Epidemiology and Control of Citrus Greasy Spot on Grapefruit in Texas

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ABSTRACT

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Greasy spot and greasy spot rind blotch (GSRB) in Texas are caused by *Mycosphaerella citri*. In a 4-yr study to determine the effect of *M. citri* on red grapefruit in the semi-arid Texas citrus area, the maximum spray program (cupric hydroxide—spring, benomyl + oil—summer) sharply reduced foliar greasy spot and somewhat reduced GSRB. The minimum program (citrus spray oils in the summer) was less effective, but significantly reduced disease incidence compared to the no fungicide control. Neither program increased yield or fruit size, or reduced defoliation compared to the control. Applications of acaricides to control the citrus rust mite, *Phyllocoptruta oleivora*, significantly reduced foliar greasy spot symptoms in 2 of 3 years in orchard studies and in a test on container-grown trees. In 1978, ascospore production by *M. citri* was low during spring, but sharp peaks occurred following flood irrigations in late June and in late July. Most ascospores were trapped during abundant rainfall in late August and early September and infection apparently occurred primarily in late summer. Symptoms did not appear until early spring and the disease did not cause excessive premature defoliation. Benefits of fungicidal control of the disease in Texas are few.

Additional key words: Citrus paradisi.

The cause of citrus greasy spot has been attributed to damage by the citrus rust mite, (*Phyllocoptruta oleivora* [Ashmead]) (1,6), a Cercospora-like fungus (2), physiological effects or combinations of these factors. Yamada (16) considered greasy spot to be another symptom produced by *Mycosphaerella horii* Hara which causes a brown spot disease of citrus leaves in Japan. Whiteside (9,11,12) described the causal agent as *Mycosphaerella citri* Whiteside and determined the epidemiology and control of the disease in Florida (9,10,13–15). Ascospores are produced in perithecia formed only on decaying leaves on the orchard floor. In Florida, they are released most abundantly during periods of high rainfall in June and July. Ascospores germinate and produce ramifying extramatricular mycelium on the underside of the leaf which penetrate through stomata. Symptoms develop slowly and do not appear until November or later. The imperfect state is a *Stenella* sp., but conidia play a minor role in the epidemiology of the disease (9). Penetration of stomata on the fruit produces a blemish, designated greasy spot rind blotch (GSRB), due to small necrotic flecks at the stomata and greening from the retention of chlorophyll in surrounding cells (11). Benomyl and copper fungicides provided the best control of the disease (10,15). Citrus spray oil, also used as an insecticide-acaricide, provides substantial control of the disease (13). Application time is not critical and a single spray in June or July provided optimum disease control in Florida (10).

Despite the established etiology of the disease, some questions remain on the possible effect of citrus rust mite on greasy spot severity. Earlier reports (2,6) on control of greasy spot by elimination of *P. oleivora* were based on use of sulfur, zineb, and citrus spray oil, all of which are known to control fungal diseases. However, van Brussel (8) reported reduction in greasy spot severity by using chlorobenzilate and trichlorofon which are not known to control fungal diseases.

Greasy spot has been reported from Texas and apparently is widely distributed in citrus areas of the world. However, all of these reports were based on symptomatology (15) and *M. citri* has been conclusively identified as the cause of the disease only in Florida.

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The purposes of the present study were to verify the cause of greasy spot disease on Texas citrus, to determine the effect of the disease on defoliation and fruit yield under semi-arid Texas conditions, to describe its epidemiology under semi-arid conditions, and to determine the effect of rust mite on greasy spot severity. A preliminary report of this study was published (7).

MATERIALS AND METHODS

Identification of the causal organism. Grapefruit leaf and fruit tissue with greasy spot-like symptoms were surface-disinfested by swabbing with 0.05% NaOCl, then with 70% ethanol. The surface layers were removed and bits of leaf mesophyll tissue and tissue from the substomatal areas on the fruit were transferred aseptically to corn meal agar (CMA).

Grapefruit leaves with abundant greasy spot lesions were collected from the field, dried on the greenhouse bench, and moistened daily for 3 mo. They were examined periodically for production of perithecia and ascospores.

