Overwintering of *Alternaria mali*, the Causal Agent of Alternaria Blotch of Apple

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ABSTRACT

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Survival of *Alternaria mali* in lesions on leaves on grass or bare ground, in lesions on terminals, and as conidia in buds of Delicious apple trees was examined in three distinct orchard environments in North Carolina. No lesions were observed on terminals. Leaves on the ground were a more important overwintering site than buds at all three locations. Mean number of conidia detected per leaf averaged over all three locations and 2 years was 66.7, whereas an average of only 2.8 conidia per bud were detected. Conidia from buds also had lower germination (6.5%) than conidia from leaves (38.8%). Number of conidia per leaf and conidial germination did not differ consistently among locations. Number of overwintering conidia detected per leaf did not decrease from November 1991 to May 1992, but was less in May 1993 than in November 1992. Treatment of leaves with urea in the fall had minimal effects on amount of leaf area remaining in May. Overwintering on grass or bare ground or treatment with urea did not affect the number of conidia detected per leaf or their ability to germinate.

Alternaria blotch (caused by *Alternaria mali* Roberts) has become a serious disease of Delicious apple (*Malus* × *domestica*) in the southeastern United States. The disease was first observed in western North Carolina in 1988 (6). Since then it has been reported from all four neighboring states. The fungus causes circular brown lesions on leaves that can result in up to 70% defoliation. Fruit quality and yield also can be reduced (10).

Reports from Japan and South Korea indicate that *A. mali* overwinters as conidia on leaves, buds, and twig lesions of the susceptible cultivar Indo; however, no studies have been conducted on cultivars widely grown in the United States, such as Delicious (11,13). Furthermore, studies in Japan and South Korea did not quantify inoculum production in the overwintering sites.

Various treatments, including the use of urea (4) and biocontrol agents (1), may reduce the inoculum of *Venturia inaequalis* (Cooke) G. Wint. in apple orchards. Burchill (4) found that 5% urea sprayed in the fall enhanced leaf decomposition, which resulted in a 49 to 96% reduction in ascospore production and a 46 to 59% reduction in the number of apple scab lesions the following spring. The use of urea is an attractive option to reduce initial inoculum of pathogens because it is inexpensive, can be applied with conventional equipment, does not select for resistant strains of pathogens, and is safe. However, to our knowledge no one has investigated the use of urea sprays to reduce the inoculum of any apple pathogen except *V. inaequalis*.

The objectives of this study were to i) determine the primary source of overwintering inoculum of *A. mali* under conditions in North Carolina, ii) determine the effectiveness of urea in reducing leaf area and number and viability of overwintering conidia and iii) investigate the effect of grass or bare ground on the amount and viability of overwintering inoculum on leaves. A preliminary report has been published (8).

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MATERIALS AND METHODS

Locations for the study. Three orchards of Delicious apple located in different environments in North Carolina and with different Alternaria blotch histories were used: i) the McKay orchard, located in Henderson Co. in western North Carolina at 600 m elevation. The environmental conditions in this orchard are typical of the principal apple-growing areas in the mountains of North Carolina. Very severe Alternaria blotch, with up to 70% defoliation, was recorded in the orchard from 1989 to 1993; ii) the Saylor orchard, also located in western North Carolina (1,100 m elevation). The cooler temperatures in this orchard are similar to those in the midwestern and mid-Atlantic apple growing regions of the United States. The orchard has a history of moderate to severe Alternaria blotch intensity from 1989 to 1993; iii) the Central Crops Research Station (CCRS), located in Clayton, N.C. (100 m elevation). Temperature and rainfall at this location are characteristic of the warmer growing areas in the Piedmont of North Carolina, South Carolina, and Georgia. The disease does not occur naturally in the orchard, but was induced by inoculation of apple foliage with spore suspensions of *A. mali* each year from 1989 to 1993.

