Detection of Latent Infections in Apple Fruit with Paraquat

Alan R. Biggs, Professor, West Virginia University, University Experiment Farm, P.O. Box 609, Kearneysville 25430

ABSTRACT

Biggs, A. R. 1995. Detection of latent infections in apple fruit with paraquat. Plant Dis. 79:1062-1067.

This research was conducted to assess the effect of paraquat on the breakdown of apple fruit (pre- and postharvest), to identify the fungi recovered from paraquat-treated fruit, and to determine the potential use of paraquat in a rapid quantitative measure of the pathogenic component of fruit storability. In 1992, a greater incidence of Golden Delicious and Nittany apple fruit developed acervuli of *Colletotrichum acutatum*, conidiophores of *Alternaria alternata*, and pycnidia of *Botryosphaeria dothidea* after treatment with paraquat than without treatment. In 1993, fungi observed on both cultivars were *C. acutatum*, *B. dothidea*, *Phoma* spp., *Phyllosticta solitaria*, *Penicillium expansum*, and *A. alternata*. Treatment of asymptomatic fruit sections with paraquat facilitated the detection of only *B. dothidea*, *Phoma* spp., and *P. solitaria* on Golden Delicious fruit. Exposure of Nittany fruit to paraquat facilitated the detection of *B. dothidea*, *P. expansum*, *Phoma* spp, and *P. solitaria*, but not that of *C. acutatum* or *A. alternata*. Golden Delicious fruit inoculated with *B. dothidea* or *C. acutatum*, harvested when asymptomatic, and treated with paraquat, yielded 80 and 20% infection, respectively, compared with only 6.7 and 0%, respectively, for the untreated controls. Following exposure to paraquat, naturally infected symptomatic Golden Delicious fruit exhibited signs of *B. dothidea*, *P. expansum*, *A. alternata*, *Phoma* spp., and *P. solitaria*. The incidence of *C. acutatum* on paraquat-treated fruit was positively correlated with the incidence after cold storage ($r = 0.98$) and after cold storage followed by a 4-week incubation at 22 ± 2°C ($r = 0.79$). The incidence of *B. dothidea* on paraquat-treated fruit was not correlated with the incidence after cold storage; however, there was a positive correlation after fruit removed from cold storage were incubated at 22°C for 4 weeks ($r = 0.95$). The incidences of *P. expansum* and *A. alternata* after paraquat treatment were correlated with their incidences after only cold storage ($r = 0.81$ and $r = 0.85$, respectively). The incidences of *Phoma* spp. and *P. solitaria* on paraquat-treated fruit were not correlated with their respective incidences after any storage and incubation conditions.

In the combined mid-Atlantic region of Pennsylvania, Virginia, West Virginia, and Maryland, apples are grown on approximately 28,000 ha and command a market value of about $124.3 million annually. West Virginia grows approximately 6,120 ha of apples with Delicious being the leading cultivar, and York and Golden Delicious ranking second and third, respectively. With increased consumer awareness of food safety issues and the probable loss or withdrawal of fungicides for postharvest use on fruits and vegetables in the United States, the influences of preharvest management practices on crops in the postharvest environment must be better understood. Storability of apples following harvest is a major concern of the industry in the mid-Atlantic region.

Storage quality is determined by measuring parameters that include fruit firmness and acid content (measured as titratable acids), incidence and severity of superficial scald, and decay. One aspect of rating fruit for decay that is not generally practiced is the identification of the fungal-decay organisms established in the preharvest period. Such information may help improve postharvest management of pathogens because the identity of the pathogens reflects on the preharvest programs, providing information necessary for making changes in these programs to achieve optimum storage time.

Studies to determine the incidence of decay in postharvest fruit require storage periods of 3 to 6 months to allow decay fungi to develop. Shortening the period to 7 to 21 days would be an important improvement in assessing postharvest pathogens. In addition, the development of such a technique for stored fruit could provide an opportunity to make informed decisions on the proper storage time and conditions for particular lots. Isolation of fruit pieces onto agar media could be used to screen fruit for fungal organisms; however, no one medium is adequate to detect all important fruit pathogens and, if practiced, such isolation techniques tend to be overly labor intensive. Hastening the breakdown of fruit with the herbicide paraquat may hold promise for accelerating the appearance of decay fungi and their fruiting structures.

Scientific Article No. 2479 of the West Virginia Agricultural and Forestry Experiment Station.

Accepted for publication 20 July 1995.

Paraquat was first used to detect latent infections by *Colletotrichum truncatum* (Schwein.) Andrus & W. D. Moore, *Phomopsis phaseoli* (Desmaz.) Sacc., and *Cercospora kikcuchii* (Matsumoto & Tomoyasu) M. W. Gardner in field-grown soybean stem and pod tissues (7). Later, this method was used in a field study that determined the effect of environmental conditions on infection and latent colonization of soybean plants by *Phomopsis longicolla* T. W. Hobbs (25), in studies of the epidemiology and latent colonization of soybean and weed tissues by *Colletotrichum* spp. (18,21,22), and of lupin in western Australia by *Phomopsis leptostromiformis* (Kühn) Bubák in Kab. & Bubák (9). The herbicide also enhanced the recovery of *Cercospora canescens* Ellis & G. Martin from bean tissue in Brazil (10) and *Monilinia fructicola* (G. Wint.) Honey in symptomless plum fruits following severe blossom blight (23).

