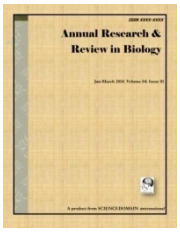

Annual Research & Review in Biology
4(9): 1397-1405, 2014

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Good Orchard Maintenance and Agronomic Practices as Working Components in Management of Dieback Disease on Passion Fruit (*Passiflora* sp.) in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author CW designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MM and RG managed the analyses of the study. Author RM managed the literature searches edited the first draft. All authors read and approved the final manuscript.

Original Research Article

Received 1st June 2013
Accepted 2nd September 2013
Published 13th January 2014

ABSTRACT

Passion fruit is of high economic importance in Kenya. In the recent past, diseases have led to decline in fruit production. This study aimed at identifying disease management practices that could be adopted to mitigate disease impacts. Passion fruit varieties used include purple passion (*Passiflora edulis* Sims) and the KPF hybrid. Field maintenance practices assessed include frequency of orchard sanitation through regular weeding and pruning, pesticide application, nutrient and water provision to plants during the dry season. Agronomic factors assessed include grafting, prunning and effect of wounding in increasing plants' susceptibility to dieback infections. Assessments were done on-farm and on station; 3 research sites were identified in the field and 1 site was set up on-station. For each of the 3 on-farm sites, a farmer's performance in orchard maintenance was rated for all the factors on a score of 1-5 and compared to disease severity (1-5 dieback scale) and incidence (0-100%). Experiments were set up to assess role of grafting on 4 months

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old purple passion seedlings, while effects of prunning and wounding were assessed on 9 months old passion fruit plants of both varieties. Disease establishment was observed and severity was assessed based on the 1-5 dieback scoring chart. Results showed that proper field maintenance reduced disease incidence and severity (12% and 1.6, respectively) in site 1, while poor maintenance yielded higher levels (55% and 4.0, respectively) in site 3. Agronomic practices important in transmission of dieback pathogens on the purple variety were pruning, grafting and wounding. These were found to enhance disease establishment and severity ($P \leq 0.05$) on the purple variety while KPF 12 was tolerant to dieback infections. The study established that proper agronomy and field maintenance practices are important and should be integral in control of dieback disease.

Keywords: Passion fruit; dieback disease; disease management.

1. INTRODUCTION

Kenya relies primarily on agriculture, which represents 24% of the GDP and hence invests in this sector [1]. About 5 million smallholders in rural areas rely on farming as a source of livelihood [2]. Approximately one-third of Kenya's agricultural produce is exported and this accounts for 65% of Kenya's total exports [3]. However, production of most horticultural crops including passion fruit has declined since the turn of the century due to effects of climate change [4]. Climate change has had an adverse effect on rainfall amount and regime leading to prolonged droughts. It has also led to emergence of new pest species whose populations are increasing gradually. These pests have also been adapted to a wide geographic range leading to high disease incidences affecting all types of crops [5].

Passion fruit was introduced to Kenya in the early 20th century [6]. It was the third most important fruit in Kenya (8%) after avocado (62%) and mango (26%) in terms of foreign exchange earnings in the mid 2000's [7,3]. Currently its production has slumped due to various factors, among them effects of pests and diseases [8]. The fruit is a pro-poor crop, grown mostly by farmers owning 0.5 – 2 acres of land [8]. Farmers prefer it due to its fast maturity period of 9 months and it also requires minimum labor and land space [7]. It therefore has enormous potential to generate wealth and improve livelihoods of its value chain actors.

Passion fruit production decline has adversely affected the livelihoods of growers and industrial processors [9]. Farmers have shifted to low income earning activities like vegetable growing, lumbering and charcoal burning. Industrial processors are operating below capacity. Some companies import pulp from South Africa and Brazil [8] for production of passion fruit juice. Importation of raw materials increases the cost of production which in turn leads to increase in prices of the finished products [2]. The area under passion fruit cultivation has also declined. In 2007, about 5,193 ha were cultivated, yielding 71,000 tons worth Ksh 2.1 Billion while in 2008 less than 2,800 ha were cultivated yielding an estimated 49,000 tons worth about Ksh 1.05 Billion.

