Journal of Applied Biosciences 20: 1146 - 1152
ISSN 1997–5902

Incidence, prevalence and severity of passion fruit fungal diseases in major production regions of Kenya

Amata, R. L[1*]., Otipa, M. J[1]., Waiganjo M[1]., Wabule, M[1]., Thuranira E.G[1]., Erbaugh M[2]., Miller S[2].
[1]Kenya Agricultural Research Institute, P.O. Box 57811-00200 Nairobi, Kenya.
[2]Ohio State University, 2120 Fyffe Road, Columbus , Ohio 43210 USA

*Corresponding author e-mail: amata_ruth@yahoo.com

Published at www.biosciences.elewa.org on 7 August 2009

ABSTRACT

Objectives: To determine incidences, prevalence and severity of fungal diseases of passion fruit in major growing regions in Kenya and identify fungi causing the diseases.

Methodology and results: Surveys were carried out in passion fruit growing regions of Central, Eastern, Rift Valley, Western and Nyanza provinces of Kenya in 2008. A total of 85 farms were surveyed. Data was collected using a questionnaire, field observation and laboratory analysis of diseased plant materials. Dieback disease was the most important and its incidence on individual farms ranged between 0-100%. Fungi isolated from stems affected by dieback were *Fusarium* spp and *Phytophthora nicotianae* var *parasitica*. Incidence of wilt disease caused by *F. oxysporum* was between 0 - 33%, while that of leaf stem and fruit spots caused by *Alternaria passiflorae* and *Septoria passiflorae* ranged between 2-100%. Anthracnose disease caused by *Colletotrichum passiflorae* and *Glomerella cingulata* ranged between 5-60%. There was a significant difference in the pathogens isolated across the growing regions. *Phyrtophthora nicoatianae* var *parasitica* was most frequently isolated in Meru followed by Embu district while *Fusarium* spp., *Colletotrichum passiflorae* and *Alternaria passiflorae* were isolated most frequently from Thika district.

Conclusion and application of findings: Diseases observed were generally the same across the districts but incidences, severity and prevalence varied depending on control measures employed by individual farmers. Dieback disease, which was most devastating on majority of the farms, urgently needs to be addressed and the role of the individual species in aggravating the disease needs to be evaluated. Management of fungal diseases require that farmers scout for initial disease symptoms so as to apply timely control measures. Accurate diagnosis is a prerequisite to proper disease management in order to enhance sustainable production and empower rural communities through wealth creation and employment.

Key words: passion fruit, fungal diseases, incidence, prevalence, severity

INTRODUCTION

Passion fruit (*Passiflora edulis* Sims.) is a major crop grown in Kenya for both local and export markets. It is currently ranked third among fruit exports and has great potential since demand for both fresh fruit and processed juice is on the increase (MOA, 2003, Njuguna *et al*., 2005). The crop is grown commercially throughout the tropics and subtropics mainly by small-holders who comprise about 80% of all growers, majority of whom are women (Vieira & Carneiro, 2005). Major passion fruit growing areas in Kenya include Eastern (Meru and Embu districts), Central (Thika, Muranga and Maragwa districts), Rift Valley (Nakuru, Molo and Uasin Gishu districts), Nyanza (Kisii district) and Western (Bungoma and Lugari districts). Passion fruit is particularly rich in minerals and vitamins A, C and D. Increased production will lead to improved health and nutritional status particularly in vulnerable groups such as children, the aged and HIV/AIDS infected persons.

Pest and diseases are highly rated among the principal constraints to sustainable crop production in East Africa (Sutherland & Kibata, 1993). Global losses attributed generally to plant diseases are approximated at 24.8 million dollars annually (Olanya *et al*., 2001). Unlike in the temperate regions where crops are attacked by one or two economically important pests or diseases, multiple pest infestations and/or disease infection are the norm within the tropics and subtropics thus compounding their management. Yield losses of upto 80% of passion fruit have been attributed to a combination of diseases and pests including root rots (*Fusarium* spp.), nematodes (*Meloidogyne* spp.), passion fruit woodiness disease (PWD) complex, aphids, thrips and stink bugs (KARI, 2001). The combination of root-rot causing pathogens, dieback and nematodes is greatly responsible for the reduction of passion fruit lifespan from 5 to 2 years.