Paraquat was first used on apple in our laboratory to determine the occurrence of latent infections on Nittany apple fruit by *Alternaria alternata* (Fr.:Fr.) Keissl., a serious pre- and postharvest pathogen causing significant losses in this cultivar (3). In these studies, latent infections due to other fungi were detected. Of particular interest was the occurrence of bitter rot, caused by *Colletotrichum* spp. and white rot, caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. The objective of this research was to assess the use of paraquat for the rapid qualitative and quantitative identification of latent preharvest and storage decay pathogens of apple.

MATERIALS AND METHODS

Preharvest and postharvest studies with asymptomatic and symptomatic whole fruit. In 1992, Nittany apple fruit at various stages of maturity (described below) were selected. This cultivar is highly susceptible to bitter rot, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and *C. acutatum* J. H. Simmonds, and Alternaria rot, caused by *A. alternata*. Fruit selected for treatment had received biweekly sprays of captan + benzimidazole at label recommended rates. Fruit were observed for any disease symptoms, marked with an indelible pen, washed in tap water, dipped in 70% ethanol for 2 min, in 0.5% NaOCl for 7 min, and then rinsed in sterile distilled water for 1 min. Whole, surface-disinfested fruit were then dipped for 1 min in 2,900 µg/ml paraquat (filter sterilized 1,1′-dimethyl-4,4′–bipyridinium dichloride in sterile

distilled water), and placed on autoclaved paper trays enclosed in plastic Ziploc bags with moistened paper towels (autoclaved) to provide high humidity. Fruit were incubated at 22°C) under natural diurnal light conditions and were observed after 1, 2, 3, and 6 weeks for rate of breakdown and appearance of fungi. After fungi appeared, they were transferred aseptically to 2% potato-dextrose agar (PDA, Difco, Detroit, Mich.) in 9-cm-diameter plastic petri dishes. Control fruit received no paraquat and were either surface disinfested with ethanol and 0.5% NaOCl or received no treatment. Control fruit were incubated and observed concomitantly with the paraquat-treated fruit. Fruit were observed weekly until the fruit could no longer be manipulated (6 weeks usually). The experiment was conducted five times with groups of 20 replicate fruit per treatment collected at 6 weeks preharvest, 4 weeks preharvest, harvest maturity, 4 weeks postharvest (from cold storage), and 8 weeks postharvest (from cold storage).

Studies with asymptomatic fruit sections and assessment of the predictive capability of the paraquat test results. In 1993, symptomless Golden Delicious and Nittany apple fruit (160 of each) were selected and brought into the laboratory. Fruit selected for the treatments described below had received no fungicide sprays after second cover (mid-June). Sixty fruit were washed in tap water, dipped in 70% ethanol for 2 min, then in 0.5% NaOCl for 7 min, and rinsed in sterile distilled water for 1 min. Thirty fruit were cored and sliced with a sterile metal template, and a single fruit section from each was dipped for 1 min in 6,000 µg/ml paraquat, and placed in sterile 1-pint Mason jars with filter paper lids (Whatman No. 4). Jars were then placed within Ziploc storage bags with a 7-mm-diameter hole punched near the top to provide air exchange. Fruit were incubated in the dark at 22 ± 2°C for 5 days, then hydrated with 1 ml of sterile distilled water applied to the filter paper lid, moved into constant fluorescent light, and observed weekly for 3 weeks for tissue breakdown (90% fruit surface with necrotic, water-soaked appearance) and signs of fungi. Sections from the same 30 fruit as above were treated identically except for the paraquat dip and served as controls. Thirty untreated whole fruit from each cultivar were incubated at 22 ± 2°C to observe the natural development of symptoms.

One hundred untreated fruit was placed in storage at 2 ± 1°C to assess the predictive capability of the paraquat test results. Fruit were assessed visually for signs and symptoms of rots after 3 and 5 months. After 5 months, fruit were incubated at 22°C and assessed after 2 and 4 weeks. The experiment was repeated five times with Golden Delicious fruit and four times with Nittany fruit at varying stages of maturity. For Golden Delicious, fruit were harvested at Julian dates 215, 228, 243, 264, and 284 (7, 5, and 3 weeks before maturity, optimum harvest maturity, and 3 weeks post-optimum maturity, respectively). For Nittany, fruit were harvested at Julian dates 228, 264, 284, and 299 (8 and 3 weeks preharvest, optimum harvest maturity, and 2 weeks post-optimum maturity, respectively). Optimum harvest maturity was determined with the starch-iodine test (8).