In Kenya, the major passion fruit diseases include Fusarium wilt, Phytophthora root rot and canker, brown spot [6,10], Woodiness viral disease and dieback [11,8]. Considerable progress in research has been made on these diseases (except dieback) and effective management practices developed. However, challenges exist in the ability of the small

holder farmer to access, adopt and/or effectively follow the stipulated guidelines for effective disease management [12].

Dieback was first recorded in 2004 in Central Kenya, but is currently widespread in all passion fruit producing areas in Central and Eastern regions [13]. The disease is highly virulent, spreads rapidly and reduces orchard lifespan to less than 2 years [8]. It is estimated that dieback has contributed to over 80% of the total fruit decline. Stakeholders have prioritized it as the most serious constraint to the passion fruit value chain, hence requiring highest priority in terms of management [4].

Dieback is a disease complex involving numerous fungal pathogens which include *Fusarium* spp. (*F. oxysporum*, *F. semitectum*, and *F. solani*), *Phytophthora nicotianae*, *Alternaria passiflorae* and *Ascochyta passiflorae*. Dieback is more severe during dry seasons [8]. Its symptoms start during the pre-flowering stages (9-12 months after transplanting). The most notable symptoms of dieback include death of shoots, tendrils, branches, leaves, and fruits from the tips downwards. Other symptoms of dieback include failure of flowers to set coupled with premature fruit and leaf falls [14]. No single Management measure has been identified that can effectively control dieback. For this reason, combinations of disease management measures are required to reduce its impacts. This study aimed at identifying field maintenance and agronomical measures that can be used to curtail disease establishment and decrease in severity and incidence at nursery level and in the orchard.

2. METHODOLOGY

Three sites were identified in Eastern (Meru County - Meru A & Meru B and central (Murang'a County) Kenya. Field maintenance practices assessed included frequency of orchard sanitation through regular weeding and pruning, nutrient provision, pesticide application and water provision to plants during the dry season. For each site, the owner carried out the maintenance practices evaluated without the input of the researcher. Five field visits (once a month from March to July 2011) were made to collect data on dieback disease incidence (at orchard level) and severity and on how appropriate the farmer was able to maintain his orchard. Disease incidence and severity were assessed using the dieback scoring chart as developed by Wangungu and Mwangi [14] as shown in Table 1. Data on the appropriateness of the orchard maintenance practices were rated using a scale of 1-5 as shown in Table 2.

To assess role of wounding in predisposing passion fruit to dieback infections, 9 months old passion plants of two varieties (KPF 12 and P. edulis) were used. Injuries on plants resulting from farmer practices such as pruning, weeding and harvesting were simulated by artificially wounding 10 mature plants of each variety. Ten wounds were inflicted by piercing the plants' leaves with sterile scalpel blades and sufficient wounding was done on roots (mimicked weeding). Wounded plants were then inoculated by spraying with an inoculum (a spore suspension of 10^5 per mililitre of water) of each of the dieback pathogens. Ten unwounded plants were also sprayed with inoculum while the control included 10 plants that were neither wounded nor inoculated. Five milliliters of inoculum were also added into the soil to enhance the role of soil borne dieback pathogens. Disease development was observed over a 4 months period. Data on disease development was recorded using the 1-5 dieback scoring chart and compared to that of the control plants.