Preparation of the leaves in the field. Four hundred and twenty leaves of similar size and disease severity were collected on 15 October 1991 and 1992 at the McKay and Saylor orchards, and on 16 October 1991 and 1992 at the CCRS orchard. Leaves averaged 25.8 cm^2 and approximately 5 to 15% of each leaf was covered with lesions of Alternaria blotch. One half of the detached leaves were dipped into a urea solution (6 g per liter) for 5 min and allowed to dry. Urea-treated and nontreated leaves were placed in groups of five each between two 20 × 20 cm pieces of 1.2 × 1.2 cm mesh wire that were fastened together. Fourteen five-leaf packages (seven of which contained leaves dipped into urea solution) were placed together on 1-m^2 pieces of Saran cloth, which were used to prevent destruction of samples by earthworms and matting of the leaves to the soil surface. Three replicate sets were placed on bare ground or on grass, except at the Saylor orchard where the entire orchard floor was covered with grass. Urea-treated and untreated five-leaf packages were collected from each set every month beginning on 15 November 1991 and continuing until 15 May 1992 (period 1), and from 15 November 1992 to 15 May 1993 (period 2). The packages were stored in a cooler at 4°C until they were processed.

Preparation of leaf samples in the laboratory. After removal of petioles, each five-leaf sample was placed into 80 ml of distilled water and ground in a blender (Waring Blender 7011, model 31BL92, Waring Production Division, New Hartford, Conn.) for 90 s (30 s at slow speed + 30 s at fast speed + 30 s at slow speed with 15 s pause between each cycle). The entire suspension was filtered through four layers of cheesecloth. Twenty milliliters of the suspension was placed

into plastic tubes and centrifuged in an automatic superspeed centrifuge (Sorvall SS-3, Ivan Sorvall Inc., Norwalk, Conn.) at 6,000 rpm for 15 min. Eighteen milliliters was drawn off with a pipette, and the remaining pellet was resuspended and filtered through a double layer of cheesecloth. Two 50-µl drops from each sample were placed on a glass slide and incubated in a moist chamber at 24°C to allow conidia to germinate. After 48 h incubation, one drop of cotton blue in lactophenol was added to each 50-µl drop, and semipermanent slides were made by placing a 20 × 20 mm cover slip over each drop. The number of conidia in each drop and the number that germinated were recorded. A conidium was considered to have germinated when a germ tube was visible at 200×. Conidia of *A. mali* were differentiated from other *Alternaria* spp. by characteristic conidial morphology and size (3).

Preparation of bud samples. In the first year of the study, terminals were examined visually in all three orchards for the presence of Alternaria blotch lesions. A sample of five arbitrarily selected terminals from each of five trees with severe disease the previous season was brought into the laboratory and terminals were examined for presence of lesions under a dissecting microscope.

Bud samples were obtained from terminals collected from each of five trees at each location. Collection dates were the same as those for leaves except that no bud samples were taken in May because all buds had initiated growth. Terminals were placed in plastic bags and stored at 4°C until processed. Twenty arbitrarily selected buds of a similar size were excised from each five-terminal group and cut into pieces (ca. 0.5 mm). The entire sample was placed into 20 ml of distilled water, sonicated (Branson Sonifier 450, Branson Ultrasonics Corp., Danbury, Conn.) at 60% duty cycle for 3 min (six times 30 s with 15 s pause between each cycle), and filtered through a double layer of cheesecloth. The suspension was placed into a plastic tube and centrifuged for 15 min at 6,000 rpm. Eighteen milliliters of supernatant was drawn off and the pellet was resuspended in the remaining 2 ml. Two 50-µl drops were placed on each slide, and incubated. Germinated and nongerminated conidia were enumerated as described for the leaf samples.

Leaf area measurements. A photocopy was made of each five-leaf group before leaf samples were processed. Leaf area was measured using the Optimas ver. 3.01 software connected to a high resolution camera (COHU Solid State Camera, Model 4815-5000-0000). Leaf area was measured only for leaves that were collected in November and May of each year.

Climatological data. Mean daily temperature and precipitation were recorded at the meteorological stations nearest to the orchards used in this study. Hendersonville 1 NE is located 8 km from the McKay orchard, Banner Elk, 25 km from the Saylor orchard, and Smithfield, 16 km from the CCRS station.