Experimental design. *Experiment 1.* The effects of fungicide spray programs for greasy spot control were compared from 1975–1978 in a 25-yr-old grapefruit (*Citrus paradisi* Macf. 'Webb Redblush') orchard with a 6.1 × 7.7-m tree spacing, primarily to determine the effect of greasy spot on yield. In the maximum spray program, cupric hydroxide (Kocide 101®; Kocide Chemical Corp., Houston, TX 77045) was applied at 4.5 kg Cu (actual)/ha in April of each year and benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazole-carbamate) (Benlate®; E. I. duPont de Nemours & Co., Wilmington, DE 19898) at 1.7 kg (a.i.)/ha plus citrus spray oil (Sunoco Superior Spray Oil 11N; Sun Petroleum Products, Philadelphia, PA 19103) at 115 L/ha was applied in late June or early July of each year. In the minimum spray program, citrus spray oil was applied in late June or early July. No fungicide or oil was applied to the nonsprayed plots. Chlorobenzilate (ethyl 4,4-dichlorobenzilate, Acaraben®; Ciba-Geigy, Greensboro, NC 22409) for rust mite control and methidathion (0,0-dimethyl-*S*-[2-methoxy-1,3,4-thiadiazol-5(4*H*)-onyl-(4)-methyl] dithiophosphonate) (Supracide®; Ciba-Geigy, Greensboro, NC 27409) for armored scale control was applied as needed to all plots. All sprays were applied with a John Bean Model F-357CP Speed Sprayer

(FMC Corp., Jonesboro, AR 72401) in about 2,400 L/ha. Each spray program was replicated six times on eight-ten tree plots arranged in a randomized complete block design. Buffer rows to which no fungicide was applied were maintained between each plot.

Experiment 2. An experiment to determine the effect of rust mite control on the incidence of greasy spot was conducted in a 25-yr-old red grapefruit orchard planted on a 9.2 × 12.3-m spacing. Four treatments were used: acaricide, fungicide, acaricide plus fungicide, and an unsprayed control. Treatments were replicated three times on two-tree plots arranged in a randomized complete block design. Acaricide plots received chlorobenzilate at 0.18 g/L and plots treated with fungicide received cupric hydroxide at 0.48 g of actual Cu per liter in late April and late June of each year. Dicofol [1,1-*bis*(4-chlorophenyl)-2,2,2-trichloroethane] (Kelthane® MF; Rohm & Haas Co., Philadelphia, PA 19105) was applied to the acaricide plots in September 1976; September 1977; and in July and August 1978 for late-season mite control. All applications were made with the handgun of a Hardie hydraulic sprayer (Hardie Manufacturing Co., Wilkes-Barre, PA 18701) at 35 kg/cm^2. Trees were sprayed to run-off with ~50 liter/tree.

Experiment 3. In 1978, the same treatments used in Experiment 2 were applied to 3-yr-old Webb Redblush grapefruit trees in 8-L containers growing outdoors under 50% shade. The four treatments were replicated twice on nine-tree plots. Acaricide plots received chlorobenzilate at 0.24 g/L and fungicide plots received cupric hydroxide at 0.48 g of actual Cu per L in April, June, and September. Trees were sprayed to run-off with a CO_2-pressurized hand sprayer.

Data collection. All fruit from four trees in each plot in Experiment 1, ie, 24 trees per treatment were harvested, weighed, and graded into three sizes on a commercial sizer. Data were expressed as the percentage of the total weight of fruit 9.2 cm in diameter or larger, ie, fruit sufficiently large to be marketed fresh. In the 1975 and 1977 crop years, the percentage of the fruit completely free of GSRB was determined on a randomly selected 25-fruit sample from each yield tree. Fruit were harvested in February 1976, 1977, and 1978 and in November 1978.

To assess greasy spot severity, the foliage on the southern quadrant of each tree was rated in November or December of each year on the following scale: 0 = no visible symptoms; 1 = one-to-two shoots with greasy spot symptoms; 2 = three-to-five shoots with greasy spot symptoms; 3 = six-to-10 shoots with symptoms; 4 = most shoots with symptoms; 5 = severe infection on all old leaves.