Grafting was carried out on 10 P. edulis seedlings under a green house. Seedlings were obtained by sowing seeds of P. edulis Sims and P. edulis var flavicarpa in pots. These were

raised until 4 months old. The scion of P. edulis Sims was grafted on a root stock of P. edulis var flavicarpa. During grafting, the scalpel blade was each time subjected to artificial infection by cutting dieback infected plant materials. Pruning was carried out on 1 year old plants of two passion fruit varieties (KPF 12 and P. edulis Sims) grown under field conditions. Ten healthy plants were selected and pruned using secateurs that were subjected to dieback infections (by artificially severing dieback infected passion fruit materials). Disease development was monitored every 14 days on grafted seedlings and on pruned plants for a period of 4 months. The plants status in regard to dieback disease development was assessed using the dieback disease scoring chart (Table 1).

Table 1. Dieback disease scoring chart

Score	Percentage incidence	Descriptions
1	0	No infection: There are no infections and no signs of disease observed in the orchard; no infection, no spots, on all vines, all leaves are healthy, flowers and tendrils healthy.
2	≤ 15	Low: small portions of most symptomatic plants in the crop field are affected; spots formation just starting on the vine no more than 1 spot per vine; tendrils showing infection on at least 1 vine (either main vine or on the auxiliary vines); one flower showing infection with initiation of dieback; one branch showing dieback initiation.
3	16-40	Moderate: significant portions of most symptomatic plants in the crop field are affected; tendrils dead and infection entering into the adjoined vines; at least 1 auxillary vine completely blighted and disease entering main vine; spots on more than 3 places on the main vine, with spots coalesced to form a lesion of at least 1cm long or wide.
4	41-75	Severe: large portions of most symptomatic plants in the crop field are affected; one main vine dead at least 2/3 of the length or to the graft union level; leaves on the auxillary and main vines blighted and falling and fruits on the infected vine withering but may be still attached.
5	76-100	Very severe: all portions of most symptomatic plants in the crop field are affected; Infection on both main vines (where 2 are retained); plant >75% defoliated; >50% of fruits shrivelled and dropping prematurely; entire plant drying up.

3. RESULTS

Results showed that the site in Murang'a County (site 3) had the lowest level of orchard maintenance based on farmer's practices that were assessed and correspondingly had highest dieback incidence and severity. Meru County's site 1 recorded the best maintenance levels and had the lowest disease incidence and severity (Table 3).

There was a significant difference (P = 0.002) between disease severity on the wounded and the unwounded plants of the purple variety. Wounded plants showed a higher rate of infection (3.0) compared to the unwounded plants (2.3).There was no significant difference (P = 0.092) between the wounded and unwounded plants of variety KPF 12 where infection

scores for both treatments were 1.3 and 1.2, respectively. This corresponds to no infection according to the dieback disease scoring chart (Table 1).

Dieback disease levels between plants pruned using sterilized and unsterilized secateurs on the purple variety showed a significant difference (P = 0.007) (Fig. 1) with the latter getting diseased (2.2) while the former remained healthy (1.1). No difference was noted in the case of cv. KPF 12 (P = 0.134) where both wounded and unwounded plants remained healthy. There was a significant difference between plants grafted using unsterilized scalpel blades and those grafted using sterilized scalpel blades (P = 0.038). The mean infection score for purple passion plants grafted using sterile scalpel blades (1.5) remained within level 1 while that of plants grafted using unsterilized scalpel blades was 2.1.