Fusarium wilt disease has made it necessary to graft susceptible cultivars onto *Fusarium* resistant rootstocks (Ssekyewa *et al.,* 1999; Vieira & Carneiro, 2005). The yellow passion fruit is used as the root stock due to its resistance to *Fusarium* wilt disease, while the purple is used as the scion due to its relatively sweeter taste and hence higher market demand (Lippmann 1978; Njuguna *et al*., 2005). Management of root rot and PWD requires that growers start their orchards with disease free seedlings, practice field sanitation and control insect vectors (KARI 2001; Njuguna *et al.,* 2005; Vieira & Carneiro 2005).

Pathogens infecting passion fruit in Kenya have not been fully characterized, although diseases affecting the crop in the country have been reported based mainly on field symptoms. In addition, the current distribution, severity and relative importance of passion fruit diseases is not known. This information is needed for appropriate monitoring and development of management strategies including determining priority areas in breeding for resistance to these diseases.

An effective strategy for combating plant diseases requires a thorough knowledge of the pathogens including their biology, ecology and their variability. Since passion fruit diseases significantly reduce yields and hence incomes of resource poor farmers, a better understanding of the causal agents is essential. The main aim of this study was to assess the geographical distribution of passion fruit diseases in Central, Eastern, Rift Valley, Western and Nyanza provinces of Kenya, by determining their incidence, prevalence and severity and to isolate and identify fungi associated with the diseases.

MATERIALS AND METHODS

Surveys were carried out in Western, Nyanza, Rift valley, Central and Eastern Provinces to determine the distribution, incidence and severity of passion fruit fungal diseases in different agro-ecological zones. Passion fruit leaves and fruits were sampled from farmer's fields exhibiting different disease symptoms. The fields were randomly selected using a predetermined criterion, where distance between the fields ranged from 5 to 20 Km. Farm size ranged between ¼ to 6 acres. The survey included orchards as young as 1 year to 4 years old.

For each disease the incidences was determined by counting the number of visibly diseased plants per farm in relation to the total number of plants on each farm. Prevalence was recorded as the percentage of fields in which the disease symptoms were observed to the total number of fields visited in each district. Severity among 20 plants in each field was recorded using a scale 0 to 3 according to Sutherland *et al.*(1996). For leaf, stem and fruit spots the scale used was; 0= no spots; 1= Few spots (1-10%) on <50% of leaves/stems/fruits; 2= 1-10% spots on >50% of leaves/stems/fruits or 10-30% spots on <50% of leaves/stems/fruits; 3=>30% spots on >50% of leaves/stems/fruits. For wilt and dieback, severity was also recorded on a scale of 0-3 where 0= No wilt and no dieback; 1= < 10% of plant showing symptoms; 2= 10-30% of plant showing symptoms; 3=>30% of plant showing symptoms.

During the survey, a detailed questionnaire was used to collect information on acreages under the crop, agronomic practices and symptomatic plants were collected and brought to the laboratory for isolation and identification of different pathogens. To isolate leaf and fruit spot pathogens, 25 pieces each of diseased leaves fruits collected randomly per farm were sterilized in 75% ethanol for 1 minute then incubated in moist dishes. In addition, 25 pieces each of sterilized leaf and fruit diseased material per farm were plated initially on tap water agar and later single spored onto carnation leaf agar and potato dextrose agar. Pieces of stems with dieback, brown spot and wilt symptoms collected randomly from 50% of the diseased plants per farm were washed in tap water, and 1 cm sections cut at the diseased /healthy border region. The sections were washed in sterile water and

surface sterilized in 75% ethanol for 1 minute, then incubated in moist dishes and also plated on Peptone PCNB agar and V8 juice agar. Cultures were purified through single spore and hyphal tipping onto carnation leaf agar, potato dextrose agar, V8 agar and Lima bean agar according to Burgess *et al.* (1994) and Ohio Agricultural Research Development Centre, Circular no. 143 (1994).

Morphological characterization of pathogens was done using microscopy according to Burgess *et al.,* (1994), Ohio Agricultural Research Development Centre Circular no. 143 (1994), and Commonwealth Mycological Institute descriptions. Cultures were stored in 15% glycerol at –20°C at KARI, National agricultural Research Laboratories, Nairobi.

