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Research Article

Post-harvest assessment of infectious fruit rot on selected fruits in Lafia, Nasarawa State Nigeria

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Abstract

The post-harvest health and microbial safety of plant products and foods continue to be a global concern to farmers, consumers, regulatory agencies and food industries. A study was carried out to evaluate the pathogenicity of fungi associated with post-harvest rot of oranges, watermelons and bananas in Lafia, Nasarawa State, Nigeria. Healthy fruits inoculated with fungal spores obtained from rotted fruit tissues were incubated at ambient temperature conditions and observed daily for the appearance and development of tissue rot. Oranges and Watermelons had the highest number of fungal isolates (3) compared to banana (2). Fungi belonging to the genus *Curvularia* were the most isolated (37.50%), followed by both *Aspergillus* and *Colletotrichum* (25.00% respectively) and lastly *Alternaria* (12.50%). The highest tissue rot diameter of sweet orange (2.40 cm) was induced by *Alternaria sp.* followed by *Curvularia geniculate* (1.40 cm) and lastly *Colletotrichum sp.* (1.28 cm). The highest rot of banana fruit tissues was produced by *A. niger* (3.90 cm), followed by *Curvularia geniculate* (3.40 cm). *Aspergillus sp.* produced the highest tissue rot diameter on watermelon fruits (1.93 cm), followed by *Colletotrichum sp.* (1.30 cm) and lastly *Curvularia geniculate* (1.20 cm). Differences in the susceptibilities of different fruits to rot by fungal pathogens were significant ($p \leq 0.05$). There is need for improved handling of fruits after harvest to prevent losses due to bacterial and fungal rots in the study area.

More Information

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Introduction

Infections from pathogens such as bacteria and fungi are the main causes of postharvest rots of fresh fruits and vegetables during storage, transport and cause significant economic losses in the commercialization phase [1,2]. Infections caused during postharvest conditions lower the shelf life and adversely affect the market value of fruits [3]. Contamination of fruits with microbial toxins does not only account for various health hazards but also results in economic losses, especially for exporting countries [2,4,5].

In Nigeria, cultivation of fruit crops and vegetables is prominent in the North-Central Region where the soils are well drained and rainfall is sufficient for efficient growth and reproduction of a number of fruit crops and vegetables. However, after harvest and storage, the post-harvest phase from the farm gate to major market outlets within and outside the country is often faced with challenges of microbial rots leading to substantive crop losses [6].

Fruit rots caused by fungal and bacterial pathogens result to huge annual losses during storage and transportation of harvested fruits in the study area. Identifying the rot causing organisms is crucial in the mitigation of post-harvest yield losses in the study area.

Citrus (*Citrus sinesis* L.) which originated from south-eastern Asia, China and the east of Indian Archipelago from at least 2000 BC [7-9] is one of the most important fruit crops known by humans since antiquity and is a good source of vitamin "C" with high anti-oxidant potential [10]. About 50% of the harvested crop is lost on transit in developing countries [11] and therefore development and use of alternative postharvest control options involving biological agents are critically important [12-15]. Moreover, natural plant extracts may provide an environmentally safer, cheaper and more acceptable disease control approach [16-18].

Banana is also one of the oldest fruits known to mankind. Its antiquity can be traced back to the Garden of Paradise where Eve was said to have used its leaves to cover her modesty. According to [19], banana is essentially a humid tropical plant, coming up well in regions with a temperature range of 10 °C to 40 °C and an average of 23 °C. In cooler climate,

the duration is extended, sucker production is affected, and bunches are smaller. Low temperatures (less than 10 °C) are unsuitable since they lead to a condition called choke or impeded inflorescence and bunch development. In their study [20] reported that bananas require a fairly humid climate, moist deep rich soil with perfect drainage, protection from wind, full sun and much heat. For successful growth, banana demands plenty of warmth and moisture in the air throughout the year. Heavy rainfall and high temperatures which vary little throughout the year are suitable for bananas. The two most important diseases of bananas are Panama disease and leaf spot [19].