Studies with naturally infected symptomatic fruit and asymptomatic, inoculated fruit. In 1993, attached fruit were inoculated with a spore suspension of 1×10^3, 1×10^4, or 1×10^5 conidia/ml of either *C. acutatum* or *B. dothidea*. Cultures of both fungi were maintained on PDA. Sporulating cultures were flooded with sterile, distilled water and scraped with a rubber spatula. The suspension of conidia and mycelial fragments was passed through three layers of cheesecloth to remove the majority of mycelial fragments. Concentration of conidia was determined with a hemacytometer and then adjusted to the desired level. Strips of autoclaved cheesecloth, 2.5 cm wide and three layers thick, were dipped in the conidial suspensions and wrapped around the intact fruit. Fruit were then wrapped completely with aluminum foil and kept for 7 to 12 days. Control fruit for the inoculations were prepared as above except the inoculum was excluded from the cheesecloth wrap. Approximately 15 days after inoculation, symptomless fruit were collected, treated with paraquat, and incubated as described above. Controls for the paraquat procedure included fruit that were surface disinfested but not exposed to paraquat, and whole untreated fruit allowed to incubate at 22 ± 2°C. The experiment was conducted three times with Golden Delicious and once with Nittany apples. For Golden Delicious, samples of fruit exhibiting small (≤3 mm), red superficial lesions, often around lenticels, were collected at Julian date 284 and treated with paraquat or allowed to incubate at room temperature.

Data analysis. Data were analyzed for each cultivar separately with the general linear models procedure, using type I sums of squares for balanced data and type IV sums of squares for unbalanced data (SAS Institute, Cary, N.C.). For Golden Delicious, noninoculated, asymptomatic fruit data were analyzed separately (with type I sums of squares) from the symptomatic fruit and the inoculated, asymptomatic fruit, which were analyzed together (with type IV sums of squares). For Nittany, data for all fruit were analyzed together using type IV sums of squares. Means were separated with the Waller-Duncan *k*-ratio *t*-test. Contrast comparisons also were made among treatment pairs for each cultivar to determine the utility of the paraquat treatment to detect latent pathogens. Correlation analysis was conducted on the incidences of the various fungi from the paraquat-treated fruit sections with their respective incidences after 5 to 6 months of cold storage, and again after an additional 4 weeks at 22°C.

RESULTS

Preharvest and postharvest studies with asymptomatic and symptomatic whole fruit. Approximately 13% of paraquat-treated apples developed acervuli of *C. acutatum*, 36% showed conidia of *A. alternata*, and 18% exhibited pycnidia of *B. dothidea*. Surface-disinfested control fruit exhibited fungal activity, with 14% of fruit showing acervuli of *C. acutatum* and 20% showing conidia of *A. alternata*. *Botryosphaeria dothidea* was not observed in the control samples. The incidence of *A. alternata* and *B. dothidea*, but not *C. acutatum*, was significantly greater in the paraquat-treated fruit than in the control fruit ($P \leq 0.05$). The rate at which whole fruit senesced after exposure to paraquat declined with prolonged cold storage. Several other genera of fungi were detected on the fruit used in the 1992 tests, including *Penicillium*, *Cladosporium*, *Pullularia*, *Pestalotia*, *Arthrobotrys*, *Phoma*, and *Fusarium*.

Studies with asymptomatic fruit sections and assessment of the predictive capability of the paraquat test results. In 1993, fewer genera of fungi were observed in our samples than in 1992, mainly because the use of a sterile metal coring and slicing template resulted in the elimination of stem and calyx tissues that may be more difficult to surface disinfest. Predominant fungi observed on both cultivars were *C. acutatum*, *B. dothidea*, *Phoma* spp., *Phyllosticta solitaria* Ellis & Everh., *Penicillium expansum* Link, and *Alternaria* spp. *Botryosphaeria obtusa* (Schwein.) Shoemaker, the cause of black rot of apple, was not observed during the course of these experiments.

For Golden Delicious fruit, the brown, water-soaked appearance of fruit sections after 3 weeks was consistently associated with the paraquat-treated fruit sections, which showed significantly greater necrosis and water-soaking (97.3%) than the paraquat control (9.3%) or the whole fruit (7.0%) after 8 weeks (Table 1). The effect of the date on which the fruit were collected for the tests was significant for water-soaking ($P \leq 0.05$) but was not significant for detection of any of the fungal pathogens (data not shown). Fruit harvested later in the season (Julian date 284) showed a greater percentage of water-soaked fruit sections than fruit harvested earlier in the season (Julian dates 215 and 243) (data not shown).

Treatment of noninoculated, asymptomatic fruit sections with paraquat facilitated the detection of *B. dothidea* (27.3%) and *P. solitaria* (23.8%) on Golden Deli-

cious fruit, whereas control fruit sections had 0.7 and 0 %, and whole fruit 0 and 0%, respectively (Table 1). There were no significant differences among treatments for any of the other fungi.

For Nittany fruit, the paraquat-treated fruit sections showed significantly greater necrosis and water-soaking (76.2%) than the control (6.7%) or the whole fruit (13.7%) (Table 2). The effect of the date on which the fruit were collected for the experimental runs was not significant for water-soaking, but was significant ($P \leq 0.05$) for detection of *C. acutatum* and *P. expansum*. No *C. acutatum* was detected on the earliest Julian date (228), and the amounts detected on the other three dates were significantly higher but were similar to each other (data not shown). The greatest amount of *P. expansum* (31.1%) was detected on the latest Julian date (299), and this was significantly greater than the levels observed on the other three dates (data not shown).