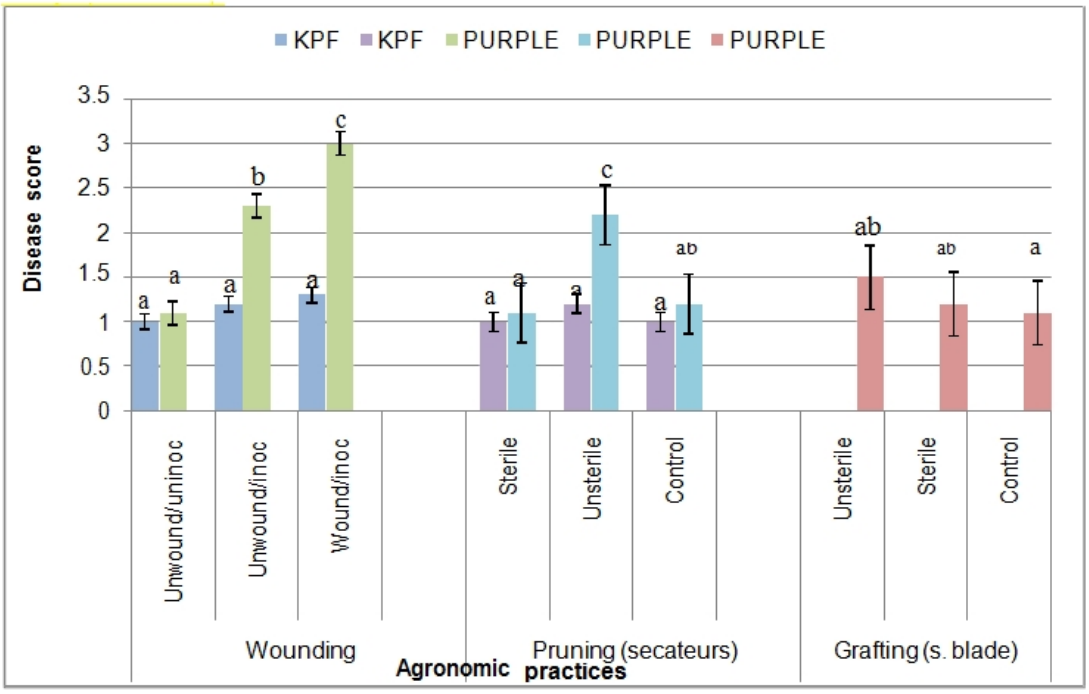

Fig. 1. Effect of wounding, grafting and pruning in increasing plants susceptibility to dieback infections.

Key: For each variety, S.E bars with same letter in the same cluster are not significantly different at P=0.05 by Tukey's B test. For all aspects level 1 = worst; 5=best.

Table 2. Scoring key used for assessing appropriateness of agronomic practices

Score	Prunning	Fertilizer application	Weeding	Water application	Pest control
1	More than 2 main vines retained while auxillary vines lying on the ground.	Manure applied but no fertilizer applied.	Weeds present and intertwined with auxillary vines.	Exclusively rain-fed.	None
2	Too many auxillary vines retained; plants not pruned for more than 10 months.	Fertilizer applied once since planting.	Weeds covering entire ground.	Only during dry seasons.	Pest control exclusively cultural.
3	Average number of auxillary vines retained; plants pruned at 8 months.	Fertilizer applied once a year.	Weeds covering about 50% of the ground.	Once a week.	Pesticide application Only done during heavy pest infestation.
4	Ideal number of auxillary vines retained hanging about 20cm from the ground.	Once every six months	Weeds covering about 20% of the ground.	Three times a week.	Pesticide application only.
5	Each auxillary vine can be identified and is retained; 45cm from the ground.	Manure, fertilizer application every six months and foliar feed application.	No weeds present	Irrigated exclusively (except during seasons with ample rainfall).	Integrated pest control.

Key: 1 = Worst while 5 = Best

Table 3. Dieback disease incidence and severity in relation to the appropriateness of the maintenance practice

	Site	Percent Incidence	Severity		Prunning	Fertilizer	Weeding	Watering	Pest control
1.	Meru B	12%	1.6a		3.40a	3.40a	4.40a	5.00a	4.00a
2.	Meru A	32%	2.6b		2.60b	2.60b	1.40c	2.60b	3.80b
3.	Mathioya	55%	4.0c		2.40c	1.00c	2.20b	1.00c	1.20c
	LSD		0.69	LSD	2.14	1.77	1.38	1.53	1.90
	P. value		≤ 0.001						

Means followed by same letter in the same column are not significantly different at P = 0.05 by Tukey's B test. On all aspects 1 = Worst, 5 = Best.