The watermelon (*Citrullus lanatus)* is a flowering plant that originated in North East Africa, where it is found growing wild [21]. It has sometimes been considered to be a wild ancestor of the watermelon; its native range extends from north and West Africa to west India. Evidence of the cultivation of both *C. lanatus* and *C. colocynthis* in the Nile Valley has been found from the second millennium BC onward, and seeds of both species have been found at Twelfth Dynasty sites and in the tomb of Pharaoh Tutankhamun [22].

Citrullus lanatus is a plant species in the family Cucurbitaceae, a vine-like (scrambler and trailer) flowering plant originally from Africa. It is cultivated for its fruit. Watermelon fruit is 91% water, contains 6% sugars, and is low in fat. In a 100 gram serving, watermelon fruit supplies 30 calories and low amounts of essential nutrients. Only vitamin C is present in appreciable content at 10% of the daily value. Watermelon pulp contains c□arotenoids, including lycopene [23]. The amino acid citrulline is produced in watermelon rind [24].

Watermelon is rich in carotenoids. Some of the carotenoids in watermelon include lycopene, phytofluene, phytoene, beta-carotene, lutein, and neurosporene. Lycopene makes up the majority of the carotenoids in watermelon.

Different approaches have been used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers have often relied heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years [25,26]. However, increasing use of chemical inputs has caused several negative effects, ranging from the development of pathogen resistance to the applied agents to their non-target environmental impacts. The growing cost of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has as also called for a search for substitutes to these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or non-existent [25,27,28]. Today, apart from the strict regulations on chemical pesticide use and increasing political pressure to remove the most hazardous chemicals from the market, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such application might have to be applied.

In attempt to tackle the growing challenge of pesticide hazards, pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls [29].

Materials and methods

The study was carried out in Lafia, Nasarawa State, Nigeria located on $8^0$32'N $8^0$18'E in the Southern Guinea Savannah region of North-Central Nigeria. It is home to abundant fruit crops such as *Mangifera indica* (Mango), *Anacadium occidentale* (Cashew), *Citrus sinensis* (Sweet orange), and *Musa acuminate* [30].

Healthy fruit samples (not showing visible signs of rot) of Sweet orange, Mango and Banana were obtained from Lafia Main Market and refrigerated in the Plant Science and Biotechnology Laboratory of Federal University of Lafia, at 4 ^{0}C prior to pathogenicity tests.

The rotted fruit samples were collected from retailers and merchants of the fruit products in different locations within Lafia metropolis, namely; Mararaba-Akunza (In the South), Lafia Main Market (In the East), Bukan-Sidi (In the North) and New Tomato Market (In the West). Sampled fruits were conveyed in sterile polyethylene bags to the Laboratory of Federal University of Lafia, for further processing.

For the isolation of fungi, partially rotted fruits were cut at intersections between rotten and healthy portions into smaller pieces (2 cm × 2 cm), surface sterilized by soaking in 5% Sodium Hypochlorite solution for 2 minutes. Three surface sterilized tissues each of plant materials collected from different locations were plated separately on sterile solidified Potato Dextrose Agar (PDA) and incubated at room temperature (28 °C ± 2 °C) for 4 days. Pure cultures obtained were identified on the basis of spore characteristics and nature of mycelia, using a compound microscope and taxonomic keys [31].

For bacterial isolation, the method described by the International Commission on Microbiological Specification of Food [32] was employed as follows:

Each diseased fruit was first surface sterilized by mopping the entire surface with cotton wool moistened with 1% sodium hypochlorite solution (bleach). The disinfected fruits were further cut open with a flamed knife to reveal boundary areas between diseased and healthy portions. Smaller pieces of about 2 cm^2 were cut and teased out in 10 ml of sterile water in a beaker. A loopful of the inoculum was subsequently

collected from the suspension and aseptically inoculated evenly and streaked onto sterile nutrient agar plates. The inoculated plates were kept in the incubator at 37 °C for 48 hr and observed daily for colony growth. Singly formed colonies were collected and transferred aseptically onto sterile nutrient agar plates and incubated as earlier stated. Pure cultures obtained were used for further identification and subsequent tests.

The characterization and identification of bacterial isolates was based on macroscopic and microscopic examination of growth features as well as biochemical reaction tests, as enumerated below by [33-35].