Data from symptomatic and asymptomatic fruit were combined and analyzed to determine the utility of paraquat exposure for detecting latent infections (Table 3). For Golden Delicious, exposure of fruit sections to paraquat facilitated the detection of *B. dothidea*, *Phoma* spp., and *P. solitaria* (Table 3). Exposure of Nittany fruit sections to paraquat facilitated the detection of several fruit pathogens. There were significant differences for detection of *B. dothidea*, *P. expansum*, *Phoma* spp, and *P. solitaria*. No significant differences were observed for *C. acutatum* or *Alternaria* spp., pathogens to which the cultivar Nittany is highly susceptible. For *P. expansum*, paraquat-treated fruit exhibited an overall mean of 17.6% fruit infection, compared with 0.4% for the controls.

The incidences of the various fungi detected with the paraquat test were examined for the presence of correlation with the incidences observed after 5 to 6 months in cold storage and again after an additional 4-week incubation. The incidence of *B. dothidea* from paraquat-treated fruit was not correlated with incidence after cold storage, except after incubation ($r = 0.95$, $P \leq 0.003$). The incidence of *C. acutatum* from paraquat-treated fruit was positively correlated with the incidence after cold storage ($r = 0.98$, $P \leq 0.0001$) and after cold storage plus incubation at 22°C ($r = 0.79$, $P \leq 0.06$) (Table 4). Incidences of *P. expansum* and *A. alternata* after paraquat treatment were correlated with their incidences after cold storage ($r = 0.81$, $P \leq 0.05$, and $r = 0.85$, $P \leq 0.03$, respectively), but they were not correlated after fruit had incubated for 4 weeks. Incidences of *Phoma* spp. and *P. solitaria* from paraquat-treated fruit were not correlated with their respective incidences after cold storage only nor after cold storage plus 4-week incubation at 22°C.

Studies with naturally infected symptomatic fruit and asymptomatic, inoculated fruit. For Golden Delicious fruit inoculated with *B. dothidea* or *C. acutatum*, those treated with paraquat yielded 71 and 90% infection, respectively, from symptomless fruit, compared with 30 and 38%, respectively, for the controls (Table 5). For inoculated fruit, differences between paraquat-treated and the control were apparent only for *C. acutatum*. Fruit subjected to the inoculation procedure, but without fungal conidia included, showed no *C. acutatum*; however, 35% yielded *B. dothidea* (Table 5). This level of *B. dothidea* was similar to the amount detected in noninoculated, asymptomatic fruit (Table 1). In inoculated fruit, levels of *B. dothidea* ranged from 0 to 90%, with the paraquat-treated fruit exhibiting significantly more *B. dothidea* than incubated

Table 1. Percentage of fruit exhibiting surface breakdown and fungi observed 3 weeks following treatment of Golden Delicious apple fruit slices with paraquat, surface disinfestation with no paraquat (control), or incubation of whole fruit at 22°C for 8 weeks

Treatment	≥90% fruit surface water-soaked	*Colletotrichum acutatum*	*Botryosphaeria dothidea*	*Penicillium expansum*	*Alternaria* spp.	*Phoma* spp.	*Phyllosticta solitaria*
Paraquat-treated	97.3 a[y,z]	0.7 a	27.3 a	2.7 a	11.1 a	4.7 a	23.8 a
Control	9.3 b	0.0 a	0.7 b	4.0 a	5.3 a	1.3 a	0.0 b
Whole fruit	7.0 b	0.0 a	0.0 b	2.7 a	0.0 a	0.0 a	0.0 b

[y] Each value is the mean of 150 observations from 30 fruit collected on each of five sample dates (Julian dates 215, 228, 243, 264, and 284).
[z] Different letters in columns denote significant differences according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$). Means separations conducted on transformed data (arcsine square root of the percentage).

Table 2. Percentage of fruit exhibiting surface breakdown and fungi observed 3 weeks following paraquat treatment of noninoculated, asymptomatic Nittany apple fruit slices, slices from asymptomatic fruit that had been inoculated with *Botryosphaeria dothidea* or *Colletotrichum acutatum*, slices surface disinfested with no paraquat (control), or incubation of whole fruit at 22°C for 8 weeks

