International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2018 IJFAS Journal-2018-7-5/137-144/ 28 Oct, 2018
ISSN 2322-4134 ©2018 IJFAS

Brown spot disease of yam tubers (*Dioscorea* spp.): Diagnosis and control method

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ABSTRACT: Tubers of some varieties of Bètè-bètè and Sao (*Dioscorea alata*) are damaged by circular brown spots called *Internal Brown Spot Disease* or IBSD. IBSD depreciates the market value and organoleptic characteristics of tubers. One or several viruses have been associated with these spots. The etiology of this disease is not known. Given the enormous losses caused by this virus, it is necessary to identify the virus or viruses responsible for this disease in Côte d'Ivoire in order to propose appropriate control methods. To this end, diagnostic surveys, evaluation of epidemiological parameters and molecular analyzes were performed on symptomatic or asymptomatic leaf and tuber samples collected in Toumodi and Bouaké areas. The effectiveness of some control methods has also been determined. The diagnostic survey revealed an incidence of 80 % for IBSD in both production areas with a severity 4 (scale of 1 to 5).There is no close relationship between the presence of foliar symptoms of viruses and that of brown spots in tubers. In addition, for plants with symptoms of viral diseases, the severity of leaf symptoms changes with that of brown spots. Molecular analysis of tubercles with brown spots revealed the presence of bacilliform bands resembling those of *Badnaviruses*. However, the implication of this virus as agent responsible for the disease has not yet been proved. Thermotherapy, carbofuran and callicuivre have reduced the severity of IBSD. Thermotherapy (soaking seeds for 15 min in water at 50 ° C) reduces the severity of symptoms and gives satisfactory results.

***Keywords*:** Badnavirus, control method, Dioscorea alata, etiology, IBSD

INTRODUCTION

The Yam, *Dioscorea* spp., is a food crop consumed by thousands of people living in the tropical and subtropical areas (Coursey, 1967a). Yams provide huge sources of income and important carbohydrates, proteins and vitamins for consumption. In addition, they also play a determining role in socio-cultural activities (Degras, 1993a). Several viruses have been isolated from yam plants or tubers. The most widespread is the yam mosaic virus (YMV), a member of the *Potyvirus* group (Thouvenel and Fauquet 1979; Goudou-Urbino, 1995; Asiedu et al., 1998; Séka et al., 2009b). The symptoms of this disease vary according to the variety of the host plant and can be characterized by discoloration of the leaves or veins, deformation of the leaves, green vein banding, leaf curl and leaf veins as well as plant regrowth (IITA, 1993). Other viruses are associated with yam in the West African sub region. It is the *Dioscorea* marbling virus (DMoV), a possible member of *Comovirus*; *Dioscorea alata virus* (DAV), Potyvirus; Bacilliform Virus of *Dioscorea alata* (DaBV), *Badnavirus*; *Dioscorea dumetorum* virus (DdV), of the *Potyvirus*; the *Dioscorea dumetorum* (DdV), *Potyvirus*; among others (Mantell, 1978). High genetic diversity has been found in yam potyviruses from West Africa (Goudou-Urbino et al., 1996). In addition to YMV, whose production losses vary from 27 to 45 % (Thouvenel and Dumont 1990; Asiedu et al., 1998; Séka et al., 2009a), another virus, that of cucumber mosaic (CMV), a member of *Cucumoviruses*, causing severe leaf chlorosis has also been identified (Fauquet and Thouvenel, 1987). The viruses responsible for this disease are transmitted by

sucking biting insects but also by infected tubers and / or by rubbing infected leaves on healthy leaves (Thouvenel et al., 1989; Thouvenel and Dumont, 1990; Goudou-Urbino, 1995). Internal brown spots are also present in the tubers of some varieties of *Dioscorea alata* in Cote d'Ivoire (Mantell, 1978). Unlike other viruses where the characteristic symptoms are visible on the leaves, those of IBSD are visible only in the tubers (Mantell, 1978). According to Mantell (1978), the presence of circular brown spots of variable size (0.2 - 0.5 mm in diameter) in the tubers of Bete-Bete and Sao varieties of D. alata affects their quality. The virus particles observed have a diameter of 25 nm in diameter (Mantell, 1978). The virus responsible for brown spots in yam tubers are not transmitted by insect vectors. Tools for early detection of the disease are not available. An incidence of IBSD ranging from 70 to 75 %, with severity up to 4 on the scale of 1 to 5 in Mignouna (2001) was recently recorded in the Toumodi area (Diallo, personal communication). In addition, no means of preventive control has been adopted in the peasant environment to limit yield losses caused by IBSD. In collaboration with IITA in Nigeria, trials were set up to propose methods of controlling IBSD. However, due to the socio-political crisis these have been destroyed. Preventive control methods involving the use of healthy seeds is an alternative for the control of brown spots in yam tubers (Mantell 1978, Séka et al., 2009b). The meristem culture and thermotherapy allow to produce healthy seeds (Mantell, 1978; Filloux et al., 2007) but they are very expensive for the producer. The establishment of sustainable control methods requires a good knowledge of the epidemiology and tools for early detection of IBSD in yam tubers. Technologies for the control of IBSD through thermotherapy, carbofuran deposition and callicuivre have been generated to reduce the incidence of IBSD in yam tubers.