Statistical analysis. Mean temperature and precipitation were calculated using the PROC MEANS procedure in Statistical Analysis Systems (SAS Institute, Cary, N.C.). Effects of treatments on numbers of conidia per leaf and bud and conidial germination were analyzed using PROC

Table 1. Mean daily temperatures recorded at meteorological stations located near apple orchards in three regions of North Carolina

	November 1991 to May 1992				November 1992 to May 1993			
	Mean		Departure from normal		Mean		Departure from normal	
Location[z]	Temp (C)	Precip (cm)	Temp (C)	Precip (cm)	Temp (C)	Precip (cm)	Temp (C)	Precip (cm)
McKay	9.2	11.5	+1.1	–0.1	8.5	14.9	+1.3	+5.6
Saylor	5.7	10.1	+0.8	–0.3	4.6	10.0	+0.7	–1.4
CCRS	10.2	6.8	–1.0	–2.4	10.3	11.0	+0.1	+1.1

[z] The McKay orchard is located in western North Carolina at 600 m elevation; the Saylor orchard is located in western North Carolina at 1,100 m elevation; the Central Crops Research Station (CCRS) orchard is located in Clayton, N.C., at 100 m elevation.

Table 2. Mean number of conidia of *Alternaria mali* detected per leaf or bud overwintering in three locations and the percentage that germinated after incubation in water at 24°C for 48 h

Year/Location	Mean number of conidia per leaf	Germination (%)	Mean number of conidia per bud	Germination (%)
1991-1992				
McKay[y]	120.2 a[z]	54.9 ab	4.2 NS	24.2 NS
Saylor	68.2 b	60.2 a	7.2	14.9
Central Crops Research Station	109.4 a	50.9 b	0.8	0.0
1992-1993				
McKay	21.1 b	24.3 NS	2.0 NS	0.0 NS
Saylor	44.3 a	21.8	1.0	0.0
Central Crops Research Station	37.2 ab	20.8	1.6	0.0

[y] The McKay orchard is located in western North Carolina at 600 m elevation; the Saylor orchard is located in western North Carolina at 1,100 m elevation; the Central Crops Research Station orchard is located in Clayton, N.C., at 100 m elevation.

[z] Letters are significantly different ($P = 0.05$), according to the Waller-Duncan k-ratio t test. NS = not significant ($P = 0.05$).

Table 3. Mean number of conidia of *Alternaria mali* detected per leaf overwintering in three locations and the percentage that germinated after incubation in water at 24°C for 48 h

	Period 1						Period 2					
	November 1991		April 1992		May 1992		November 1992		April 1993		May 1993	
Location	Conidia per leaf[w]	Germination (%)	Conidia per leaf[w]	Germination (%)	Conidia per leaf[x]	Germination (%)	Conidia per leaf[w]	Germination (%)	Conidia per leaf[w]	Germination (%)	Conidia per leaf[w]	Germination (%)
McKay[x]	89.1 ab[y]	53.5 NS	78.9 b	35.0 NS	172.6 a	60.5 a	59.6 NS	36.1 NS	12.0 NS	28.6 NS	8.6 NS	38.9 NS
Saylor	37.4 b	63.3	36.0 b	42.5	91.6 b	77.5 a	113.4	35.0	. . .[z]	. . .[z]	12.0	6.7
CCRS	97.7 a	58.5	320.0 a	31.6	50.6 b	26.3 b	100.6	36.1	13.4	0.0	15.4	13.3

[w] Number is based on three replications of five leaves from each site and month.

[x] The McKay orchard is located in western North Carolina at 600 m elevation; the Saylor orchard is located in western North Carolina at 1,100 m elevation; the Central Crops Research Station (CCRS) orchard is located in Clayton, N.C., at 100 m elevation.

[y] Numbers within the same column followed by different letters are significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t test. NS = not significant ($P = 0.05$).

[z] Missing data.

