
n = number of stalks having a common value of disease symptom (in 3 sample rows)
v = value of symptom (0, 1, 2, 3, or 4), see Table 1 for classification
N = total number of stalks (in 3 sample rows)
V = highest value of symptom (=4)

Table I—Classification of symptom value.

Symptom length : stalk diameter (x)	Symptom value (v)
x = 0 (no symptom)	0
$0 < x \leq 1$	1
$1 < x \leq 2$	2
$2 < x \leq 3$	3
x > 3	4

Ten sample stalks from each plot were weighed and subsequently crushed by using a laboratory mill for juice pol analysis. Differences of stalk weight and pol % juice between controls (without artificial inoculation) and treated plots (with artificial inoculation) would represent yield and sucrose losses.

Results and discussion

Characterisation of disease infection in the fields

In general, the characteristics of disease infection were similar to those described in Taiwan. In plant crops, disease infection is visible on mature plants of 9 months or older. When the rotten section at the basal stem becomes so extensive and reaches ground level, the leaves would start yellowing and drying. At this condition, the plants are easily pulled out of the ground because the roots and the basal stem have already rotted and dried. Upon splitting, the infected tissues looked pale and many lateral lines or spots, dirty white in colour, are clearly visible, representing fungal mycelia.

In ratoon crops, the drying leaves could be found on younger canes, namely on 5–7 month old plants. The symptoms here appear earlier because the infection is actually a continuation of

previous infection (in plant crops), developing from old stubbles to the newly growing tillers (secondary infection). There will be patches of depleted tiller population under very severe infection. These patches are visible as early as on 1.5 to 4 month old canes. The size of the patches varies from very small to 100 m^2 or even wider, wherein invasion of weeds would easily take place.

The key indicator of the infection of root and basal stem rot disease in the field is the occurrence of white tipped black stromata protruding from the dead stubbles or from underground parts around the sugarcane stool. Upon splitting, the diseased tissues smell like rotten wood. A strong sour smell is never associated with this disease.

Under closer observation, there are actually two types of stroma found in the fields. The first one has multiple branches with abundant white conidia easily visible on their tips, which is easily found in the contaminated fields.

The second one has a sausage-like fruiting body, a single structure with no branches. Both stroma have black external appearance but they are white inside. A detailed morphological description was obtained after microscopic examination.

Morphological description and pathogen identification

The multiple-branched stroma is the anamorphic state and may be considered as sterile stroma (Fang *et al.*, 1994). Conidia isolates from GMP consisted of single cells, hyaline, and have an oval form. The conidia size here is bigger compared to those reported from Taiwan. On average they are 5.0–13.0 µm long by 2.5–8.0 µm wide, compared to 4.0–8.0 µm by 1.8–2.1 µm of the Taiwanese (Fang *et al.*, 1994).

The second type with sausage-like fruiting body is the teleomorphic state. It is clear under the microscope that the rough surface of the fruiting body actually consists of perithecia, and reported as perithecial-stroma by Fang *et al.* (1994). The size of the ascospores is also slightly bigger compared to those found in Taiwan. They are 13.8–15.8 µm by 4.6–5.3 µm while, in Taiwan, they are 11–12 µm by 4–5 µm (Fang *et al.*, 1994).

Based on the above morphological description, supported by available references particularly from Taiwan, it is suggested that the pathogen associated with root and basal stem root disease in GMP, Indonesia, is a *Xylaria sp.* Alexopoulos and Mims (1979) stated that the Xylariaceae family are mostly saprophytic or weakly parasitic of fungi on woody plants.

Their stromata are usually epixylous, stipitate, filiform to sausage-shaped, black outside but mostly white internally, and produce their perithecia over the entire fertile portion above the stipe.

Specimens for identification have been sent to CABI (Centre for Agriculture and Bioscience International) Identification Services, England. The Centre's taxonomist at first had some difficulties in making identification when only the anamorphic state was available.

After the teleomorphic state was made available, he reported that the specimens were almost certainly the same species as the sugarcane pathogen referred to in Taiwan as *Xylaria* cf. *warburgii.*

This species was originally described from New Caledonia from rotten fruit of *Sloanea* (Elaeocarpaceae), and is unlikely to be conspecific with the sugarcane fungus. ITS (internal transcribed spacer) sequence analysis confirmed that the fungus was a species of *Xylaria*, but there are no publically accessible ITS sequences of *X.* cf. *warburgii* (Cannon, 2009a, b).