Gram stain

Bacteria smear were heat-fixed and flooded with crystal violet on glass slides for one minute, after which the primary stain was washed off with water. The heat-fixed smear was further flooded with iodine for one minute and washed off with water. This was followed by flooding the smear with 95% alcohol for 10 seconds and safranin for one minute. Flooded smears were again washed off with water, air dried and observed for either the presence of a purple colouration as an indication of the presence of Gram-positive bacteria, or pink colouration for Gram-negative bacteria.

Catalase test

A sterile wire loop was used to transfer a small amount of colony onto the surface of a clean dry glass slide, after which a drop of 3% H_2O_2 was added. Evolution of oxygen bubbles was regarded as a positive result.

Oxidase test

A bacterial colony was picked with a sterile wire loop and smeared onto a filter paper soaked with the substrate (tetramethyl-p-phenylenediaminedihydrochloride) and moistened with sterile distilled water. The inoculated area was observed for a colour change to deep blue or purple within 10 – 30 seconds.

Indole test

The test organism was inoculated into Tryptone broth and incubated for 18 to 24 hours at 37 °C. Fifteen drops of Kovacs reagent were then added down the inner wall of the tube and observed for the presence of a bright red colour at the interface as an indication of the presence of indole.

Triple sugar iron test

The organism to be tested was inoculated into Triple sugar iron agar and incubated for 18 to 24 hours at 37 °C. After incubation it was observed for red slant, yellow butt, hydrogen sulphide production and gas production.

The pathogenicity of fungi isolated from diseased fruit tissues was determined using the method reported by [36] as follows:

Healthy fruits surface sterilized by swabbing with 5% Sodium hypochlorite solution were aseptically wounded by the removal of 7 mm diameter flesh tissue to a depth of 3 mm, with the aid of a sterile 7 mm diameter cork borer. Seven milliliter (7 mm) agar discs obtained from actively growing mycelial regions of 7 days old cultures of potential rot fungi were aseptically plugged into wounded spots and incubated for 5 days at 28 °C. Artificially inoculated fruits were observed every 48 to 72 hours for the development of rot symptoms. Rot diameters were measured in millimeters (mm) with the aid of a meter rule.

The ability of bacterial isolates to cause rot in healthy fruit tissues was evaluated using the methods of [37]. Surface sterilization was carried out by swabbing entire fruit surfaces with cotton wool moistened with 1% sodium hypochlorite solution. Holes bored into fruit surfaces with the aid of a flamed 5 mm cork borer were aseptically inoculated with 0.5 ml of 48 hr old cultures of bacterial isolates, and covered by replacing the removed fleshy core and finally sealed sterile petroleum jelly. The inoculated fruits were labeled accordingly, kept at room temperature and observed daily for appearance of symptoms such as rot like colour change, softening, characteristic and foul odour. A control experiment was set up by opening and closing the core in fruits without introducing any organism except 0.5 ml sterile water. Inoculated fruits were examined two weeks after inoculation, and rot diameters were measured in millimeters (mm) with the aid of a meter rule.

Treatments were administered in a Completely Randomized Design (CRD) with 3 replicates in a 3 × 5 × 2 layout in the laboratory. Data obtained from the treatments were subjected to Analysis of variance (ANOVA) at 5% level of probability.

Results and discussion

Biochemical identification of bacteria associated with rotted fruits in Lafia

Results of the biochemical identification of bacteria associated with rotted fruits in Lafia are presented in Table 1. Banana had the highest number of bacterial colonies [4], followed by both watermelon and sweet orange (3 each). Bacteria belonging to the genus *Streptococcus* had the highest occurrence on sampled fruits (40.0%), followed by *Staphylococcus* (20%). *Proteus, Micrococcus, Enterobacter* and *Bacillus* all had 10.00% occurrences respectively on the sampled fruits.

Pathogenicity of bacterial isolates on *Musa acuminata* (Banana) fruits

Results of pathogenicity of bacterial isolates on *Musa*

Table 1: Biochemical identification of bacteria associated with rotted fruits in Lafia.

