Latent Infection of Papaya Caused by *Colletotrichum gloeosporioides*

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ABSTRACT

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Colletotrichum gloeosporioides, the causal agent of anthracnose of papaya (*Carica papaya*), has not previously been known to cause latent infections in green papaya fruit. Evidence from field studies indicates that *C. gloeosporioides* can initially establish itself on attached immature fruits by direct cuticular penetration. Fruit were infected at early stages of maturity but the fungus remained quiescent until the fruit reached the climacteric phase. Symptoms were then expressed as anthracnose or chocolate spot lesions; thus, papaya anthracnose has a latent stage in its development.

Anthracnose of papaya (*Carica papaya* L.) caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. is considered the most important postharvest disease of papaya in the state of Hawaii, and it is important in many other tropical regions where papaya is grown (5,23). The stage at which fruit are initially infected has been the subject of considerable discussion (2,17–19, 22). Fruit show no evidence of infection when picked at the mature green stage, and symptoms develop during the postclimacteric phase of maturation. Thus, infection appears to take place near maturity or during postharvest handling.

Despite numerous attempts to demonstrate latent infections by histological examination of inoculated detached green immature papaya fruits, evidence was inconclusive (2,3,15,17,18,22). Stanghellini and Aragaki (20) pointed out that detached fruit should not be used as an indicator of field infection because the physiology of the fruit is altered after harvest. They (20) therefore studied resistance of attached immature papaya fruit to *C. gloeosporioides*. Because they failed to obtain infection on attached green unwounded fruit, they concluded that *C. gloeosporioides* is primarily a wound parasite of papaya with the ability to infect only mitotically inactive fruit (20).

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Recent spray trials (1) demonstrated that after spray initiation, a distinct lag period (8 wk) occurred before disease incidence was reduced. Similarly, when sprays were terminated, a lag period took place before disease levels again increased. This delayed response to a protective rather than an eradicant fungicide could occur if a fungus were capable of forming latent infections. In a recent ultrastructural study, Chau (9) provided evidence for the first time of direct fungal penetration through the cuticular membrane of papaya fruit. Subcuticular hyphae were also observed, corroborating the earlier observations of Simmonds (18). In a biochemical study, Dickman et al (10) demonstrated that production of cutinase by *C. gloeosporioides* was essential in its penetration of papaya fruit. Specific inhibition of the enzyme prevented penetration and subsequent infection. Although studies on penetration (9,10) were carried out on detached immature green fruit, these data suggested a possible mode of penetration for field infection.

Because the question of latency still remained unanswered, this study was undertaken to determine whether or not *C. gloeosporioides* infects immature attached papaya fruit in the field and then remains latent until after harvest.

MATERIALS AND METHODS

The following field experiments were done either at Malama-ki Experiment Station on the island of Hawaii or at Poamoho Experiment Station on the island of Oahu. In all cases, the Kapoho Solo cultivar of papaya was used. In the first field experiment at Malama-ki, a randomized complete block design was used with 54 1-yr-old trees of uniform planting, spacing of 1.85 × 3.4 m, and well-developed fruit columns. Entire fruit columns were sprayed with a conidial suspension of *C. gloeosporioides* (10^6 conidia/ml). For the control, water alone was sprayed on 27 trees.

Inoculation was done at sunset and the fruits were covered with polyethylene bags overnight to keep the fruit moist. The bags were removed the next morning. Mature green to colorbreak fruit were harvested at intervals of 1–2 wk from treated and untreated trees during the next 25 wk. The youngest fruit, at the top of the fruit column, were marked with indelible ink to indicate the last fruit that received the inoculum. When these fruit were harvested 22–25 wk later, the trial was terminated. This time interval was chosen because the time required between anthesis and harvest was previously established to be 22–26 wk (1). After harvest, fruit were immediately surface-sterilized by immersion for 5 min in a 0.5% sodium hypochlorite solution, placed in clean cartons, sent to the laboratory to ripen at 24 C for 6–14 days, and observed for evidence of *C. gloeosporioides* infection. Chi-square analysis was performed on paired data points for statistical evaluation of differences.

In a second experiment at Poamoho, inoculations were made at specific sites on 80 attached immature fruits on four trees. After surface-sterilization of the fruit with 0.5% sodium hypochlorite, flexible plastic tubing cut into small cylinders was affixed to the papaya fruit surface with high-vacuum grease to help ensure localization of the inoculum. Inoculum was prepared by removing conidia from the surface of a V-8 juice agar petri dish, washing twice, and suspending in sterile distilled water (10^6 conidia/ml). Fifty microliters of the suspension was placed in each cylinder. Previous comparisons of inoculation methods showed that this method was most reliable for producing uniform infection at specific sites. Again, water alone served as the control. Fruit were incubated by covering them in plastic bags overnight as described previously.