Confirmation of pathogen

Fungus mycelia grew more profusely on mango twigs compared to on sugarcane slices. At the time of inoculation into the poly-bag, the mycelia have nearly covered the entire surface of the mango twigs while, on sugarcane slices, the mycelia did not grow on the sugarcane rind surface.

Disease infection was observed on the 75^{th} day on maize plants, where initial symptoms have been visible at the basal stem and the leaves turned into straw colour. On sugarcane plants, the typical symptoms at ground level developed after 5.5 months. The extent of infection on the two test plants is presented in Table 2.

Table 2—Number of infected plants after inoculation.

Inoculants	Infected plants	
	Maize	Cane
Inoculated mango twigs	6 of 6	10 of 10
Inoculated cane stalk slices	4 of 11	8 of 10
Sterilised mango twigs	0 of11	0 of 10
Sterilised cane stalk slices	0 of 12	0 of 10

It is shown that the infection took place only on plants inoculated with either mango twigs or sugarcane slices which were already colonised by the fungus, and there was no infection on plants 'inoculated' by sterilised materials. The infected maize and sugarcane plants in the poly-bags showed the same symptom as those on naturally infected plants in the fields. Because of restricted environment in the poly-bags, disease symptoms on sugarcane plants were developed earlier, on 5.5 month old plants, compared to 9 month old plants under natural environment in the fields.

The infected maize plants were chopped and some 20 pieces of infected tissues were then isolated in the laboratory. The culture produced the same fungus as originally inoculated. It confirmed that this particular fungus species, having such typical stromata which is easily found with or around the diseased plants in the fields, is the causal pathogen of the root and basal stem rot disease in GMP, Indonesia.

Cane seedpieces (setts) were used in this poly-bag experiment. Upon splitting the infected stalk, it could be suggested that the infection started from the sett roots or from root primordia encircling the nodes. Lines of mycelia were clearly visible on the nodes but not on the internodes. The infection was then expanded from the setts toward basal parts of the newly growing tillers.

Yield loss assessment

The two varieties used did not cause any significant effect on infection severity, stalk population, stalk weight, and pol % juice. There was also no significant effect on all parameters related to interaction of variety x inoculum dosage.

Table 3—Effect of inoculum dosage on plant crop.

Dosage	Severity (%)	Stalk population (/ m row)	Stalk weight (kg)	Pol% juice
0 g/metre	0.3 a	11.6 a	1.38 a	18.43 a
250 g/metre	25.1 b	9.7 a	1.21 b	17.79 b
500 g/metre	26.2 b	9.5 a	1.21 b	17.78 b
LSD.05	*5.34*	*2.35*	*0.10*	*0.60*

Inoculum dosage, however, gave significant effect on infection severity, stalk weight, and pol % juice, but not on stalk population (Table 3). The infection on sugarcane plants through artificial inoculation under a field environment took place effectively. The infection severity was only 0.30% on the controls (without artificial inoculation), significantly lower than on 250 g and 500 g inoculum treatments (25.09% and 26.22% respectively). There was no significant difference between 250 g and 500 g inoculum treatments. (The smaller dosage should be sufficiently effective for use in any future studies involving field inoculation).

The 'healthy' plants (controls, without artificial inoculation) had significantly higher stalk weight compared to the diseased ones of the other two treatments, which suffered from 25.1% and 26.2% infection severity. Compared to the controls, the stalk weight of the other two treatments was 12.3% lower. Similarly, the pol % juice (representing sucrose content) on the diseased plants was

significantly lower (around 3.5% in percentage) compared to the controls. Roughly, with infection severity of 25–26%, the root and basal stem rot disease could potentially reduce the cane and sugar yield on plant crops by 12.3% and 15.4%, respectively.

Conclusions

The root and basal stem rot disease found in GMP sugarcane plantation, Indonesia, is almost certainly caused by *Xylaria* cf. *warburgii*, similar to the causal pathogen of root and basal stem rot disease in Taiwan. However, the size of fungus conidia and ascospores here is somewhat larger compared to the Taiwanese.

