Seasonal variation in the incidence of yeast rotters of tomato fruit in soil and on various parts of tomato

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Abstract

GEOTRICHUM CANDIDUM and PICHIA KLUYVERI, the two important yeasts which incite rots of tomato fruits in southern Nigerian were frequently isolated from field/soil and various parts of the host plant during the wet season but rarely during the dry season. However, the frequency of their isolation remained uniformly high in forest soil in both seasons. G. CANDIDUM was isolated from the shoots and roots of seedlings as well as the flowers and fruits; it was also a frequent contaminant of tomato seeds extracted during the wet season. P. KLUYVERY on the other hand, was more prevalent on the stems and leaves of mature plants.

Additional Index Words: GEOTRICHUM CANDIDUM, PICHIA KLUYVERI.

Introduction

In 1973, GEOTRICHUM CANDIDUM Lin ex Pers. caused a rapid extensive damage to fruits of tomato (LYCOPERSICON ESCULENTUM Mill.) in the Western State of Nigeria (Ladipo & Amosu, 1975). This fungus has also been reported to cause very heavy losses in canning tomatoes in California (Butler 1959, 1960). In a study carried out at Ile-Ife, Nigeria, Onesirosan and Fatunla (1976) attributed approximately 40% of tomato rots in the field to G. CANDIDUM and PICHIA KLUYVERI Bedford. They also found that these two yeasts were responsible for the majority of rots in storage and in the market.

The high incidence of yeast-incited rots of tomato fruits has stimulated interest in the ecology of these organisms. Studies at Ile-Ife (Onesirosan and Fatunla, 1976) indicated the presence of G. CANDIDUM and P. KLUYVERI in soil and various parts of the tomato plant. Butler (1960) reported a similar finding for G. CANDIDUM in California. The present study was done to learn about the seasonal variation in the incidence of these fruit rotters in forest and field soil as well as on various parts of the host plant.

MATERIALS AND METHODS

Soil Samples

For 13 months, starting from June 1976, soil samples were collected from various areas with known cropping histories at the University of Ife Teeaching and Research Farm. The first set were collected from tomato plots at a Dam Site where availability of irrigation made cropping possible all the year round. The second set of samples were collected under cassava, MANIHOT ESCULENTA Crantz and pigeon pea, CAJANUS CAJAN. (L.) Mill. in an area where tomato had not been grown for 3 years. Samples were also taken from three locations in a forest area which, as far as could be ascertained, had not been cropped in the previous ten years. Samples taken from crop stands were collected from around the plant roots, between plants within rows and between rows. All sampling was done to a depth of 15 to 20 cm with a soil auger and were taken from 25 to 30 locations in each area. Samples from each set were thoroughly mixed and a portion of each of the three composites was taken to the laboratory and assayed for the yeasts within one hour of collection to minimize the possibility of multiplication of the propagules prior to assay.

Sampling of tomato plant parts

Seeds were extracted from the variety, Ife 1, grown at the University of Ife Teaching and Research Farm during both the wet and the dry seasons. The wet season seeds were obtained from six harvests made in July and September, 1976 from two plantings made in the arable area of the farm. Following extraction and "fermentation", the seeds were dried on filter paper in an oven at 30°C for 48 hours and then assayed immediately for the yeasts; the remaining seeds were stored at 4°C and each batch of stored seed was subsequently separately assayed at 2-weekly intervals. Following the extraction and assaying of the harvest, all the left-over seeds from the wet season seed lots were bulked, stored at 4°C and assayed at regular monthly intervals till June, 1977. The dry season seeds were also obtained from six harvests taken in February and March, 1977 from two plantings at the old Dam Site area of the farm. The seeds were treated in the same way as indicated for the wet season seed samples.