Treatment	≥90% fruit surface water soaked	*Colletotrichum acutatum*	*Botryosphaeria dothidea*	*Penicillium expansum*	*Alternaria* spp.	*Phoma* spp.	*Phyllosticta solitaria*
Asymptomatic fruit							
Paraquat-treated	76.2 abc[y,z]	19.2 cd	4.2 bc	20.4 abcd	52.9 abc	7.1 a	25.0 a
Control	6.7 d	9.2 cd	0.8 c	1.7 cd	20.8 abcd	0.0 a	2.5 a
Whole fruit	13.7 d	31.7 bc	1.7 bc	12.9 bcd	3.8 cd	0.0 a	1.7 a
Asymptomatic, *Botryosphaeria*-inoculated fruit							
Paraquat-treated	100.0 a	30.0 bc	30.0 a	30.0 ab	10.0 bcd	0.0 a	0.0 a
Control	20.0 cd	0.0 d	0.0 c	0.0 d	80.0 a	0.0 a	0.0 a
Whole fruit	0.0 d	25.0 bc	0.0 c	25.0 abc	0.0 d	0.0 a	0.0 a
Asymptomatic, *Colletotrichum* inoculated fruit							
Paraquat-treated	90.0 ab	80.0 a	20.0 ab	10.0 bcd	10.0 bcd	10.0 a	20.0 a
Control	20.0 cd	80.0 a	0.0 c	0.0 d	30.0 abcd	0.0 a	0.0 a
Whole fruit	28.6 bcd	92.9 a	0.0 c	53.6 a	0.0 d	0.0 a	0.0 a
Asymptomatic, inoculation control fruit							
Paraquat-treated	90.0 ab	60.0 ab	0.0 c	10.0 bcd	20.0 abcd	0.0 a	20.0 a
Control	10.0 d	20.0 bc	0.0 c	0.0 d	20.0 abcd	0.0 a	10.0 a
Whole fruit	0.0 d	32.2 bc	0.0 c	0.0 d	66.7 ab	0.0 a	33.0 a

[y] Each value for asymptomatic fruit is the mean of 120 observations from 30 fruit collected on each of four sample dates (Julian dates 228, 264, 284, and 299). Each value for inoculated (and inoculation control) paraquat-treated and control fruit is the mean of 10 observations. Each value for whole fruit (8 weeks) is the mean of 28 observations. All inoculated fruit collected and treated on Julian date 299.
[z] Different letters in columns denote significant differences according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$). Means separations conducted on transformed data (arcsine square root of the percentage).

whole fruit. Date of harvest was not significant for any of the fungi examined.

For inoculated Nittany fruit collected at 2 weeks after optimum harvest maturity, *Botryosphaeria* isolates were recovered, after treatment with paraquat, from 30% of fruit inoculated with *B. dothidea* (Table 2). This was significantly greater than levels of *B. dothidea* recovered from paraquat-treated asymptomatic fruit (4.2%), and from paraquat and inoculation controls (both 0%) (Table 2). For fruit inoculated with *C. acutatum*, the fungus was recovered from 80% of paraquat-treated fruit as well as from 80% of fruit that were surface disinfested but not treated with paraquat (Table 2). In addition, 60% of the fruit not exposed to conidia yielded *C. acutatum* after paraquat treatment. This was not significantly different from the recovery levels from inoculated fruit, although it was significantly higher than that obtained from noninoculated, asymptomatic fruit (19.2%) (Table 2).

Fruit deemed symptomatic exhibited faint to pronounced reddish lesions with dark centers, usually associated with a lenticel. Following exposure to paraquat, symptomatic Golden Delicious fruit sections exhibited signs of *B. dothidea* (70%), *P. expansum* (20%), *A. alternata* (40%), *Phoma* spp. (20%), and *P. solitaria* (20%). No significant differences were observed among treatments for incidence of the various fungi (Table 5).

DISCUSSION

Treatment of apple fruit with paraquat hastened the breakdown of fruit and facilitated the detection of probable latent infections of *B. dothidea*, *Phoma* spp., and *P. solitaria* on Golden Delicious; and *A. alternata*, *P. expansum*, *Phoma* spp., and *P. solitaria* on Nittany. While latent infections caused by *C. acutatum* could be detected with paraquat, its use did not improve detection beyond that achieved by incubating whole fruit at 22°C. Occurrences of symptomless or latent infections of apple fruit have been either reported or conjectured for several of the pathogens included in the present study; however, definitive studies on the nature of latent infections on apple have not been conducted. Although the present findings do not conclusively demonstrate the latent nature of the pathogens detected with paraquat, for some of the pathogens included here these findings are the strongest evidence to date in support of latency.

For blue mold, caused by *P. expansum*, Baker and Heald (1) obtained the first positive evidence in the U.S. that, even though the majority of infections occur at wounds (14), a significant number of infections originate at lenticels and may remain undetected until storage. Further studies (2,19), showed that delayed maturity is associated with increased incidence of lenticel infection by blue mold, and our finding (with Nittany only) of increased *Penicillium* detection with fruit age corroborates these earlier reports.

For *Colletotrichum* spp. on apple, including *C. acutatum* and *C. gloeosporioides*, there are no reports of latent infections. These fungi usually establish infections of apple fruit through wounds, especially insect punctures, but occasionally can establish themselves directly through the epidermis (27). *Colletotrichum* spp. are known to incite quiescent infection of mango (11) and certain field crops (6).