RESULTS AND DISCUSSION

Fungal diseases affecting passion fruit in Western, Nyanza, Eastern, Rift Valley and Central provinces included leaf, stem and fruit spots, anthracnose, dieback and wilt. All the six diseases affecting passion fruit were detected in all provinces except for wilt disease, which was not present in Kisii (Nyanza) (Table 1).

Die back disease had an incidence of 100% in most of the districts and the highest severity relative to the other diseases (Table 1). *Phytophthora nicotianae* var. *parasitica* and several *Fusarium* species (*Fusarium oxysporum, F. pseudoanthophilum, F. subglutinans, F. solani* and *F. semitectum*) (Table 2), were isolated from diseased plants exhibiting dieback symptoms. Several farmers especially in Thika, Kisii, Meru, and Nakuru districts had experienced total crop loss due to this disease. *Phytophthora nicotianae* var *parasitica* was more commonly associated with dieback of plants across the districts relative to individual *Fusarium* spp. (Table 2).

Fusarium wilt disease had relatively lower incidence than dieback at a range of 0-33% and was less severe at a mean score of 1-2. This may be attributed to the fact that most farmers planted grafted seedlings in all the districts except in Kisii, with the resistant yellow passion fruit as the root stock and susceptible purple passion fruit as the scion, hence preventing the entry of the wilt pathogen through the roots. Complete absence of *Fusarium* wilt disease in Kisii district of Nyanza province may imply that the local ungrafted purple variety predominantly grown in Nyanza is tolerant to the wilt disease or that the farmers control the disease by planting clean seedlings and ensuring high levels of field sanitation. Seventy percent of farmers in Kisii had planted the purple variety (ungrafted), started off from seeds locally, while 30% had planted purple passion fruit grafted on yellow passion fruit (personal observation and communication with farmers). It would be worthwhile to ascertain the response of these seedlings to highly virulent strains of *Fusarium oxysporum* fsp. p*assiflorae.*

Incidences of leaf stem and fruit spots caused by *Alternaria passiflorae* and *Septoria passiflorae*, and anthracnose caused by *Colletotrichum passiflorae* and *Glomerella cingulata* ranged between 0-100% and 0-60%, respectively, across the provinces (Table 1). *Alternaria passiflorae* which causes leaf, stem and fruit spots in passion fruits was isolated most frequently (52), followed by *C. passiflorae* (46) across the districts (Table 2). The severity of these diseases is bound to be influenced by differences in climatic conditions, since the survival and spread of different fungi varies with temperature and humidity. However disease management practices by farmers also played a major role in determining severity across the growing regions. The diverse cropping systems in the regions surveyed may also be facilitating the survival and propagation of these pathogens, since fungi are able to survive in alternative hosts.

Thika had the highest frequency (54) of fungal pathogens isolated and was significantly different (χ^2 =91.800, d.f=45, p=0.000), from all other districts (Table 2). Bungoma had the lowest frequency (15). This may be due to the fact that Bungoma had less passion fruit farmers than Thika. Meru district had the highest frequency (11) of *P. nicotianae* var. *parasitica* isolates from samples with dieback symptoms (Table 2), than all the other districts. Thika had the highest frequency of *Fusarium* spp. isolated from plants with dieback and wilting symptoms (Table 2).

The association of several *Fusarium* species (*F. oxysporum, F. solani, F. pseudoanthophilum, F. semitectum, F. subglutinans*) and *P. nicotianae* var *nicotianae* with dieback symptoms mainly originating from above ground stem parts affecting the scion (purple passion fruit) indicates a possibility of these fungi being disseminated through splash or handling of plants with infected tools. In such a case the fungus may invade the plant stem through wounds or openings and kill the tissues thus disrupting water uptake beyond the affected part, which wilts. The fungus then continues to kill the stem tissues backwards towards the base of the plant or the rootstock, hence causing the dieback. Observations made during the survey revealed that the disease ravages through the purple passion fruit (scion) stem but in cases of where grafted seedlings had been planted the tolerant yellow root stock remains alive.

