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Chemical Control of Shoot Blight [*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.] Infecting *Ricinodendron heudelotii* Seedlings in Cameroon

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A b s t r a c t

Shoot blight of *Ricinodendron heudelotii* caused by *Lasiodiplodia theobromae* is a newly identified disease on this species in Cameroon. Seedlings showing typical symptoms of shoot blight were collected from forest and nursery of the applied research farm (ARF) of the Faculty of Agronomy and Agricultural Science, University of Dschang, Cameroon. Four homologated fungicides, Copper oxide (60%) + Metalaxyl (12%), Metalaxyl (8%) + Mancozeb (64%), Maneb (80%) and Copper oxide 86% often used to control cocoa black pod disease were assayed *in vivo* against shoot blight on 35 days old seedlings of *R. heudelotii* by spraying on the leaves at the recommended dose, and the disease severity and incidence were recorded. Formulations based on copper oxide offered the highest protection of *R. heudelotii* seedlings against shoot blight compared to the other fungicides tested ($p \leq 0.05$). Disease severity and leaf disease incidence were 2 and 10% in Yaounde and 18 and 31% in Dschang respectively, in the presence of Copper oxide (60%) + Metalaxyl (12%). This fungicide mixture could be used for the control of shoot blight of *R. heudelotii* seedlings in order to reduce damage in nurseries. Such a control strategy is very significant in preventing complete shoot infection which might hamper the ongoing domestication program for this species in Cameroon.

K e y w o r d s

Disease severity
Fungicide
Lasiodiplodia theobromae
Ricinodendron heudelotii
Shoot blight

Introduction

Ricinodendron heudelotii (Baill.) Pierre ex Heckel is an endemic tree of the Congo basin belonging to the family Euphorbiaceae. Natural regeneration of the species in the forest is difficult because of seed dormancy, incidences of pests and diseases (Plenderleith, 1997; Messi et al., 1998; CIFOR, 2010; Djeugap et al., 2014). People living in the Congo basin forest use *R. heudelotii* for food (seeds) and medicine (roots and bark). Trading some of the seeds provide them additional income (Ngwasiri et al., 2002; Tchatat and Ndoye, 2006). National and international trade of *R. heudelotii* seeds is high. In 2004, the World Centre for Research in Agroforestry (ICRAF) launched a domestication program for this species in Cameroon. Unfortunately, this domestication process is largely unsuccessful owing to the incidence of pests and disease that attack seedlings in the nursery (Djeugap, 2013; Djeugap et al., 2013), thus limiting ICRAF efforts to establish plantations of this species in Cameroon. Seedling shoot blight caused by *Lasiodiplodia theobromae* was first observed on *R. heudelotii* in 2013 in ICRAF nurseries (Djeugap et al., 2013; Djeugap et al., 2015). Unfortunately, information on viable control measures for the disease is scarce to find and highly undocumented. One of the quick methods to control diseases is by using synthetic fungicides. However, regular use of unisite fungicide caused most often environmental and biological problems. Those problems could be avoided by proper selection and use of fungicides. The objective of this work was to determine the efficiency of some fungicide mixtures (often used to control cocoa black pod disease) in controlling shoot blight of *R. heudelotti* seedlings in Cameroon.

Materials and methods

Study area

The study was carried out in two different agro ecological zones of Cameroon, the High Plateau zone in the Application and Research Farm (ARF) of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang and the bimodal rain forest zone in the experimental station of the Institute of Agricultural Research for Development (IARD) in Nkolbisson, Yaounde. The ARF is situated at 5.5° latitude north, 10.05° longitude east and altitude of 1400 m while the IARD station is situated at 3.86^o latitude north, 11.46^o longitude east and an altitude of 756 m.

Production of seedlings and inoculation technique

Scarified seeds were used for seedlings production in polyethylene bags of 20 cm diameter filled with a mixture of soil and sand in a proportion of 3:1 (Djeugap et al., 2014).Top soil was taken directly from each site where the tests took place. Seedlings were then placed in a plywood box with a permanent electric lighting source and each seedling was sprayed with a 50 ml conidial solution obtained from a mature pure culture of *Lasiodiplodia theobromae*. The control lot was not inoculated. Seven days later seedlings with young shoot blight symptoms were transferred to the nurseries where fungicides were applied following a well-documented approach (Djeugap, 2013). To control insects, the insecticide Deltamethrin (Decis ®) was applied twice (1 and 2 months after planting) on the seedlings.