S/N	Isolate Code	Source	Micromorphology	Gram Stain	Catalase Test	Indole Test	Citrate Test	Triple Sugar Ion	Suspected Organisms
1	B_1	Banana	Diplococci	+	-weak	-	+	K/K	*Streptococcus sp.*
2	B_2	Banana	Cocci	+	-weak	-	+	K/K	*Streptococcus sp.*
3	B_3	Banana	Rod	-	+	-	+	K/K	*Proteus sp.*
4	B_4	Banana	Streptococci	+	-	-	+	K/K	*Streptococcus sp.*
5	Wm_1	Watermelon	Rod	+	+	-	+	K/A, H_2S	*Bacillus sp.*
6	Wm_2	Watermelon	Rod	-	+	-	+	A/A	*Enterobacter sp.*
7	Wm_3	Watermelon	Cocci	+	+	-	+	K/K, H_2S	*Staphylococcus sp.*
8	O_1	Orange	Cocci	+	+	-	-	K/A	*Micrococcus sp.*
9	O_2	Orange	Cocci	+	- weak	-	+	K/K	*Streptococcus sp.*
10	O_3	Orange	Cocci	+	+ weak	-	+	K/K	*Staphylococcus sp.*

N/B: K/K: Alkaline slant/Alkaline butt i.e Red/Red–Non fermenter of glucose, lactose and sucrose; K/A: Alkaline slant/Acidic butt i.e Red/Yellow – Only glucose is fermented; A/A: Acidic slant/Acidic butt i.e Yellow/Yellow – Fermenter of glucose, lactose and sucrose + H_2S B = H_2S = Black

acuminata (Banana) fruits are presented in Table 2. Banana fruits inoculated with *Proteus sp.* resulted in more tissue rot (2.33 cm) compared to *Streptococcus sp.* (0.90 cm). Differences in tissue rot among inoculated bacteria, and between inoculated bacteria and the control experiment were not significant ($p \leq 0.05$).

Pathogenicity of bacterial isolates on *Citrus sinensis* (sweet orange) fruits

Results of pathogenicity of bacterial isolates on *Citrus sinensis* (Sweet orange) fruits are presented in Table 3. Of all the tested bacteria, only *Micrococcus sp.* yielded tissue rot in inoculated sweet orange fruits (Plate 1). Differences in tissue rot among inoculated bacteria, and between inoculated bacteria and the control experiment were not significant ($p \leq 0.05$).

Pathogenicity of bacterial isolates on *citrulluslanatus* (watermelon) fruits

Different bacterial genera yielded different proportions of rot on inoculated watermelon fruits (Table 4). The highest tissue rot diameter of watermelon was induced by *Enterobacter sp.* (5.98 cm), followed by *Bacillus sp.* (5.13 cm) and lastly *Staphylococcus sp.* (4.88 cm). Differences in tissue rot among inoculated bacteria, and between inoculated bacteria and the control experiment were not significant ($p \leq 0.05$).

Susceptibility of different fruits to tissue rot caused by bacterial pathogens

Results of the susceptibility of different fruits to tissue rot caused by bacterial pathogens are presented in Table 5. Watermelons were the most susceptible to rot (5.33 cm) by the tested bacterial pathogens, followed by Banana (0.94 cm), and lastly Sweet Orange (0.18 cm). Differences in the susceptibilities of different fruits to rot by bacterial pathogens were significant ($p \leq 0.05$).

Fungi associated with rotted fruits in Lafia

Results of identification of fungi associated with rotted fruits in Lafia are presented in Table 6. All fungi isolated from the studied fruits belonged to the Division *Ascomycota.* Oranges

Table 2: Pathogenicity of bacterial isolates on *Musa acuminata* (Banana) fruits.

Bacterial Isolates	Tissue Rot Diameter (cm)
Proteus sp.	2.33
Streptococcus sp.	0.90
Control	0.00
LSD	1.10

Table 3: Pathogenicity of bacterial isolates on*Citrus sinensis* (Sweet orange) fruits.

Bacterial Isolates	Tissue Rot Diameter (cm)
Micrococcus sp.	0.55
Streptococcus sp.	0.00
Staphylococcus sp.	0.00
Control	0.00
LSD ($P \leq 0.05$) = NS	

Table 4: Pathogenicity of bacterial isolates on *Citrullus lanatus* (Watermelon) fruits.