MATERIALS AND METHODS

Diagnostic survey and epidemiological study of the disease

A diagnostic survey and prospections enabled to collect information on the incidence and severity of IBSD in these areas. Tubers with or without brown spots were harvested and then cut transversely into five equal parts. The symptoms were described. The incidence and severity of symptoms were recorded using the Mignouna (2001) scale. Each seed is identified and the severity of the symptoms from 1 to 5 is mentioned before planting for regular monitoring of the different symptoms during the vegetative phase and at harvest. Trials were set up in the forest zone (Toumodi) and in the savannah zone (Toumodi and Yamoussoukro) to better understand the epidemiology of the disease.

1=Absence of symptoms; 2=Symptoms on 25 % of the surface; 3=symptoms on 26-50 % of the surface; 4=symptoms on 51-75 % of the surface; 5=presence of the symptoms on more than 75 % of the surface

Figure 1 : Evaluation of the severity of IBSD in the infected yam tuber

Implementation of a method to fight against IBSD

Some tests were set up in the forest zone (Toumodi) and in the savannah zone (Toumodi and Yamoussoukro). Four treatments were performed: T1 (ash); T2 (Carbofuran); T3 (Callicuivre); T4 (Thermotherapy). Fifty grams (50 g) of each of these substances were used separately to control either against nematodes (carbofuran, ash) or against fungi and bacteria (callicuivre, ash) in the holes prior to planting. For thermotherapy, the seeds were soaked for 15 minutes in a water solution at a temperature of 50 ° C.

RESULTS

Diagnostic investigation

The diagnostic survey revealed two IBSD-sensitive varieties in the study areas targeted by the project. These are Bètè-bètè and Sao varieties of *Dioscorea alata* type (Table I). The tubers of these varieties had brown spots characteristic of IBSD. A variety of sizes of brown spots (small-scattered spots and large localized spots) was observed.

Incidence and severity of the disease in both production areas

The average incidence of the disease was 72.5 % in Toumodi with an average severity of 3.5. In the Bouaké area, the average incidence of the disease was 82.5 % with an average severity of 4.5 on the scale of 1 to 5 in Mignouna (2001). Sixty Sao infected tubers of 80 and 192 infected Bètè-bètè tubers out of 240 collected were noted (Table I).

Table 1: Incidence and Severity of brown spots in the various areas of production

Zone	Location	Bètè-bètè tubers collected	Sao Tubers collected	Number infected tubers	Incidence (%)	Severity (1 à 5)
Bouaké	Assouakro	40	0	32	80	5
	Bokassou	40	0	28	70	4
	Minankro	40	0	36	90	5
	Selakro	40	0	36	90	4
Toumodi	Bendressou	20	20	32	80	4
	Ouaouakro	20	20	28	70	4
	Mougnan	20	20	28	70	3
	Zékouakro	20	20	28	70	3

Epidemiology of IBSD

There is a relationship between the severity of foliar symptoms and that of the spots present in the tubers at harvest. On the other hand, plants with apparently healthy leaves produced tubers with brown spots (Figure 2).

Foliar Symptoms	Brown spots in the tuber
	
Mosaic	Large brown spots
	
Vein-banding	Scattered mild spots
	
Mosaic, embossing and leaves covering	Small scattered spots
	
Plant without virus symptoms	Small irregular spots

Figure 2 : Relation between foliar symptoms and the presence of spots in the tuber

Dynamics of evolution of brown spots inside tubers

In yam tubers harvested six (6) months after planting, no brown spots were observed in those from healthy, infected seed (Figure 3). Serological and molecular tests confirmed the absence of virus particles in the tuber at this phenological stage of the plant. The seed tuber of 2.83 severity (with a scale of 1 to 5) grown in the forest zone produced a tuber of 1.79 severity. A significant difference in the severity of brown spots was recorded between

seed tubers and harvested in the forest zone. In the savannah, however, the severity of the tuber was only reduced from 2.11 to 1.83. No difference in severity of brown spots was observed between seed and harvested tubers (Table II).