The synergy of the two fungal species (*Phytophthora* and *Fusarium*) may aggravate the dieback disease in passion fruit growing areas. Pathogenicity tests are underway to establish the

virulence of the individual species. *Phytophthora nicotianae* var. *parasitica* has been reported to cause fatal blight or stem rot of passion fruit in South Africa (Mc Kenzie *et al.,*1989). *Fusarium oxysporum* f.sp. *passiflorae* and *F. solani* have been reported to cause *Fusarium* wilt and crown rot, respectively, in passion fruit in Kenya (Njuguna *et al.,* 2005). In Zimbabwe, both *F. solani* and *P.nicotianae* var. *parasitica* were reported to cause wilting of passion fruit seedlings (Cole *et al.* 1992). *Fusarium subglutinans* f.sp. *pini,* has been reported to cause pitch canker disease of pines in South Africa, California, Florida, Japan and Mexico (Britz *et al.,* 1999).

Table 1: Symptoms, incidence, prevalence and severity of fungal diseases in various passion fruit growing areas in Kenya and fungi isolated.

Disease symptoms and Fungi isolated	District/Province	Incidences %	Severity	Prevalence %
Leaf spot	Bungoma (Western)	25-95	1-2	82
Alternaria passiflorae	Kisii (Nyanza)	5-100	0-2	72
Septoria passiflorae	Thika (Central)	20-95	1-3	85
	Molo (Rift valley)	20-100	1-2	95
	Nakuru (Rift valley)	10-95	1-2	80
	Embu (Eastern)	5-80	0-2	75
	Meru (Eastern)	5-80	0-2	73
Fruit spots	Bungoma (Western)	25-75	1-2	73
Alternaria passiflorae	Kisii (Nyanza)	5-80	1-2	60
Septoria passiflorae	Thika (Central)	20-70	1-2	80
	Molo (Rift valley)	45-80	1-2	65
	Nakuru (Rift valley)	20-80	1-2	65
	Embu (Eastern)	5-70	1-2	62
	Meru (Eastern)	5-60	1-2	60
Brown spot on stems	Bungoma (Western)	20-85	1-2	75
Alternaria passiflorae	Kisii (Nyanza)	0-85	0-2	73
	Thika (Central)	10-90	1-2	90
	Molo (Rift valley)	20-100	1-3	70
	Nakuru (Rift valley)	10-70	1-2	86
	Embu (Eastern)	2-80	1-3	64
	Meru (Eastern)	2-70	1-2	60
Anthracnose	Bungoma (Western)	5-40	1	60
Colletotrichum passiflorae,	Kisii (Nyanza)	0-40	1-2	60
Glomerella cingulata	Thika (Central)	10-60	1	71
	Molo (Rift valley)	20-50	1	66
	Nakuru (Rift valley)	5-20	1	60
	Embu (Eastern)	5-50	1	52
	Meru (Eastern)	5-30	1	55
Dieback	Bungoma (Western)	20-50	1-3	50
Phytophthora nicotianae	Kisii (Nyanza)	0-100	1-3	80
Fusarium oxysporum	Thika(Central)	10-100	1-3	100
F. semitectum	Molo (Rift valley)	70-100	1-3	100
F. pseudoanthophilum	Nakuru districts	40-100	1-3	100
F. subglutinans	Embu	0-100	1-3	100
F. solani	Meru	0-100	1-3	100
Wilt	Bungoma	0-20	1-2	10
Fusarium oxysporum	Kisii (Nyanza)	-	-	-
	Thika (Central)	0-10	1-2	7
	Molo	0-13	1-2	10
	Nakuru	0-33	1-2	10
	Embu	0-14	1-2	6
	Meru	0-13	1-2	10

Fig. 1A: Brown spot disease affecting fruit.

Fig. 1B: Brown spot disease affecting passion fruit leaf.

Fig. 1C: Dieback disease affecting passion fruit stem. .

Fig.1D: Cross section of passion fruit stem showing brown colouration of vascular tissue

Fig. 1E: Late stages of dieback disease.

Fig. 1F: Anthracnose disease of passion fruit

Fig. 1G. Brown spot disease affecting passion fruit stem.

Fig. 1H. Passion fruit plant affected by *Fusarium* wilt.

Figure 1 A- H: Symptoms of various passion fruit diseases observed in Kenya.

Table 2: Frequencies of fungal pathogens isolated from diseased passion fruit samples collected in Kenya.