4. DISCUSSION

Cultural practices are integral in the management of many plant diseases. Meru B (site 1) had the highest level of orchard maintenance and lowest disease incidence and severity (Table 3). The maintenance level for the orchard such as weeding, irrigation, fertilizer application, pruning and pest control were all done properly. In contrast, Mathioya (site 3) had most of these practices maintained at low standards and correspondingly registered high dieback incidence and severity. Although the independent contribution of each practice was not quantified, it can be concluded that proper field maintenance reduces rate of disease infection and spread.

Wounds on passion fruit plants are usually caused by pests feeding and during human activities. Wounds are avenues through which pathogens gain entry into plants as confirmed by this study (Fig. 1). Wounds have been shown to heighten infections on passion fruit by diseases such as collar rot caused by F. solani [15] and cankers caused by Phytophthora species [16]. Agronomic practices that result in wounding such as weeding, grafting, prunning and harvesting are necessary in passion fruit production. Therefore, measures ought to be taken to reduce infections after plants have been subjected to heavy wounding. These measures could include the use of fungicides (suppress pathogen establishment) and fertilizer application (enhance vigorous growth and nutrient supplementation) after prunning and harvesting.

Although prunning and grafting are more often linked to transfer of viruses in passion fruit, this study showed that the practices are important avenues in the spread of fungal infections. The practices cause injuries on plants while tools used if not properly sterilized may harbor pathogenic inoculum in the form of spores and mycelia. The propagules will then penetrate easily into the plant tissues from the point of injury [17].

As confirmed, the highly susceptible purple passion fruit variety (Fig. 1) is at a higher risk of dieback infection through wounding and prunning than the KPF 12 variety. The KPF 12 hybrid offers hope to passion fruit production in that it portrays tolerance to dieback infections. However, the hybrid is not adaptable to all agroecologies within the study areas. To reduce rate of infection on the purple variety after heavy wounding has occurred, Fischer and Rezende [16] suggested the use of copper oxychloride as a drench after weeding to minimize plant infection by F. solani. Spraying appropriate fungicides after prunning and harvesting has also been recommended in the control of Phytophthora blight and brown spot disease caused by *Alternaria* spp. [18,19]. Proper disinfection of prunning and grafting tools has been recommended to avoid spread of diseases from infected to healthy plants [10].

Tool sterilization during pruning and grafting is important in that it ensures that pathogens are not transferred from one plant to another via plant sap. This is a basic disease control measure that is highly recommended in passion fruit production to control *Fusarium* infections [20,10]. However, nursery operators may face challenges in terms of disinfectant strength required and on number of dips allowed before the disinfectant loses its sterilization capacity. Farmers may be ignorant or unaware of the consequences of using unsterilized tools during grafting and prunning or they may find the practice cumbersome [21]. Grafting and prunning may have enhanced dieback establishment and spread in the study regions.

Seedlings grafted using unsterilized or poorly sterilized tools serve as a source of dieback infections. As the survey confirmed, farmers and local nursery operators are substantially contributing to propagation of planting materials despite the low skills and poor production environment. In this case, the low quality seedlings they produce present a higher risk of spreading infections.

5. CONCLUSION AND RECOMMENDATION

Poor field maintenance and agronomic practices encourage and aggravate dieback infections. There is need therefore for extension service providers to emphasize good hygiene during pruning and grafting in order to reduce dieback infections.

The KPF 12 hybrid is tolerant to dieback infections but it is not adaptable to all agroecologies. It should, however, be promoted in the adaptable agroecologies in order to boost national passion fruit production.

ACKNOWLEDGEMENT

The authors wish to thank Kenyatta University, Regional Universities FORUM for Capacity Building in Agriculture (RUFORUM), Kenya National Council for Science and Technology (NCST) and FACT Ltd for supporting this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=397&id=32&aid=3302