Shoots were tagged in all experiments to assess greasy spot severity, leaf loss, and time of infection. Ten shoots on each of two trees within each plot were tagged on the spring growth flush in April 1976, 1977, and 1978 in Experiments 1 and 2. Two shoots per tree were tagged in April 1978 on container-grown trees in Experiment 3. In addition, a variable number of shoots, depending on availability, were tagged on trees in the unsprayed control plots of all three experiments from the early July and late August flushes of growth to assess greasy spot effects on summer flushes. Percentage leaf loss and the percentage of the leaf area affected by greasy spot were assessed on all tagged flushes. The spring growth flush from 1976 was evaluated in September 1977; spring flush from 1977 was evaluated in February 1978; and all flushes in 1978 were evaluated in April 1979.

In November or December of each year a 20-fruit sample selected at random from each tree in Experiment 2 was evaluated for russetting to assess the prevalence of rust mite in the orchard that year. Each fruit was rated as russet-free, mild, moderate, or severe. Data were expressed as the percentage of the fruit with moderate or severe damage; ie, fruit unacceptable for U.S. No. 1 grade. The number of mites in a 1-cm^2 area on each of 10 leaves and 10 fruit on each tree in Experiment 2 were counted at about monthly intervals from April to August 1978. Similar counts were made on 10 leaves and all of the fruit present on each tree in Experiment 3.

Analysis of variance was performed on all data from Experiments 1 and 2 and means were separated by Duncan's multiple range test. Means in Experiment 3 were separated by Students' *t*-test.

Ascospore trapping. A Burkard 7-day recording spore trap (Burkhard Scientific Sales, Ltd., Rickmansworth, Herts, England) was operated continuously from early March to late November 1978 in a control plot of Experiment 1. The trap was located under the tree canopy with the orifice about 0.7-m above the ground. Spore trap tapes were coated with silicone stopcock grease. The 7-day tapes were cut into daily segments (48 mm) and mounted with the trapping surface upward. Spores were counted at ×1,000 under oil immersion after staining with cotton blue in lactophenol. The number of spores counted in two passes across each slide was defined as the daily total. The data presented in Fig. 1 are the relative total spore numbers per week. Time of day of all spore releases was noted. A rain gauge, which was read daily, was located in an open area about 30-m from the spore trap. Dates of flood irrigations in the orchard also were recorded.

RESULTS

Identification of the causal organism. Most tissue pieces plated on CMA yielded slow-growing, grayish-green colonies of *M. citri.*

TABLE 1. Effect of fungicide spray programs on greasy spot incidence, leaf loss, and fruit yields of grapefruit in Texas

Year	Spray program[u]	Greasy spot severity rating[v]	Leaf loss (%)[w]	Leaf area affected (%)[w]	Yield (kg/tree)	Fruit ≥9.2 cm (%)[x]	Fruit free of GSRB (%)[y]
1975	Maximum	0.3 b[z]	···	···	150 a	86 a	40 a
	Minimum	0.3 b	···	···	148 a	88 a	38 a
	None	1.4 a	···	···	147 a	86 a	30 b
1976	Maximum	0.7 c	64 a	0.3 c	234 a	62 a	···
	Minimum	1.6 b	54 b	7.8 b	220 a	54 a	···
	None	2.2 a	51 b	12.5 a	208 a	57 a	···
1977	Maximum	0.0 b	70 a	0.3 b	165 a	75 a	72 a
	Minimum	0.6 a	68 a	1.3 b	162 a	65 a	52 b
	None	0.7 a	78 a	5.3 a	159 a	67 a	40 c
1978	Maximum	0.0 c	86 a	0.0 b	195 a	61 a	···
	Minimum	1.4 b	75 a	0.0 b	181 a	55 a	···
	None	2.1 a	74 a	0.7 a	189 a	62 a	···

[u] Maximum = cupric hydroxide applied in April each year and benomyl + citrus spray oil applied in late June or early July each year; minimum = citrus spray oil applied late June or early July each year; none = no fungicide applied.

[v] Foliar greasy spot symptoms rated in November–December each year on a scale of 0 = none to 5 = severe.

[w] Percentage of leaves lost and percentage of the area on remaining leaves affected by greasy spot on tagged shoots of the previous year's spring flush were determined in September 1977, February 1978, and April 1979.

[x] Percentage of the total fruit weight composed of fruit 9.2 cm in diameter or larger.

[y] Percentage of the fruit completely free of greasy spot rind blotch (GSRB).