It was widely believed for many years that *Botryosphaeria* spp. on apple, including *B. obtusa* and *B. dothidea*, entered fruit only through wounds (5,13,16,17). Kohn and Hendrix (20), using unwounded fruit and inoculum concentrations of *B. dothidea* as high as 3×10^6 conidia/ml, concluded, as others have, that *B. dothidea* is primarily a wound pathogen (only 8% of the inoculated fruit developed infections around the lenticels). In contrast, our data show relatively high levels of latent fruit infection by *B. dothidea*, and when stored fruit were incubated at room temperature for 2 to 4 weeks, the majority of these infections emerged to cause rot. The present study is the first to suggest the potential importance of latent apple fruit infections by *B. dothidea*; however, Wiehe (28), in 1952, described the life cycle of the fungus on tung and proposed the occurrence of latent infections on tung fruit.

There is scant literature on the infection of apple fruit by *Alternaria* or *Phoma* spp., or *P. solitaria* (26). In an earlier study, *A. alternata* was detected in symptomless fruit by plating fruit tissues on agar medium (4). On other fruits, latent infections by *Alternaria* have been well documented (12). The present study is the first to provide evidence of latent infection of apple fruit by these pathogens.

Growth of fungi from the stem and calyx end of the apples in the 1992 study limited the conclusions that could be made regarding the presence of latent infections. The observations of *C. acutatum* after 1 week from the middle portions of fruits are good evidence of latent infection. Only two of the *Alternaria* isolates emerged from middle portions of fruits, and because these isolates did not sporulate until 3 weeks, rapid colonization of fruits by other fungi emerging from the stem and calyx ends limited our ability to identify organisms associated with middle portions. The use of fruit sections, rather than whole fruit, effectively solved this problem. Latent infections by several genera of fungi on both cultivars were observed regularly in 1993. For some fungi, such as *A. alternata*, fruit past maturity also may be less useful for applying the paraquat procedure, given that paraquat-treated and control fruit yielded similar numbers of isolates.

Colletotrichum acutatum, *B. dothidea*, and *Phoma* spp. generally are regarded as postharvest pathogens that initiate incipient or quiescent infections during the growing season (5,15,24). Symptom expression in cold storage is considered minimal due to the inhibitory effect of low temperatures on fungal growth (5,15). The effects of low temperatures may account for the lack of correlation after storage for *B. dothidea* (17), and the subsequent significant correlation after 4-week incubation at 22°C. It is noteworthy then that the incidence of *C. acutatum* prior to storage, as determined with the paraquat test, was correlated with its incidence immediately

Table 3. Contrast comparisons for percentage of fruit exhibiting surface breakdown and fungi observed 3 weeks following treatment of Golden Delicious and Nittany apple fruit slices with paraquat, surface disinfestation with no paraquat (control), or incubation of whole fruit at 22°C for 8 weeks

Treatment	≥90% fruit surface water-soaked	*Colletotrichum acutatum*	*Botryosphaeria dothidea*	*Penicillium expansum*	*Alternaria* spp.	*Phoma* spp.	*Phyllosticta solitaria*
Golden Delicious							
Paraquat-treated	100.0 a[y,z]	19.7 a	49.7 a	9.7 a	14.5 a	10.9 a	14.8 a
Control	29.1 b	8.2 a	13.3 b	6.1 a	13.3 ab	0.6 b	0.0 b
Whole fruit	19.8 b	0.0 a	2.9 b	5.2 a	0.3 b	0.0 b	0.0 b
Nittany							
Paraquat-treated	83.6 a	35.2 a	9.5 a	18.8 a	36.0 a	5.5 a	20.0 a
Control	11.9 b	19.5 a	0.9 b	0.9 b	30.5 a	0.0 b	2.9 b
Whole fruit	11.0 b	39.7 a	0.5 b	18.6 a	11.7 a	0.0 b	5.7 b

[y] Each value is the mean of 285 (Golden Delicious) or 150 (Nittany) observations from fruit collected on each of five (Julian dates 215, 228, 243, 264, and 284, for Golden Delicious), or four (228, 264, 284, and 299, for Nittany) sample dates.

[z] Different letters in columns denote significant differences according to matrix analyses of univariate hypotheses ($P \leq 0.05$). Means separations conducted on transformed data (arcsine square root of the percentage).

following storage. This result suggests some ability of this fungus to develop within the fruit during storage. This is in contrast to previous studies that addressed apple bitter rot incited, presumably, by *C. gloeosporioides* (5,15).

For *P. expansum* and *A. alternata*, both of which are regarded as primarily postharvest decay fungi, significant correlation of their incidence after the paraquat test with incidence after cold storage demonstrates that symptomless infections can enlarge and cause losses during storage. The influence of cold temperatures on limiting disease development is largely due to the effect of temperature on the infection process rather than in limiting the growth of established (albeit symptomless) lesions (5). Although correlations of fungal detection prior to cold storage with fungal detection following cold storage were significant in some instances, the paraquat procedure tended to overestimate the incidences of *P. expansum* and *A. alternata* after cold storage; however, for *B. dothidea* and *C. acutatum*, the procedure provided accurate estimates of rot after cold storage and incubation.