Pathogen	Disease symptom	District						Total isolates
		Thika	Embu	Meru	Nakuru /Molo	Kisii	Bungoma	
F. oxysporum	Dieback	2	4	1	2	1	1	11
	Wilt	1	1	1	1	0	1	5
F. solani	Dieback	6	2	2	2	1	0	13
F. subglutinans	Dieback	4	1	1	1	1	0	8
F. semitectum	Dieback	4	0	0	0	0	0	4
F. pseudoanthophilum	Dieback	5	1	1	2	0	0	9
P. nicotianae var. *parasitica*	Dieback	4	6	11	5	4	0	30
Septoria passiflorae	Leaf & fruit spots	0	0	0	0	0	2	2
Alternaria passiflorae	Leaf, stem & fruit spots	10	7	6	15	9	5	52
Colletotrichum passiflorae	Anthracnose	18	9	1	4	9	5	46
Glomerella cingulata	Anthracnose	0	0	0	0	1	1	2
Total		54	31	24	32	27	15	183

Strains of *F. subglutinans* have also been reported to cause malformation of mangoes, stalk rots in maize and sugarcane and pineapple rots (Steenkamp *et al*. 2000). Despite being morphologically similar, strains of *F. subglutinans* have been found to be phylogenetically distinct and belong to different mating populations in accordance with the diseases they cause (Steenkamp *et al.* 2002). *Fusarium solani* also causes severe crown and root rots of a wide range of plants including peas, beans and tomatoes (Burgess *et al.* 1994).

A species such as *F. semitectum* is not regarded as an important pathogen but has been implicated in storage rot problems of bananas and other fruits, whereas little is known about the world wide distribution, host range and pathogenicity of *F. pseudoanthophilum* which has been previously isolated from asymptomatic maize plants in Zimbabwe (Burgess *et al*., 1994; Nirenberg *et al*., 1998). This limited information on the association between *Fusarium* species isolated and diseased passion fruits necessitates pathogenicity tests for all the isolated species. The hotspot regions for dieback included Meru, Embu, Thika, Nakuru, Kisii and Molo.

Most farmers lacked knowledge on disease identification and had limited resources for disease management as they indicated that they do not use any strategies to control diseases. Incidences and severity of diseases across the farmers' fields depended on management. Those actively controlling the diseases through field sanitation and chemical control had lower disease incidences and severity compared to those who did not. Control of these disease calls for integrated disease management strategies that would enhance high levels of field sanitation leading to reduced disease incidences and severity, hence high yields. There is need to educate farmers on management of passion fruit diseases through field demonstrations and farmer field schools, to minimise on losses attributed to diseases. One other major key constraint that needs to be addressed is the issue of middlemen who greatly reduce the farmers' income. Formation of farmer groups or cooperatives may minimise the activity of middlemen hence reduce the players involved in the passion fruit production and marketing.

In conclusion, despite the diseases observed being generally the same across the districts surveyed (except for *Fusarium* wilt), incidences and severity varied across the farms depending on control measures employed by the individual farmers. The absence of *Fusarium* wilt disease in Kisii district suggests that variety may influence disease incidence and severity. However the ungrafted purple variety grown predominantly in Kisii district needs to be evaluated in other agro-ecological zones to determine its stability as a wilt tolerant variety. Management of fungal diseases on passion fruit farms requires that farmers exercise high levels of field sanitation to reduce disease inoculum in their fields and avoid wounding plants as these serve as entry points for the pathogens. Farmers should also use fertilizers and manure at planting and during growth to ensure a healthy vigorous crop that would endure disease pressure and produce high yields. Scouting for initial disease symptoms is also important because it enables timely control. This would minimise losses attributed to fungal diseases in passion fruit production.

ACKNOWLEDGEMENTS: The authors thank the United States Agency for International Development (USAID), through the Integrated Pest Management Collaborative Research Support Program (IPM CRSP) for financial support.

Table 2: Frequencies of fungal pathogens isolated from diseased passion fruit samples collected in Kenya.