GLM in SAS. Treatment means were separated using the Waller-Duncan *k*-ratio *t* test. The same test was performed to separate means for reduction of leaf area.

RESULTS

Climatological data. Temperatures during the two sampling periods revealed three distinct environments (Table 1). CCRS was the warmest, Saylor the coldest, and McKay was the wettest site during the study. Temperatures during both overwintering periods were slightly above normal except for CCRS in period 1. In period 1, precipitation was below normal at all locations. In period 2, precipitation varied among the sites; precipitation at McKay was well above normal (Table 1).

Comparison of overwintering sites. No lesions were observed on any of the terminals examined. In period 1, more conidia of *A. mali* were recovered from leaves from the McKay and CCRS orchards than from Saylor (Table 2). In period 2, more conidia were detected on leaves from the Saylor and CCRS orchards. A larger percentage of the conidia recovered from leaves germinated in period 1 than in period 2, but differences in conidial germination among orchards were not great during either period (Table 2). Number of conidia per bud and their germination did not differ among the three locations. Conidial germination generally was lower in all samples in 1992–93 than in 1991–92. The most conidia were detected per bud at the Saylor and McKay orchards in periods 1 and 2 (Table 2). The number of conidia of *A. mali* detected per leaf and the percentage that germinated in November, April, and May of both years are presented in Table 3.

The effects of overwintering sites and urea on number of overwintering conidia and their germination. Number of conidia of *A. mali* detected per leaf did not differ on leaves overwintering on grass or bare ground, nor were there differences between leaves treated and not treated with urea. Similarly, treatments did not affect conidial viability, although germination in samples collected on bare ground in period 2 was lower but not significantly different from that in samples on grass (Table 4). The reduction in area of urea-treated leaves averaged over both periods was 57.8% from November to May, compared with 55.4% for leaves not treated (Table 5).

DISCUSSION

In this study, apple leaves on the orchard floor were the most important source of overwintering conidia of *A. mali*. No lesions were observed on any of the terminals examined. Although some conidia of *A. mali* were found in buds, they may not be of much epidemiological significance because temperatures at the bud break phenophase generally are not favorable for infection (7). This is different from some other apple diseases such as apple scab and frogeye leaf spot (caused by *Botryosphaeria obtusa*) in which conidia, found in bud scales, are believed to initiate early-season infections (2,3).

The abundance and viability of overwintering inoculum did not differ greatly among the three orchards with distinctly different environments. Survival in the coolest environment (i.e., Saylor) indicates a potential for *A. mali* to overwinter successfully in more northerly apple-growing areas of the eastern United States once it becomes established.

Although *A. mali* overwintered equally well at the three sites, disease intensity differed little in 1992 and 1993 from the previous 2 to 4 years at each location. Alternaria blotch was severe at McKay, moderate to severe at Saylor and was introduced each year at CCRS. Therefore, the ability of conidia of *A. mali* to overwinter does not appear to be the major influence on the intensity of Alternaria blotch the following season. Number of conidia detected per unit of leaf area and their germination was much lower at McKay and Saylor orchards from one winter to another, but disease intensity decreased very little at McKay and increased at Saylor in 1993. In addition, number of conidia per leaf was greatest at CCRS in period 2, but disease failed to develop to epidemic proportion in 1993 without artificial inoculation. The environmental conditions in the late spring determine the extent of epidemic development because *Alternaria* spp. are polycyclic pathogens with high apparent infection rates. In previous studies we determined that infection can occur in as little as 5.1 h at 20.3°C, and the incubation period is only 24 h (7).

It is not clear what factors influence the amount of overwintering inoculum in orchard and its viability. Temperature and precipitation decreased from the first winter to the second, although values of both parameters did not differ greatly between the two seasons. Precipitation was slightly greater than normal from November 1992 to February 1993 at two of the three locations, and the increased moisture may have favored competitive microorganisms in the leaves.

The number of conidia detected in leaf samples in April and May 1992 increased from those detected in other months (all data not presented). This suggests that in the spring during favorable weather, lesions on overwintered leaves resume sporulation and, if favorable conditions persist, inoculum could build to high levels and initiate primary infection when conditions become favorable in mid to late May under conditions in North Carolina.