These nuances in the pathology of apple postharvest rots are especially important as investigators are under pressure to develop disease management alternatives that place less reliance on fungicides. It is apparent from the present study that symptomless fruit with a significant proportion of latent infections are not suitable for long-term, cold storage if they are destined for the fresh market. However, a management strategy that permitted some level of latent infection to occur might be acceptable for processing fruit that is only utilized immediately upon removal from cold storage.

These results demonstrate the utility of the paraquat technique for apples in detecting latent infections of pre- and postharvest pathogens. In addition, the technique appears to have practical utility to aid in the early identification of rot organisms, the initial symptoms of which are often very similar.

ACKNOWLEDGMENTS

The technical assistance of Barbara Bergman is gratefully acknowledged. I also thank K. S. Yoder and K. D. Hickey for helpful discussions during the preparation of the manuscript. Partial financial support for this study was provided by the Research Committee of the State Horticultural Association of Pennsylvania.

LITERATURE CITED

1. Baker, K. F., and Heald, F. D. 1932. The importance of lenticel infection by *Penicillium expansum*. Wash. Agric. Exp. Sta. Bull. 264:1-15.
2. Baker, K. F., and Heald, F. D. 1934. An investigation of factors affecting the incidence of lenticel infection of apple by *Penicillium expansum*. Wash. Agric. Exp. Sta. Bull. 298:1-48.
3. Biggs, A. R. 1994. Determining the pathogens present in pre-harvest apple fruit. (Abstr.) Phytopathology 84:541.
4. Biggs, A. R., Ingle, M., and Solihati, W. D. 1993. Control of *Alternaria* infection of fruit of apple cultivar Nittany with calcium chloride and fungicides. Plant Dis. 77:976-980.
5. Brooks, C., Cooley, J. S., and Fisher, D. F. 1935. Diseases of apples in storage. USDA Farmers' Bulletin No. 1160, Washington, D.C.
6. Cerkauskas, R. F. 1988. Latent colonization by *Colletotrichum* spp.: Epidemiological considerations and implications for mycoherbicides. Can. J. Plant Pathol. 10:297-310.
7. Cerkauskas, R. F. and Sinclair, J. B. 1980. Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70:1036-1038.
8. Chu, C. L. 1988. Starch-iodine test for determining maturity and harvest dates of Empire, Idared, and Spartan apples. Ont. Minist. Agric. Food, Toronto, Ontario, Canada. AGDEX 211/50 (88-090).
9. Cowling, W. A., Wood, P. McR., and Brown, A. G. P. 1984. Use of paraquat-diquat herbicide for the detection of *Phomopsis leptostromiformis* infection in lupins. Australas. Plant Pathol. 13:45-46.
10. Dhingra, O. D., and Asmus, G. L. 1983. An efficient method for *Cercospora canescens* in

Table 4. Incidence of various apple rot pathogens following treatment with paraquat, after 5 months of cold storage, after 5 months cold storage plus 4 weeks at 22°C, and the correlation of incidence after paraquat treatment with incidence after cold storage

Pathogen	Incidence (%) after paraquat treatment	Incidence (%) after storage and correlation		Incidence (%) after storage plus 4 weeks at 22°C and correlation	
Botryosphaeria dothidea	25.6[y]	0.5	r = NS[z]	34.2	r = 0.95
Colletotrichum acutatum	13.3	1.8	r = 0.98	16.0	r = 0.79
Penicillium expansum	14.4	0.5	r = 0.81	20.7	r = NS
Alternaria alternata	30.6	3.0	r = 0.85	2.3	r = NS
Phoma spp.	7.8	3.7	r = NS	3.0	r = NS
Phyllosticta solitaria	25.6	0.7	r = NS	6.2	r = NS

[y] All data from Golden Delicious and Nittany combined. Data from paraquat test are from Julian dates 243, 263, and 284 (Golden Delicious), and Julian dates 263, 284, and 299 (Nittany) (180 combined observations). Cold storage data represent 600 combined observations.
[z] Not significant.

Table 5. Percentage of fruit exhibiting surface breakdown and fungi observed 3 weeks following treatment of symptomatic and *Botryosphaeria*-inoculated Golden Delicious apple fruit slices with paraquat, surface disinfestation with no paraquat (control), or incubation of whole fruit at 22°C for 8 weeks