Bacterial Isolates	Tissue Rot Diameter (cm)
Bacillus sp.	5.13
Enterobacter sp.	5.98
Staphylococcus sp.	4.88
Control	0.00
LSD ($p \leq 0.05$) = 1.23	

Table 5: Susceptibility of different fruits to tissue rot caused by bacterial pathogens.

Fruits	Mean Diameter of Rotted Tissues (cm)
Banana	0.94
Sweet orange	0.18
Watermelon	5.33
LSD ($p \leq 0.05$) = 1.43	

Table 6: Fungi associated with rotted fruits in Lafia.

S/No.	Isolate Code	Source	Isolate Identity	Division
1	ISO 1	Orange	*Alternaria sp.*	Ascomycota
2	ISO 2	Orange	*Colletotrichum sp.*	Ascomycota
3	ISO 3	Orange	*Curvularia geniculata*	Ascomycota
4	ISO 4	Banana	*Aspergillusniger*	Ascomycota
5	ISO 5	Banana	*Curvularia geniculata*	Ascomycota
6	ISO 6	Watermelon	*Curvularia geniculata*	Ascomycota
7	ISO 7	Watermelon	*Aspergillus sp.*	Ascomycota
8	ISO 8	Watermelon	*Colletotrichum sp.*	Ascomycota

Plate 1: Rotted tissue on orange fruit inoculated with *micrococcus sp.*

and Watermelon had the highest number of fungal isolates of 3 compared to banana which had 2. Fungi belonging to the genus *Curvularia* were the most isolated (37.50%), followed by both *Aspergillus*and *Colletotrichum* (25.00% respectively), and lastly *Alternaria* (12.500%).

Pathogenicity of fungal isolates on *Citrus sinensis* (orange) fruits

Variations were observed in tissue rot of *Citrus sinensis* induced by different fungal pathogens (Table 7). The highest tissue rot diameter (2.40 cm) was induced by *Alternaria sp.* (Plate 2) followed by *Curvularia geniculate* (1.40 cm) and lastly *Colletotrichum sp.* (1.28 cm). Differences in tissue rot produced by the fungal isolates on inoculated orange fruits were significant ($p \leq 0.05$).

Pathogenicity of fungal isolates on *Musa acuminata* (banana) fruits

The results of pathogenicity of fungal isolates on *Musa acuminata* fruits are presented in Table 8. The highest rot of banana fruit tissues was produced by *A. niger* (3.90 cm) (Plate 3), followed by *Curvularia geniculate* (3.40 cm). Differences in tissue rot induced by the tested fungal pathogens on banana fruits were not significant ($p \leq 0.05$).

Pathogenicity of fungal isolates on *Citrullus lannatus* (watermelon) fruits

Results of pathogenicity of fungal isolates on *Citrullus lannatus* (Watermelon) fruits are presented in Table 9. *Aspergillus sp.* produced the highest tissue rot diameter on watermelon fruits (1.93 cm) (Plate 4), followed by *Colletotrichum sp.* (1.30 cm) and lastly *Curvularia geniculate* (1.20 cm). Differences in tissue rot diameters yielded by the tested fungi were not significant ($p \leq 0.05$).

Table 7: Pathogenicity of fungal isolates on *Citrus sinensis* (Orange) fruits.

Isolate	Tissue Rot Diameter (cm)
Alternaria sp.	2.40
Colletotrichum sp.	1.28
Curvularia geniculata	1.40
LSD ($p \leq 0.05$) = 0.87.	

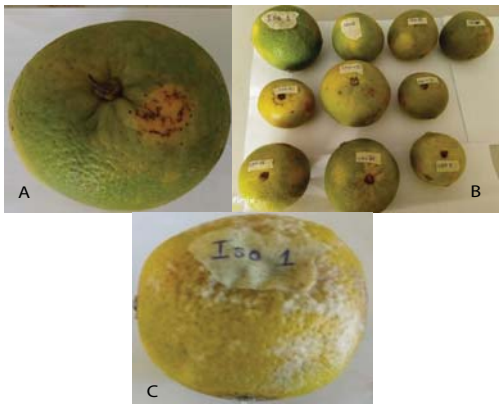

Plate 2: (a) A Healthy orange fruit; (b) Inoculated orange fruits; (c) rotted orange fruit after 4 Days of inoculation with *Alternaria sp.*

Table 8: Pathogenicity of fungal isolates on *Musa acuminata* (banana) fruits

Isolate	Tissue Rot Diameter (cm)
Aspergillus niger	3.90
Curvularia geniculata	3.40
LSD ($p \leq 0.05$) = NS.	