Beginning from July, 1976, Ife 1 tomato seeds were sown at monthly intervals in 1m x 2m beds at a spacing of *ca.* 2cm within rows and 5cm between rows. Two series of plantings were made using seeds from the wet and the dry seasons. Seeds used for the plantings made from July, 1976 to June, 1977 were obtained from harvests made in July to September, 1976 and correspond to "wet-season" seeds while those used for the second series of plantings made from February,1977 to June, 1977 were obtained from harvests made in February and March, 1977 and correspond to the "dry-season" seeds. The beds in each case were covered with a dry grass mulch that was gradually removed after seedling emergence so as to prevent the seeds from being washed away and protect the emerging seedlings from the direct force of the rain. For each month's planting, samples of roots and shoots were taken from four seedlings and assayed for the fungi when the seedlings were 2 weeks old. Also at the age of 4 weeks, 10 seedlings were transplanted to an adjacent plot and grown to maturity. From the latter, samples of leaves, stems, flowers and fruits were collected for assaying at maturity, with the samples of leaves, stems and flowers collected at the onset of flowering.

Media

An enrichment medium was used to assay for the presence of the yeasts and it comprised of 4% glucose, 1% yeast extract and 50mg/1 of aureomycin. 100 ml of this medium without the antibiotic was autoclaved in 250-ml conical flasks for 20 min and cooled before the antibiotic was added. A solid medium was used for the identification of yeasts. It consisted of 4% glucose, 1% yeast extract and agar. After it had been autoclaved as before and allowed to cool, 20 ml each of the medium was poured into petri plates containing 2 drops of 25% lactic acid and designated acidified glucose-yeast extract agar.

Assay.

10 g of each composite soil sample were added to 10ml of sterile distilled water in a 250-ml conical flask and agitated for 20 min on a shaker; 0.5 ml of the *supernatant* from this was then pipetted into each of four *flasks* of the enrichment medium. The flasks were then shaken to disperse the soil suspension, incubated near a window (Hesseltine et al., 1952)and

examined daily for the next four days for evidence of yeast growth. Pellicle formation and the characteristic odour of fermentation were used as a good indication of the presence of G. CANDIDUM while a white sediment was indicative of the presence of P. KLUYVERI.

Four flasks of the enrichment medium were also used for each plant part. The roots, stems, leaves and fruits were in each case washed free of soil in running water and dried between the folds of toilet paper which had been sterilized in an autoclave for 20 min. Root and stem samples were obtained from four seedlings, while samples were also obtained from four branches taken from four plants at the flowering stage. The leaf samples consisted of leaves taken from four flowering plants and punched into discs with a sterile 5-mm cork borer, while the flower samples consisted of 16 newly opened flowers collected from four plants. Four each of the following categories of fruits were cut into small pieces : immature green, mature green, and fully ripe. 2 g of each type of tissue and 2g of the dry tomato seeds were separately assayed using four flasks of enrichment medium in each case. All flasks were incubated and observed as indicated previously for the soil samples.

In addition, six times during the course of this study, four acidified glucose-yeast extract agar plates were exposed to the air in a tomato plot for 2 hours before being brought back into the laboratory for incubation and observation as indicated previously for the flasks.

After 96 hours of incubation, dilutions of the liquid cultures were made with sterile distilled water and plated on the solid medium for incubation at 25°C for 48 hours. The plates were then examined under the miscroscope to identify the species of yeasts present.

Pathogenicity

For each test period, the pathogenicity of 2-5 representative isolates of the two pathogens was tested on ripe fruit of Ife 1 tomato according to the method used by Butler (1960). The organisms were grown in tomato broth shake culture for 24 hours prior to inoculation. The fruits to be inoculated were first swabbed three times with absorbent cotton wool soaked in 70% ethanol and the alcohol was allowed to dry off. Four such fruits were then pierced with a flamed transfer needle which had been

dipped into the inoculum; two punctures *ca.* 2cm deep were made about 2cm from the stem end of each fruit. Another set of four fruits were also pierced with a flamed needle dipped in sterile distilled water to serve as controls. Inoculated and control fruits were placed in separate polythene bags and sealed up. Prior to being used, the polythene bags were treated with 0.5. NaOCl and rinsed four times with sterile distilled water. The fruits were incubated at 25°C and examined daily starting from the third day, for the extent of rotting.