Experimental design and fungicide application

A completely randomized design was used. Each experimental unit (EU) consisted of 10 seedlings and was replicated four times. Four fungicide formulations were tested. The frequency of spraying fungicide was 21days for systemic fungicides (PLANTOMIl 72WP and FONGISTAR 72WP) and 15 days for contact fungicides (NORDOX 75WG and TRIMANEB 80WP) (Table 1). No fungicide was applied on the control lot. The doses and frequency of application were in conformity with the prescriptions of the fungicide homologation committee. The sprays were applied in the absence of wind and a distance of 1.5m was considered between EU. Before spraying, the EU was surrounded with plywood to prevent fungicide drift from one EU to another. Weed control was manual.

Assessment of disease severity and leaf disease incidence

Disease severity and leaf disease incidence were evaluated in percentage according to the formulas described by Campbell and Madden (1990) as follows: Disease severity (%) = (infected area on the plant/total area covered by the plant organs)×100. Leaf disease incidence (%) = (number of infected leaves per plant /total number of leaves of the plant)×100.

Data analysis

Disease severity and leaf disease incidence recorded in percent were transformed by arc-sinus before analysis. Analysis of variance was carried out and the means separated using the Fisher's least significant difference (LSD) test at a probability threshold of 5% (Steel and Torrie, 1980) using SAS software (version 9.3).

Results

All the fungicides used in the present study reduced the severity and incidence of foliar diseases of seedlings in nurseries compared to the control. The disease severity decreased significantly in seedlings treated with fungicides compared to seedlings treated with sterilized distilled water (control).

Table 1. Characteristics of fungicides used in the control of shoot blight of *R. heudelotii* seedlings.

Fungicide formulation	Active ingredient	Mode of action	Chemical family	Dose	Frequency of treatments as recommended
PLANTOMIL 72WP	Copper oxide (60%) and Metalaxyl (12%)	Systemic and contact	Inorganic and Phenylamides	50 g per 15 litres of water	21 days
FONGISTAR 72WP	Metalaxyl (8%) and Mancozeb (64%)	Systemic and contact	Phenylamides and Dithiocarbamates	50 g per 15 litres of water	21 days
NORDOX 75WG	Copper oxide (86%)	Contact	Inorganic	40 g per 15 litres of water	15days
TRIMANEB 80 WP	Maneb (80%)	Contact, multi-site and polyvalent	Carbamates	60 g per 15 litres of water	15 days

Table 2. Disease severity and leaf disease incidence (means*) on 90 days old seedlings of *Ricinodendron heudelotii* after fungicides application in Dschang and Yaounde sites.

Fungicide	Dschang		Yaounde	
	Disease severity	Leaf disease incidence	Disease severity	Leaf disease incidence
PLANTOMIL 72WP (Copper oxide and Metalaxyl)	17.8 ± 8.1a	30.7 ± 12.2a	2.1 ± 1.1 a	10.3 ± 4.1a
FONGISTAR 72WP (Metalaxyl and Mancozeb)	31.2 ± 13.0 b	58.1 ± 15.3 b	10.3 ± 5.2 b	35.1 ± 10.2 b
NORDOX 75WG (Copper oxide)	20.0 ± 7.5 a	31.2 ± 8.1 a	1.7 ± 1.0 a	12.2 ± 5.0 a
TRIMANEB 80WP (Maneb)	38.6 ± 14.7 b	62.2 ± 19.8 b	19.1 ± 6.4 c	41.9 ± 12.1 b
Control (water)	65.0 ± 20.4c	85.4 ± 17.1 c	46.2 ± 11.8 d	59.3 ± 14.9 c

*Means follow by the same letter are not significantly different at the probability threshold of 5% after the Fisher's LSD test. Data in percent were transforming by arc-sinus.

Disease severity and leaf disease incidence were lower in seedlings treated with PLANTOMIL 72WP (2.1 and 10.3 respectively) and NORDOX 75WG (12.2 and 1.7 respectively) and higher in seedlings that received TRIMANEB 80WP (41.9 and 19.1 respectively). The effectiveness of PLANTOMIL 72WP was comparable to NORDOX 75 WG at p=0.05. Disease severity and leaf disease incidence were higher in Dschang than in Yaounde (17 and 30% in Dschang and 2 and 10% in Yaounde on seedlings treated with PLANTOMIL 72WP for example). In the control, disease severity and leaf disease incidence were 65 and 85% in Dschang and 46 and 59% in Yaounde respectively (Table 2). It was observed that, seedlings that received fungicides showed vigorous growth with larger leaves, greater height and collar diameter than the control plants (Table 3 and Fig. 1).

Table 3. Plant height and collar diameter (cm) on 90 days old seedlings of *Ricinodendron heudelotii* after fungicides application.