Plate 3: (a) Healthy banana fruits; (b) Rotted banana fruits After 4 Days of inoculation with *Apergillus niger.*

Table 9: Pathogenicity of fungal isolates on *Citrullus lannatus* (Watermelon) fruits.

Isolate	Tissue Rot Diameter (cm)
Curvularia geniculata	1.20
Aspergillus sp.	1.93
Colletotrichum sp.	1.30
LSD ($p \leq 0.05$) = NS	

Plate 4: (a) Healthy watermelon fruit; (b) Rotted watermelon fruit after 4 Days of inoculation with *Aspergillus sp.*

The Bacterium *Enterobacter sp.* was the major causative agent of post-harvest rot of watermelon and *Enterobacter*as was also reported as a major cause of rot in watermelons [38,39]. The excessively high moisture content of watermelons predisposes them to infection and colonization by fruit rot bacteria, resulting to significant tissue damage. Watermelons were also reported to contain rough succulent skins which allow adherence and proliferation of bacterial cells [40]. This probably accounts also for the highest occurrence of tissue rot in watermelons compared to other fruits in the study.

The highest rot of banana observed in the study was as a result of infection of banana fruits by bacteria from the genus *Proteus*. Similarly [41] reported brown rot in banana caused by *Proteus sp*. in Nigeria. The author also opined that the presence of high moisture, protein, carbohydrate and crude fiber in banana were major contributory factors to the high proliferation of *Proteus sp*. in banana tissues.

Orange fruits inoculated with fruit rot bacteria showed the least tissue rot compared to other evaluated fruits. The work of [42] also agreed that oranges are not easily degraded by fruit rot bacteria due to the presence of a resistant pericarp

that prevents the proliferation of bacterial cells. The presence of citric acid in oranges also serves as a deterrent to the proliferation of tissue degrading microorganisms as reported by [43,44].

Fungi belonging to the genus *Aspergillus, Alternaria, Colletotrichum* and *Curvularia* were isolated from the studied fruits and were found to cause extensive rot when inoculated on healthy fruits. Post-harvest fruit rots of numerous crop plants have been reported by a number of workers. In related studies by [45], *Alternaria alternata,* and *Colletotrichum musae* were reported as the major pathogens while members of the *Aspergillus* genus were classified as secondary colonizers, hastening fruit rot of banana fruits in Andhra Pradesh, India. Other authors from India [46] also reported that *Aspergillus sp., Curvuleria sp., Alternaria sp.,* and *Colletotrichum sp.* caused various degrees of rot and compromised citrus fruit quality in selected orchards of Sargodha, Pakistan. In Allahabad, India [47-49] also reported that *Alternaria sp., Colletotrichum gloesporioides Aspergillus niger,* and *Curvularia sp.,* were among the major fungi responsible for post-harvest losses of guava andbanana fruit samples. Most recently, it has been reported that fruit rot of watermelon was caused by *Aspergillus sp.* in fruit stalls in Maiduguri, Nigeria [39].

Conclusion

Rot production by post-harvest fungal pathogens of fruit crops is majorly a result of the activity of tissue macerating enzymes synthesised by various fungal cells to achieve the breakdown of complex carbon compounds for the release of nutrients required for their metabolic activity. Enzyme synthetic ability and the activity of synthesised enzymes varies from one fungal genus to another. This accounts to a large extent for the variations in the extent of rot engineered by different post-harvest rot fungi.

The bacterial and fungal rots observed on fruits in the study are an indication of appreciable microbial contamination of fruit samples in the study area. There is need for improved handling of fruits after harvest to prevent losses due to bacterial rot in the study area.

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