Using this initial field design, two experimental procedures were employed. Some of the fruit were left on two trees past colorbreak until fully ripe and observed while on the tree for signs of infection at inoculated sites. On the other two trees, green immature fruits were surface-sterilized with a 0.5% sodium hypochlorite solution while still attached to the tree, and after air drying, plugs were removed from the specific inoculation sites with a sterile cork borer (15 mm diam.). These plugs were cut into six

small pieces and plated out on either 2% water agar or 10% V-8 juice agar. Plugs were taken at biweekly intervals for 12 wk. The growth of *C. gloeosporioides* from one or more pieces of each was considered positive evidence that a fungal infection had been established at the inoculated site.

RESULTS

Three types of symptoms on papaya fruit were produced on fruit inoculated with *C. gloeosporioides*. One was the typical anthracnose lesion (Fig. 1). The second was the poorly understood "chocolate spot" (11) (Fig. 1), which usually was a superficial reddish brown lesion but occasionally developed into a typical anthracnose lesion as the fruit ripened. Laboratory studies showed no correlation between cultural characteristics, conidial morphology, conidial size, and lesion type. Single-conidial isolations made from anthracnose lesions resulted in either anthracnose or chocolate spot upon reinoculation. Likewise, single-conidial isolates from chocolate spot lesions also resulted in both lesion types. There was no evidence of strain differences. Like anthracnose, chocolate spot is usually expressed as a postharvest rather than a field problem. The third type of symptom was a stem end rot that usually occurred after harvest on severed fruit stems and thus was not considered pertinent to this study.

For statistical analysis, the field study data were divided into three categories: 1) total disease incidence due to *C. gloeosporioides*, eg, anthracnose plus chocolate spot, 2) anthracnose alone, and 3) chocolate spot alone. The results of the chi-square distribution analysis of the data showed that total disease was significantly higher for inoculated fruits than for control fruits for every harvest period until harvest 14 (Table 1). At the early harvest periods, the percentage of anthracnose symptoms was higher on inoculated fruit than on control fruit, whereas the percentage of chocolate spot symptoms was not significantly ($P = 0.05$) different from the control. In contrast, at the 7th, 9th, 10th, 11th, 12th, and 13th harvest periods, the percentage of anthracnose was lower than that of chocolate spot, suggesting a possible inverse relationship between the two types of symptom expression. At the final three harvest points (harvests 14, 15, and 16) no significant differences ($P = 0.05$) in percent disease were noted between inoculated and uninoculated fruits. Most of these fruit developed before the field inoculation and therefore were not expected to show higher incidence of disease than the controls.

In the remaining field studies, viable fungi were recovered from immature green fruit that ranged in age from 4 to 14 wk after anthesis. *C. gloeosporioides* was recovered from 95% (19/20) of the plugs taken from the inoculated sites. Controls showed only 5% (1/20) infection. In addition, when inoculated fruits were left on the tree until fully ripe (140 days), visible lesions were observed in 70% (14/20) of the inoculated sites (Fig. 2). None of the controls were infected.

DISCUSSION

Results of these field studies coupled with previous field and laboratory data (1,9,10) indicate that anthracnose of papaya is initially established in the field on attached immature unwounded fruits. These results are in contrast to earlier work (20), which questioned the existence of latent infections on papaya. Other tropical fruits such as banana (*Musa* spp.) (7), mango (*Mangifera indica* L.) (13), avocado (*Persea americana* Mill.) (4), and citrus (*Citrus* spp.) (6) have all been reported to harbor latent infections caused by this pathogen. The evidence also demonstrates that attached fruit at early stages of maturity (4–14 wk after

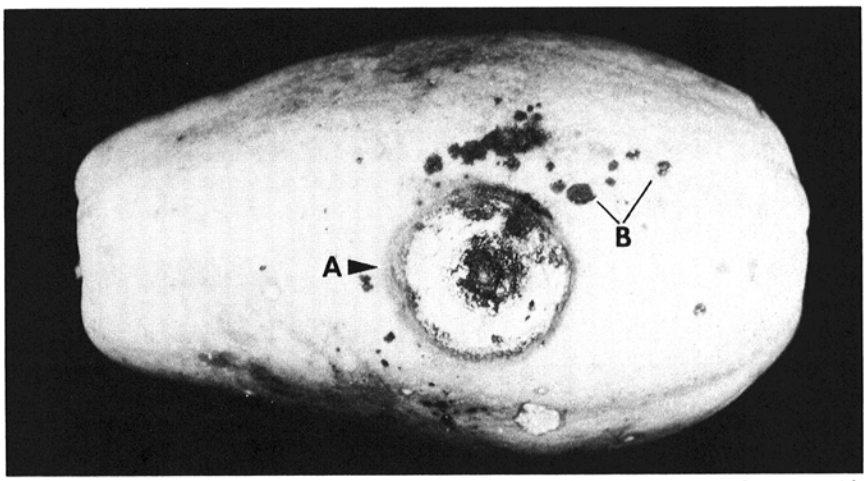

Fig. 1. Naturally infected papaya fruit showing symptoms of *Colletotrichum gloeosporioides* infection: **(A)** anthracnose and **(B)** chocolate spot in various stages of development.