Treatment	≥90% fruit surface water soaked	*Colletotrichum acutatum*	*Botryosphaeria dothidea*	*Penicillium expansum*	*Alternaria* spp.	*Phoma* spp.	*Phyllosticta solitaria*
Symptomatic fruit							
Paraquat-treated	100.0 a[x,y]	0.0 c	70.0 ab	20.0 a	40.0 a	20.0 a	20.0 a
Control	10.0 b	0.0 c	10.0 bc	0.0 a	20.0 a	0.0 a	0.0 a
Whole fruit	39.3 ab	0.0 c	14.3 bc	16.1 a	1.8 a	0.0 a	0.0 a
Asymptomatic, *Botryosphaeria*-inoculated fruit							
Paraquat-treated	100.0 a	0.0 c	90.0 a	0.0 a	0.0 a	13.3 a	20.0 a
Control	45.0 ab	0.0 c	38.3 abc	0.0 a	20.0 a	0.0 a	0.0 a
Whole fruit	14.3 b	0.0 c	0.0 c	14.3 a	0.0 a	0.0 a	0.0 a
Asymptomatic, *Colletotrichum*-inoculated fruit							
Paraquat-treated	100.0 a	71.1 a	30.0 abc	3.3 a	10.0 a	0.0 a	11.1 a
Control	26.7 b	30.0 b	11.7 bc	4.4 a	13.3 a	0.0 a	0.0 a
Whole fruit	–[z]	–	–	–	–	–	–
Asymptomatic, control for inoculation experiment							
Paraquat-treated	100.0 a	0.0 c	35.0 abc	8.3 a	30.0 a	25.0 a	6.7 a
Control	46.7 ab	0.0 c	17.7 bc	16.7 a	20.0 a	0.0 a	0.0 a
Whole fruit	35.3 ab	0.0 c	2.9 bc	14.7 a	0.0 a	0.0 a	0.0 a

[x] Each value is the mean of 45 observations from 15 fruit collected on each of three sample dates (Julian dates 243, 264, and 284). Symptomatic fruit exhibited faint to pronounced red halo symptoms around lenticels (faint = dark center with faint red halo about 3-mm diameter; pronounced = red halo very red and dark center appeared dark brown and/or purplish, whole symptom >3-mm diameter and ranged up to 10-mm diameter).
[y] Different letters in columns denote significant differences according to the Waller-Duncan k-ratio t test ($P \leq 0.05$). Means separations conducted on transformed data (arcsine square root of the percentage).
[z] Data not available.

bean seed. Trans. Brit. Mycol. Soc. 81:425-426.
11. Dods, J. C., Estrada, A. B., Matcham, J., Jeffries, P., and Jeger, M. 1991. The effect of climatic factors on *Colletotrichum gloeosporioides*, causal agent of mango anthracnose in the Philippines. Plant Pathol. 40:568-575.
12. Droby, S., Prusky, D., Jacoby, B., and Goldman, A. 1986. Presence of an antifungal compound and its relation to the latency of *Alternaria alternata* in unripe peel of mango fruits. Physiol. Mol. Plant Pathol. 29:173-183.
13. Eid, R. F. 1959. Etiology and control of *Botryosphaeria ribis* in apple. M.S. thesis, University of Delaware, Newark.
14. English, H., Ryall, A. L., and Smith, E. 1946. Blue mold decay of Delicious apples in relation to handling practices. USDA Circular No. 751. Washington, D.C.
15. Eustace, H. J. 1908. Investigations on some fruit diseases. New York Agric. Exp. Sta. Bull. No. 297.
16. Fenner, E. A. 1925. A rot of apples caused by *Botryosphaeria ribis*. Phytopathology 15:230-234.
17. Fulkerson, J. F. 1960. *Botryosphaeria ribis* and its relation to a rot of apples. Phytopathology 50:394-398.
18. Hartman, G. L., Manandhar, J. B., and Sinclair, J. B. 1986. Incidence of *Colletotrichum* spp. on soybeans and weeds in Illinois and pathogenicity of *Colletotrichum truncatum*. Plant Dis. 70:780-782.
19. Kidd, M. N., and Beaumont, A. 1925. An experimental study of the fungal invasion of apples in storage, with particular reference to invasion through the lenticel. Ann. Appl. Biol. 12:14-33.
20. Kohn, F. C., Jr., and Hendrix, F. F. 1982. Temperature, free moisture, and inoculum concentration effects on the incidence and development of white rot of apple. Phytopathology 72:313-316.
21. Manandhar, J. B., Hartman, G. L., and Sinclair, J. B. 1986. *Colletotrichum destructivum*, the anamorph of *Glomerella glycines*. Phytopathology 76:282-285.
22. McLean, K. S., and Roy, K. W. 1988. Incidence of *Colletotrichum dematium* on prickly sida, spotted spurge, and smooth pigweed and pathogenicity to soybean. Plant Dis. 72:390-393.
23. Northover, J., and Cerkauskas, R. F. 1994. Detection and significance of symptomless latent infections of *Monilinia fructicola* in plums. Can. J. Plant Pathol. 16:30-36.
24. Rosenberger, D. A. 1990. Postharvest diseases. Pages 53-54 in Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, Minn.
25. Rupe, J. C., and Ferriss, R. S. 1987. A model for predicting the effects of microclimate on infection of soybean by *Phomopsis longicolla*. Phytopathology 77:1162-1166.
26. Scott, W. M., and Rorer, J. B. 1909. Apple blotch, a serious disease of southern orchards. USDA, Bureau of Plant Industry Bull. No. 144. Washington, D.C.
27. Von Schrenk, H., and Spaulding, P. 1903. The bitter rot of apples. USDA Bureau of Plant Industry Bull. No. 44. Washington, D.C.
28. Wiehe, P. O. 1952. Life cycle of *Botryosphaeria ribis* on *Aleurites montana*. Phytopathology 42:521-526.