Pathogen	Disease symptom	District						
		Thika	Embu	Meru	Nakuru /Molo	Kisii	Bungoma	Total isolates
F. oxysporum	Dieback	2	4	1	2	1	1	11
	Wilt	1	1	1	1	0	1	5
F. solani	Dieback	6	2	2	2	1	0	13
F. subglutinans	Dieback	4	1	1	1	1	0	8
F. semitectum	Dieback	4	0	0	0	0	0	4
F. pseudoanthophilum	Dieback	5	1	1	2	0	0	9
P. nicotianae var. *parasitica*	Dieback	4	6	11	5	4	0	30
Septoria passiflorae	Leaf & fruit spots	0	0	0	0	0	2	2
Alternaria passiflorae	Leaf, stem & fruit spots	10	7	6	15	9	5	52
Colletotrichum passiflorae	Anthracnose	18	9	1	4	9	5	46
Glomerella cingulata	Anthracnose	0	0	0	0	1	1	2
Total		54	31	24	32	27	15	183

There was a significant difference (χ^2 =91.800, d.f=45, p=0.000) between the pathogens and the locations

REFERENCES

Britz H, Coutinho TA, Wingfield MJ, Marasas WFO, Gordon TR, Leslie JF, 1999. *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. Applied and Environmental Microbiology 65: 1198-1201.

Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D, 1994. 'Laboratory manual for *Fusarium* research.' Fusarium Research Laboratory, University of Sydney, Australia

Cole DL, Hedges TR, Ndowora T, 1992. A wilt of passion fruit (*Passiflora edulis* Sims) caused by *Fusarium solani* and *Phytophthora nicotianae* var. *parasitica.* Tropical Pest Management 38: 362-366.

KARI, 2001. Annual Report, Kenya Agricultural Research Institute, Nairobi, Kenya.

Lippmann D, 1978. Cultivation of *Passiflorae edulis* S. . - General information on passion fruit growing in Kenya. Germany Agency for Technical Cooperation (GTZ), Hoehl – Druck, 6430 Bad Hersfield.

Mc Kenzie CB, Staveley GW, Smith IE, 1989. Intensive Container culture of passion fruit. ISHS Acta Horticulture 275.

Ministry of Agriculture, 2003. Production and export statistics for fresh horticultural produce for the year 2003. Ministry of Agriculture, Horticulture Division Nairobi, Kenya.

Njuguna JK, Ndungu BW, Mbaka JN, Chege BK, 2005. Report on passion fruit diagnostic survey. A Collaborative Research of KARI / MOA / GTZ under promotion of private sector development in Agriculture (PSDA) programme.

Nirenberg HI, Kroschel J, Adrianaivo AP, Frank JM, Mubatanhema W, 1998. Two new species of *Fusarium: Fusarium brevicatenulatum* from noxious weed *Striga asiatica* in Madagascar and *Fusarium pseudoanthophilum* from *Zea mays* in Zimbabwe. Mycologia 90: 459-464.

Ohio Agricultural Research and Development Centre (OARDC), 1994. Useful Methods for Studying *Phytophthora* in the laboratory, Special Circular no. 143.

Olanya OM, Adipala, E, Hakiza J, Kedera JC, Ojiambo P, Mukalazi JM, Forbes G, Nelson R, 2001. Epidemiology and population dynamics of *Phytophthora infestans* in Sub-Saharan Africa: Progress and constrains. African Crop Science Journal 9: 185-194.

Steenkamp E, Britz H, Coutinho T, Wingfield B, Marasas W, Wingfield M, 2000. Molecular characterization of *Fusarium subglutinans* associated with mango malformation. Molecular Plant Pathology 1(3): 187-193.

Steenkamp ET, Wingfield BD, Desjardins AE, Marasas WFO, Wingfield MJ, 2002. Cryptic speciation in *Fusarium subglutinans*. Mycologia 94(6): 1032-1043.

Sutherland JA. and Kibata G, 1993. Technical Report II KARI/ODA Crop Protection Project, National Agricultural Research Laboratories.

Sutherland JA, Kibata G, Farell G, 1996. Field Sampling Methods for Crop Pests and diseases in Kenya; National Agricultural Research Laboratories.

Ssekyewa C, Swinburne, TR, Van Damme PLJ, Aburbakar ZM, 1999. Passion fruit collar rot disease occurrence in major growing districts of Uganda. Fruits 54:405-411.

Vieira MLC. and Carneiro MS, 2005. *Passiflora* spp. Passionfruit. In R.E. Litz (Ed.) Biotechnology of Fruit and Nut Crops. CAB International. Wallingford UK.