Results

The results are presented in Tables 1 and 2,from which the following observations are evident:

TABLE 1

Frequency of isolation of *G. candidum* and *P. kluyveri* from soil taken from three different habitats in 1976/77.

Sampling date	Frequency[§] of Isolation of Yeasts		
	Tomato Plots	Plots without tomatoes for 3 years	Forest Soil
June, 1976	4/4	4/4	4/4
July, 1976	4/4	3/4	4/4
August, 1976	3/3	2/4	4/4
September, 1976	4/4	3/4	4/4
October, 1976	4/4	4/4	4/4
November, 1976	2/4	1/4	4/4
December, 1976	1/4	0/4	4/4
January, 1977	0/4	0/4	4/4
February, 1977	0/4	0/4	4/4
March, 1977	0/4	0/4	4/4
April, 1977	0/4	0/4	4/4
May, 1977	1/4	1/4	3/4
June, 1977	2/4	1/4	4/4

[§]Four flasks of enrichment medium were inoculated with soil in each case and the figures indicate the number of flaks that yielded *G. candidum* and/ *P. kluyveri* after 96-hr. incubation

Field soil

During the wet season (June - October), G. CANDIDUM and P. KLUYVERI were isolated frequently from field soil; all or almost all of the four flasks inoculated with each composite sample yielded one or both yeasts (Table 1). This was true even for the soil on which tomato had not been grown for over three years. The frequency of isolation however, fell as the season progressed. From January to April, these yeasts were not isolated from either of the composite samples. With the start of the rainy season in 1977, the yeasts were again isolated from these soils.

Forest Soil

In forest soil, the frequency of isolation of the yeasts (especially G. CANDIDUM) remained uniformly high in both seasons. The population of G. CANDIDUM appeared to be higher in forest soils than in field soil as indicated by the fact that pellicle formation and the characteristic odour of fermentation were detected in flasks seeded with forest soil in less than 48 hours. Comparative results with field soil were obtained only after 72hr of incubation.

Tomato plant parts

Seeds

Seeds extracted from fruit during the wet season were heavily contaminated with G. CANDIDUM (Table 2); P. KLUYVERI was also occasionally isolated from them. Neither yeast was, however, isolated from seeds extracted from fruits in the dry season.

Seedling roots

Only G. CANDIDUM was frequently isolated during the wet season from roots of seedlings raised from seeds extracted during either season. It was also the only one isolated in the dry season even though it was isolated only from roots of seedlings raised from seeds extracted in the wet season.

Seedling shoots

In the wet season, shoots of seedlings yielded both G. CANDIDUM and P. KLUYVERY and in addion, a number of unidentified budding yeasts. In the dry season however, only the unidentified budding yeasts were isolated.

TABLE 2

Incidence of *G. candidum* and *P.kluyveri* on various parts of the tomato plant during the wet and dry seasons.

Plant Part	Yeast Isolated	
	Wet season	Dry season
Seeds	*G. candidum*; *P. kluyveri*	None
Seedling root *ex* wet season seeds	*G. candidum*	*G. candidum*
Seedling root *ex* dry season seeds	*G. candidum*	None
Seedling shoot *ex* wet/dry season seeds	*G. candidum*; *P. kluyveri* unidentified budding yeasts	unidentified budding yeasts only
Stems of mature plants	*P. kluyveri*; unidentified buddging yeasts	unidentified budding yeasts only
Leaves of mature plants	*P. kluyveri*; unidentified budding yeasts	unidentified budding yeasts only
Flowers	*G. candidum*; *P. kluyveri*; unidentified budding yeasts	unidentified budding yeasts only
Immature green fruit	*G. candidum*. *P. kluyveri*; unidentified budding yeasts	unidentified budding yeasts only
Mature green fruit	*G. candidum*; *P. kluyveri*; unidentified budding yeasts	unidentified budding yeasts only
Ripe fruit	*G. candidum*; *P. kluyveri*; unidentified budding yeasts	unidentified budding yeasts only