The disease typically caused yellowing or drying of leaves on mature plant crops (9 month old) and on younger plants on ratoons (5 month old). Under very severe infection the symptoms could be visible much earlier and there will be patches of dying stools across the field. The most important indicator of disease infection in the field is the emergence of multiple branched stromata off the ground, around the cane stool, and from the dead stubbles.

At 25–26% infection severity on plant crops, the root and basal stem rot disease could potentially reduce cane and sugar yield by 12.3% and 15.4%, respectively.

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POURRITURE BASALE DES RACINES ET DE LA TIGE DE LA CANNE À SUCRE À LAMPUNG EN INDONÉSIE

Par

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Résumé

LA POURRITURE basale des racines et de la tige est une nouvelle maladie en Indonésie. Elle a été observée en 1993 dans une variété commerciale au Gunung Madu Plantations, Lampung. La maladie a été subséquemment notée sur d'autres variétés et elle est devenue de plus en plus répandue. Selon les observations au champ, le niveau d'infection sur tiges pourrait atteindre 11%. Les symptômes apparaissaient dans les plantations matures (9 mois ou plus), et sur des plantes plus jeunes en repousse (5 mois ou plus), comme un jaunissement ou un dessèchement des feuilles. Sous des conditions très sévères, les symptômes pourraient apparaître plus tôt, suivis de poches de

mortalité de souches à travers le champ. De par le fait que les racines sont pourries, la plante infectée est facilement arrachée. L'indicateur clé de l'infection consiste en une apparition de branches multiples de stroma sortant de terre autour de la base de la souche et des souches mortes. En général, les caractéristiques de la maladie sont similaires à la pourriture basale des racines et de la tige comme rapporté au Taiwan. Le CABI Identification Services, en Angleterre, après examen des stades anamorphique et téléomorphique, a presque confirmé que l'agent causal en Indonésie et au Taiwan est similaire, notamment le *Xylaria* cf. *warburgii*. Une évaluation de l'effet de la maladie sur le rendement a été effectuée en inoculant les plantes artificiellement au champ. Il a été démontré qu'une infection de 25–26%, pourrait occasionner une chute potentielle en rendement de canne et en sucre de 12.3% et 15.4%, respectivement.

PUDRICIÓN BASAL DEL TALLO Y RAÍCES DE LA CAÑA DE AZÚCAR EN LAMPUNG, INDONESIA

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PALABRAS CLAVE: ***Xylaria* sp., Estroma, Pérdidas en la Producción**

Resumen

LA PUDRICIÓN basal del tallo y raíz es una enfermedad nueva para Indonesia. La afección se detectó en 1993 en una variedad comercial cultivada en Gunung Madu, Lampung. Desde entonces, se han encontrado plantas afectadas con mayor frecuencia en las nuevas variedades, convirtiéndose la enfermedad en importante para las plantaciones comerciales. Con base en las observaciones de campo, la infección en los campos afectados puede llegar a 11% de tallos enfermos. Los síntomas aparecen en las plantillas con plantas maduras (9 meses de edad o más), y en las socas, en plantas más jóvenes (5 meses o más), como un amarillamiento o secamiento de las hojas. En los casos de mayor severidad, los síntomas pueden ocurrir mucho antes, seguidos por la producción de parches de cepas muertas en el campo. Debido a la pudrición basal de los tallos y raíces, las plantas afectadas se pueden arrancar con facilidad. Un indicador clave de la infección es la aparición de estromas ramificados que aparecen en la tierra, alrededor de las cepas afectadas o brotes muertos. En general, las características de la enfermedad son muy similares a la pudrición basal del tallo y raíz registrada en Taiwán. Los servicios de identificación de CABI, Inglaterra, en los exámenes a los estados anamórfico y teleomórficos del patógeno causal, confirmaron que los agentes causales en Indonesia y Taiwán son similares, es decir, Xylaria *cf.* warburgii. La evaluación de pérdidas en la producción realizados en plantas inoculadas artificialmente en el campo mostraron que con una severidad entre el 25–26%, la enfermedad puede reducir la producción de caña y azúcar en 12.3% y 15.4%, respectivamente.