Leaf area was not reduced by urea treatment and urea did not influence the number or viability of overwintering conidia. Ross and Burchill (12) and Burchill (4) suggested that the primary effects of urea on inoculum of *V. inaequalis* were based on (i) increase in the nitrogen con-

Table 4. Mean number of conidia of *Alternaria mali* detected per leaf overwintering in three locations on bare ground or grass and the percentage that germinated after incubation in water at 24°C for 48 h

	Sample period 1[x]		Sample period 2[x]	
Treatment	Conidia per leaf (mean)	Germination (%)	Conidia per bud (mean)	Germination (%)
Bare ground	93.3 NS[y]	51.2 NS	28.6 NS	14.9 NS
Bare ground + urea[z]	126.2	47.3	41.4	14.2
Grass	106.8	55.5	32.3	26.9
Grass+ urea	106.6	60.0	25.0	27.7

[x] Period 1 was from November 1991 to May 1992; period 2 was from November 1992 to May 1993.
[y] NS = no significant difference among treatments ($P = 0.05$) according to the Waller-Duncan *k*-ratio *t* test.
[z] Leaves were dipped into a urea solution (6 g per liter) for 5 min.

Table 5. Mean percent leaf area reduction from November to May recorded at three locations for leaves treated and not treated with urea[w]

	Mean reduction in leaf area (%)				
	Period 1[x]		Period 2		
Location	McKay	CCRS	McKay	Saylor	CCRS
Urea-treated[y]	61.9 NS[z]	64.9 NS	49.8 NS	62.5 NS	50.0 NS
Nontreated	43.3	42.1	52.8	74.1	64.9

[w] The McKay orchard is located in western North Carolina at 600 m elevation; the Saylor orchard is located in western North Carolina at 1,100 m elevation; the Central Crops Research Station (CCRS) orchard is located in Clayton, N.C., at 100 m elevation.
[x] Period 1 was from November 1991 to May 1992; period 2 was from November 1992 to May 1993.
[y] Leaves were dipped into a urea solution (6 g per liter) for 5 min.
[z] NS = no significant difference between treatments ($P = 0.05$) according to the Waller-Duncan *k*-ratio *t* test.

tent in leaves, which inhibits pseudothecial formation; (ii) increase in microbial activity on leaves, especially those that are antagonistic to *V. inaequalis*; (iii) direct fungitoxic effect of urea on *V. inaequalis*; and (iv) increased disappearance of leaf litter due to preference of soil fauna for treated leaves. The use of the Saran-cloth barrier precluded us from obtaining any data on increased feeding activity of soil fauna on treated leaves, but should not have affected any direct toxic effects of nitrogen or urea or increases or shifts in microbial activity. Although leaves were not in direct contact with the soil, soil particles that had washed or splashed on overwintered leaves were frequently observed adhered to leaf samples. Additionally, Burchill (4) and Cross et al. (5) used nylon mesh envelopes in their studies on the effect of urea on *V. inaequalis*. The apparent lack of effect of urea on *A. mali* may be due to the different overwintering structures of *A. mali* and *V. inaequalis* and the effect of urea and/or antagonists on them. Studies on the effect of urea on the growth of *A. mali* in the laboratory would help clarify any effect that it may have.

Because the amount of overwintering inoculum above a certain threshold does not appear to be the most important factor in determining Alternaria blotch intensity the following year, measures to reduce inoculum of *A. mali* in orchards during the winter or early spring may not be of great value. Apparently there is a very low threshold of inoculum needed to initiate disease in spring and any measure to decrease inoculum below that level would have to be extremely efficient and probably would be quite costly. Even if that goal is achieved, conidia of *A. mali* could still arrive from external sources (9). However, fall applications of urea may be useful as a disease management tool in Integrated Pest Management (IPM) programs for control of other apple pathogens that overwinter on leaves, such as *V. inaequalis* and *Mycosphaerella pomi*, the cause of Brooks spot.

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