Stems and leaves of mature plants

G. CANDIDUM was not isolated from stemps and leaves of mature plants in either season. P.KLUYVERI was isolated only during the wet season. The unidentified budding yeasts were, however, present in both seasons.

Flowers

During the wet season, G. CANDIDUM was very frequently isolated from tomato flowers whiel P.KLUYVERI was less frequently isolated; neither yeast was isolated from the flowers in the dry season. The unidentified yeasts were isolated at all times.

Fruits

The two pathogens were isolated from tomato fruits at various stages of development during the wet season. Ripe fruits appeared to contain the highest number of propagules as evidenced by the rapidity with which pellicles of G. CANDIDUM and sediments of P.KLUYVERI formed in flasks containing pieces of ripe fruit. In addition to these, other unidentified yeasts were also isolated. In the dry season, the isolated yeast microflora was generally low and the two pathogens were not isolated.

Field Air

No yeasts were isolated from the air in the field but a number of fungi were isolated.

Pathogenicity

All isolates of G. CANDIDUM tested were highly pathogenic. All the P. KLUYVERI isolates were also pathogenic, though less virulent than those of G. CANDIDUM, as measured by the speed of symptom appearance and rate of rot progression. In fruits inoculated with G. CANDIDUM, symptoms (in the form of a soft depression around the points of inoculation) appeared within 24 hours and all four fruits inoculated with each isolate were totally rotted within five days or less. Fruits inoculated with P. KLUYVERI showed symptoms in about 48 hr but the fruits were not completely rotted until seven days. None of the control fruits were rotted within the same period.

Discussion

The results help to shed light on a number of important aspects in the epidemiology of yeast-incited rots of tomato fruit. In the first place, inoculum is not air-borne; neither in California (Butler, 1960)nor at Ile-Ife were the pathogens isolated from the air. The soil is the source of inoculum and the distribution of the propagules in the field is not necessarily associated with the tomato crop. In the present study, the two yeasts were readily isolated during the wet season both from soil under tomato and from soil on which tomato had not been grown for over 3 years; it was also isolated from forest soil at all times.

The inoculum, carried on wet season seed as surface contaminant, seemed to die out rapidly when the

seeds were planted during the dry season and it did not lead to a contamination of the soil or other plant parts. This observation, together with the very low frequency of isolation of the yeasts from field soil during the dry season, indicate that field conditions were not conducive to the survival of these pathogens in that season. In contrast, the frequency of isolation from forest soil remained uniformly high in both seasons. Differences in the two environments probably accounts for this differential survival, with the temperature perhaps being the most significant factor. The forest soil with its vegetation cover remains cool at all times whereas the cultivated field with its soil largely exposed will be very hot during the dry season.

Population build-up of these yeasts with the return of the rains very likely results from propagules carried in surface water from the forest or from newly opened land. Residual propagules in field soil itself might multiply rapidly in the rhizosphere of crops. Contaminated seeds could also help to raise the propagule density.

This study has established that G. CANDIDUM and P. KLUYVERI are part of the normal microflora of the tomato plant during the wet season. This fact may have implications in the epiphytotics incited by these organisms in the field. The presence of natural cracks or punctures made by insects such as the unidentified fruit piercing moths suspected to have been involved in the 1973 epiphytotics (Ladipo and Amose, 1975) might provide entry points into which this surface inoculum can be washed to initiate infection. Thus, an unusual increase in the population and activity of the moths might be one of the factors responsible for such epiphytotics. However, further experiments would be needed to support this argument.

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