Table 1. Distribution of anthracnose and chocolate spot symptoms of papaya harvested at successive stages of maturity after inoculations with *Colletotrichum gloeosporioides* conidia

		Fruits diseased by *C. gloeosporiodes*					
		Total[a]		Anthracnose		Chocolate spot	
Harvest period (no.)	Days after inoculation	Control (no.)	Inoculated (no.)	Control (%)	Inoculated (%)	Control (%)	Inoculated (%)
1	0	11/55	23/41	14	54***[b]	10	9
2	10	5/41	15/53	9	23*	5	6
3	26	11/67	30/101	16	30*	3	6
4	43	6/37	32/57	11	26*	5	32**
5	50	3/41	19/47	7	28**	0	17**
6	57	1/34	16/47	0	17**	3	21**
7	65	5/38	27/42	10	17	5	45***
8	72	4/33	27/43	9	28*	0	30***
9	79	2/34	29/58	6	17	0	29***
10	87	12/30	42/52	12	19	25	62***
11	95	7/34	26/46	12	20	6	35***
12	102	18/60	25/50	13	22	18	32**
13	108	4/52	33/61	8	16	0	41***
14	159	14/40	21/71	27	18	10	8
15	167	9/39	8/33	21	21	5	9
16	174	4/36	3/32	11	9	0	3

[a] No. diseased fruit/total fruit.
[b] Statistically significant differences at * $P = 0.05$, ** $P = 0.01$, and *** $P = 0.001$, according to chi-square distribution.

Fig. 2. Papaya fruit inoculated with *Colletotrichum gloeosporioides* and left on the tree until fully ripe. Black circle on light-colored fruit in the center indicates inoculation site.

anthesis) are susceptible to *C. gloeosporioides* infection, substantiating claims by Wardlaw et al (23), who used detached fruit only. Because *C. gloeosporioides* produces an extracellular cutinolytic enzyme (10) by which the pathogen gains entrance into green host tissue on detached fruit, this same mode of penetration also may occur in the field. After penetration, fungal growth is arrested until the fruit matures, with subcuticular hyphae possibly being a dormant form of the fungus (9).

The factors involved in suppressing fungal development of infected immature fruit remain unknown. Evidence for fungistatic compounds such as tannins and phenolic compounds found in unripe fruits has been reported in a number of host-parasite systems (7,14,21) but their presence at the appropriate site, at the right time, and in adequate concentrations requires further proof. Papayas are known to contain the fungal inhibitor benzyl isothiocyanate (16), but its role in latency also has yet to be demonstrated. With apple (*Malus sylvestris* Mill.) fruit rots caused by various fungi (19), the lack of a threshold concentration of sugars appears to be involved in immature fruit resistance, whereas at the onset of the climacteric phase, high concentrations of sucrose accumulate that apparently are available to the pathogens as an energy source. Immature papayas contain no starch and have about 75% sugars as a percentage of dry weight (12). At harvest, sucrose, which comprises about 80% of the total sugars, is converted to simpler reducing sugars (8). Thus, it is difficult to explain the breaking of latency in papayas by changes in nutrition or substrate availability because *Colletotrichum* sp. can utilize sucrose as well as glucose and fructose (18). As Simmonds (17) concluded, a complex interaction of factors may be responsible for the latency of *C. gloeosporioides* in papayas.

We can conclude from the data that an inverse relationship exists between the occurrence of anthracnose and chocolate spot symptoms. Chocolate spot has been recognized as a distinct disease thought to be caused by different physiological strains (11). Because strain differences did not account for differences in symptom expression in the inoculation studies, the results suggest that environmental factors or possibly fruit maturity played a role in symptom expression. A previous study (20) suggested that mitotically active fruit may form periderm and callose in response to fungal invasion. Such a defense mechanism might cause aborted anthracnose lesions, resulting in the more superficial chocolate spot symptom at early stages of fruit development. Fruit nearing maturity, however, may permit greater proliferation of the fungus, giving rise to the deep-seated anthracnose lesion. Observation of symptom expression might allow estimation of early season versus late season infection frequencies.

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