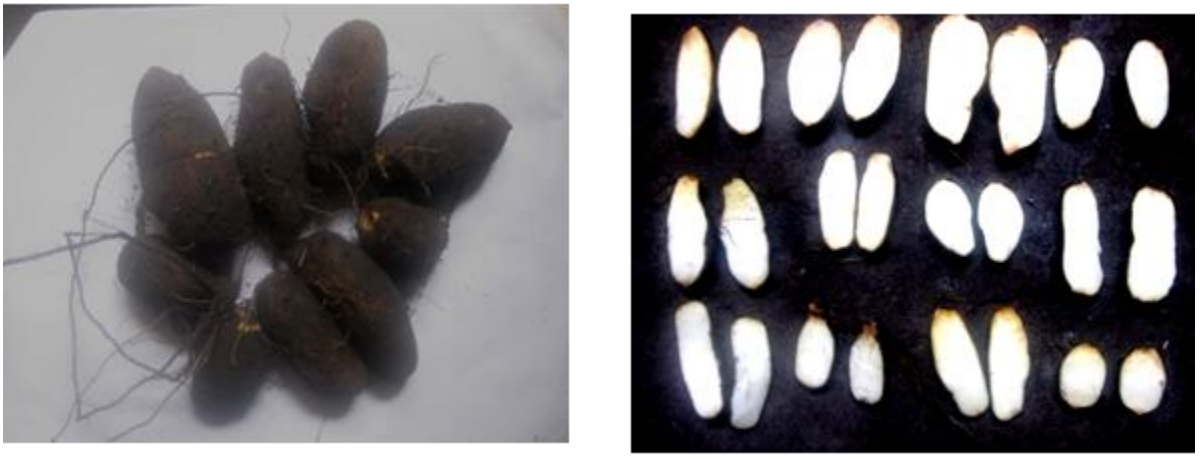

Figure 3 : Absence of brown spots in the yam tubers harvested 6 months after planting
A : Tubers harvested 6 months after planting ; **B** : Absence of brown spots in the tubers longitudinally cut

Table 2 : IBS severity in the tuber seed and harvested according to the vegetation

	Severity	
Vegetation	Tuber seed	Tuber harvested
Forest	2.83 ± 0.23^{b}	1.79 ± 0.20^{a}
Savannah	2.11 ± 0.21^{a}	1.83 ± 0.18^{a}

N = 546 tubers, Scale (1 to 5), the figures are followed by the same letter on the same line and column are not significantly different to the limit of 5% according to Newmann-keuls test

Agents responsible for IBSD

Some tubers with brown spots reacted positively to the PCR test. Analyzes performed in reference laboratories with *Badnavirus*-specific enzymes revealed the presence of bands migrating at 580 bp. However, other PCR assays for tubers with brown spots inside reacted negatively.

Existing control methods and technologies generated against IBSD

Existing IBS control methods are reported in Table III. Two adapted and accessible control technologies against brown spots of yam have been generated and are tested in farmers' environment. These are: 1) Thermotherapy, soaking seeds in hot water at 50 ° C for 15 min before planting and 2) Deposits of ash, carbofuran and callicuivre in holes prior to planting (Table III).

Table 3: Existing control method and technologies generated against brown spots of yam tubers

Classical control methods	Control method in rural area	Generated technologies to fight against brown spots on the yam's tubers
• Use of healthy seeds or resistant or tolerant varieties and the practice of double harvesting • Early identification of plants with symptoms (mosaic, shoe laces, deformation) and elimination in the field • Produce healthy seeds by meristem culture and thermotherapy but very expensive for the producer	Rubbing the surface of the tuber with the tip of the feet before planting is the only practice mentioned in the 8 localities visited	• Thermotherapy (soaking seeds in hot water at 50 ° C for 15 min before planting) • Deposit of ash in the holes before planting.

Effectiveness of treatments on the severity of brown spots and income gain associated

The severity of brown spots was reduced by 51.58 % by thermotherapy, by 22 % by ash, by 20.92 % by carbofuran and by 12.7 % by callicuivre. Significant differences in the severity of the spots were recorded between seed tubers and harvested with thermotherapy treatments, carbofuran and callicuivre. On the other hand, no significant difference in the severity of the spots was recorded between seed tubers and harvested in the absence of treatment (Table IV). Yield gains ranging from 1.10 T to 2.55 T % of the production were recorded thanks to the different treatments of the Bete-Bete variety. With thermotherapy, yield gains vary between 2.4 T and 2.55 T (Table V).