Fungicide formulations	Plant height (cm)	Collar diameter (cm)
FONGISTAR 72 WP	91.75 ± 2.75 b[z]	2.1 ± 0.11 b
NORDOX 75 WG	100.1 ± 0.82 a	3.07 ± 0.09 a
PLANTOMIL 72 WP	100.25 ± 4.64 a	2.97 ± 0.10 a
TRIMANEB 80 WP	92.3 ± 7.83 b	2.32 ± 0.27 b
Control (water)	61.5 ± 13.96 c	1.82 ± 0.24 b

[z]Means follow by the same letter in the column are not significantly different at the probability threshold of 5% after the Fisher's LSD test.

Fig. 1: Seedlings of *Ricinodendron heudelotii* ready for transplanting 90 days after sowing. A (Control seedlings severely destroyed by shoot blight), B and C (seedlings treated with PLANTOMIL 72WP (Copper oxide and Metalaxyl). Pictures were taken in the experimental site of Yaounde.

Discussion

One of the major roles of plant pathologists is to develop control measure of diseases in order to reduce losses, increase yields and consequently the income of farmers. In the absence of any control measure, losses due to diseases are more than 25% (Lepoivre, 2003). Despite some established constraints related to chemical control like toxicity and pollution, it remains one of the most effective and rapid means of controlling plant diseases .The effectiveness of a fungicide in disease control depends on its mobility in the plant tissues (systemic fungicide), its mode of action, conditions of handling, application period in relation to host phenology and biology of the pathogen and the climatic factors during the application period (Semal, 1989; Rapilly, 1991). All the synthetic fungicides were effective against shoot blight of *R. heudelotii* seedlings. However, this effectiveness varied from one fungicide to another. Indeed, due to the nature of their active ingredients, fungicides act differently on fungi. Copper oxide for example, plays a supporting role in the formation of proteins in the host and contributes directly in maintaining the balance in plant nutrient required for the development of good resilience of fungi (Ragsdale and Sisler, 1991). The fungitoxicity of Mancozeb is attributed to its ability to generate isothianate that inactivates thiol enzymes and metabolites in the pathogen cells. On the other hand, Maneb which is a contact fungicide inhibits the germination of fungal spores and is effective for disease prevention (Ragsdale, 1992). The high efficiency of PLANTOMIL 72WP and

NORDOX 75 WG when compared to TRIMANEB 80WP could be attributed to their mode of action and the nature of the active ingredient which were shown to be effective against many other Ascomycetes fungi (Lepoivre, 2003). The copper ions (Cu^{2+}) present in the copper oxide passively diffuse through cell walls of fungi (and bacteria) and accumulate until their concentration is lethal to cells. Moreover, by acting on the spores, it prevents the development of the mycelium and consequently the spread of the disease. This ion cannot be altered or degraded by heat or light and is resistant to leaching. In solution, the Cu^{2+} ions combine with various chemical groups of cells or membrane proteins inducing denaturation of proteins and enzymes (Ragsdale and Sisler, 1991; Lepoivre, 2003).

The effectiveness of Copper oxide, Mancozeb and Maneb was also established against crop pathogens such as *Helminthosporium oryzae, Pirucularia grisea, Mycosphaerella fijiensis* and *Phytophthora infestans* (Serghat et al., 2004; Vawdrey and Grice, 2005; Djeugap et al., 2011) and against pathogens of wood decay of trees (Rawal, 1998; Khanzada et al., 2005). The differences in disease severity between Dschang and Yaounde could be attributed to climate. Indeed, Dschang locality is characterized by an altitude climate which is cold compared to Yaounde which has an equatorial climate. In fact, the preference of parasitic fungi for temperature depends on the species. However, it is known that high relative humidity conditions around the plants promote spore germination, disease development and fungi sporulation (Campbell and Madden, 1990; Agrios, 2005). During our experiment, the low temperature conditions (15.9 to 20.7°C) and high relative humidity (> 85%) recorded in Dschang possibly favoured the rapid development of the disease in this area compared to Yaounde.

Conclusion

The use of synthetic fungicides in the control of plant diseases should follow homologation requirements to ensure effectively plant protection, the health of consumers and beneficial organisms of the environment. The study confirms that Copper oxide and Metalaxyl fungicides currently used in the control of black pod disease of cocoa by farmers effectively protects *R. heudelotii* seedlings against shoot blight irrespective of the locality. These fungicides should be used by farmers to control shoot blight of *R. heudelotii* seedlings in their cocoa plantations and *R. heudelotii* nurseries.

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