Table 4: Impact of the treatments on the severity of IBS in the harvested yam tubers

Substance used for treatments	Severity of spots	
	Tuber seed	Harvested tuber
Carbofuran	1.96 ± 0.22^{b}	1.55 ± 0.20^{a}
Callicuivre	1.89 ± 0.16^{b}	1.65 ± 0.14^{a}
Ash	1.87 ± 0.25^{b}	1.46 ± 0.17^{a}
Thermotherapy	2.21 ± 0.10^{b}	1.07 ± 0.13^{a}
Sample	1.50 ± 0.14^{a}	1.46 ± 0.15^{a}

Table 5: Yield gain generated by the reduction of IBS severity in the yam tubers with different treatment

Treatments	Yield (T/ha)	
	Toumodi	Yamoussoukro
Carbofuran	11.60 ± 0.97^{ab}	8.6 ± 0.70^{bc}
Callicuivre	12.30 ± 1.04^{ab}	8.9 ± 0.79^{bc}
Ash	12.58 ± 1.02^{ab}	9.0 ± 0.26^{bc}
Thermotherapy	13.05 ± 1.25^{a}	10.2 ± 1.00^{b}
Sample	10.50 ± 0.67^{b}	7.8 ± 0.83^{c}

Scale (1 à 5), the averages followed by the same letter in the same column are significantly Identical at the limit of 5 % according to the Newmann-Newmann-Keuls test

DISCUSSION

Different brown spots sizes were observed in yam tubers with Bètè-bètè and Sao de *Dioscorea alata* varieties. These are small scattered spots and localized large spots. These results are in agreement with those of Eni et al. (2008). A relationship between the severity of leaf symptoms and that of tuber stains at harvest may have existed. However, plants with apparently healthy leaves produced tubers with brown spots. The incidence and severity of

the disease are higher in the savanna zone than in the forest / savannah transition zone. This difference in epidemiological parameters could be due either to the maximum vector activity in the forest area or to the mineral element content of the forest zone soils. These results are in agreement with those of Séka et al. (2009a). Indeed, these authors showed that the incidence of viral diseases decreased from the forest area to the savanna zone. Six months after planting, no brown spots were observed in tubers from healthy and infected seeds. Serological and molecular tests confirmed the absence of virus particles in the tuber at this phenological stage of the plant. This low viral concentration in young tubers is due to the fact that the viral suspension is still divided between all parts of the plant. These results are in agreement with those of Séka et al. (2014). These authors have shown that there is at least a 46.7 % loss in the concentration of YMV and CMV in tubers harvested when the stem is still fresh.

Molecular analysis of tubers with brown spots revealed the presence of bacilliform bands resembling those of *Badnaviruses*. This virus could be either responsible or act in synergy with other viruses. However, the involvement of this virus as agents responsible for the disease has not yet been proven. The results of the work of Panattoni and Triolo (2010 and 2013) made it possible to set up new techniques to reduce their impact on crops. The different treatments applied to the yam seedlings allowed these authors to reduce the severity of the brown spots in yam tubers to variable rates depending on the treatment. Indeed, the use of chemicals such as carbofuran, could reduce the severity of a viral infection, because it has the ability to act as an insecticide to reduce the population of insect vectors as it is the case in the fight against Rice tungro virus disease (Azzam et al., 2002). However, thermotherapy enabled to reduce the severity of the symptoms of the disease to more than 50 %. Its effect could be due to the heat generated, which in contact with the organ penetrates and inhibits the multiplication of viruses present (Manganaris et al., 2003). Although the heat treatment has yielded somewhat unsatisfactory results, it would be interesting to combine it with the meristem culture in order to obtain virus-free seeds as in the case of cassava where the combination of these two treatments has produced healthy plant material (Acheremu et al., 2015).

CONCLUSION

There is no close relationship between the presence of foliar symptoms of virosis and that of brown spots in tubers. In addition, for plants with symptoms of viral diseases, the severity of leaf symptoms changes with that of brown spots. Some treatments such as thermotherapy, deposition of ash, carbofuran and callicin in pre-planting holes have reduced the severity of IBSD in tubers. Production gains ranging from 10.47 % to 19.5 % of the production were recorded thanks to the various treatments with the Bètè-Bètè variety.

ACKNOWLEDGEMENTS

We are grateful to FIRCA for their financial support

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