[z] Mean separation in columns and groups by Duncan's multiple range test, $P = 0.05$.

Conidia of *Stenella* sp., were formed sporadically on the young colonies. Immature perithecia were formed on decomposing greasy spot-affected leaves in the greenhouse in 1–2 mo and mature perithecia with asci and ascospores of *M. citri* were formed after 3 mo.

Yield effects. *Experiment 1.* The maximum spray program significantly reduced greasy spot severity over the no-fungicide control in all 4 yr and nearly eliminated greasy spot symptoms in 1977 and 1978 (Table 1). The minimum spray program reduced greasy spot severity over the no-fungicide control in years when the greasy spot incidence was highest, but was generally less effective than the maximum spray program. When greasy spot incidence was evaluated at a later date as the percentage of the leaf area affected, results were similar to the general severity rating in November or December. Greasy spot did not significantly increase leaf drop in any year (Table 1).

Although nearly complete control of greasy spot was attained, neither spray program resulted in significant increases in yield or fruit size in any year (Table 1). Analysis of data over all years also indicated that there were no significant differences in yield or fruit size among the treatments. Greasy spot rind blotch was commonly observed on the fruit, but only rarely was severe enough to reduce fruit quality from U.S. No. 1 grade. In the 2 yr in which fruit was closely examined, the maximum spray program significantly increased the percentage of fruit free of GSRB and generally improved the brightness of the fruit.

Interaction with citrus rust mite. *Experiment 2.* Neither acaricide nor fungicide applications greatly affected leaf loss (Table 2). However, in 1976, when rust mite infested 70–80% of the leaves in mid-summer, the acaricide treatment significantly reduced defoliation. Except in 1977, when greasy spot incidence was low, the fungicide applications reduced the percentage of the leaf area affected by greasy spot in all years. In most years, the acaricide alone significantly reduced the percentage of the leaf area affected by greasy spot (Table 2). In 1977, fungicide application significantly increased rust mite populations at one count date and damage to the fruit at the end of the season. When acaricides and copper fungicides were applied together, mite populations and mite damage to fruit were not significantly different than in plots that received acaracide alone (Table 2).

Acaricide treatments effectively reduced greasy spot incidence in 1976 and 1978, when rust mite activity was greatest as indicated by the mite damage on fruit (Table 2). Observations and limited counts in 1976 indicated that leaves and fruit were heavily infested with rust mite. However, in 1978, in the orchard the number of rust mites counted at monthly intervals from April-August averaged only $2.8/cm^2$ on the leaves in the unsprayed control and $0.7/cm^2$ in the acaricide-treated plots. Numbers of mites on fruit in the unsprayed control were four or five times higher than those on leaves.

Experiment 3. On the container-grown trees, acaricide treatments similarly reduced the percentage of the leaf area affected by greasy spot to a value below that of the unsprayed control trees (Table 2). The percentage of leaf area affected by greasy spot on container-grown trees evaluated in November 1978 was: acaricide—1.9; fungicide—0.0; acaricide-fungicide—0.0; no spray—7.5 and may be compared to the figures on the same shoots in April 1979 (Table 2). On these trees, rust mite populations on leaves never exceeded 0.2 mites per square centimeter in any treatment in six counts made monthly from April to September. However, populations developed on fruit late in the season and counts on fruit in the unsprayed control averaged 170 mites per square centimeter in September.

Epidemiology. Generally, few symptoms appeared on tagged spring flush growth until February to April of the following year. Symptoms developed by November 1978 on tagged spring flush growth on container-grown trees in the shaded area, but were considerably more severe by April 1979. Greasy spot symptoms were severe by April 1979 on the July and August 1978 flushes in one orchard and on the container-grown trees where no fungicides, insecticides, or acaricides were applied (Table 3). Where mites and insects were controlled in the orchard in Experiment 1, little greasy spot developed on any of the flushes even though no fungicide was applied.

Nearly all ascospores were released during or following rainfall and no diurnal pattern was distinguished. Occasionally, a few spores were trapped in the early morning hours during nights with heavy dews. Rainfall was generally low through July. Few ascospores were trapped following rains in early April and early June (Fig. 1). However, an ascospore release followed a flood irrigation in late June and greater numbers were released following another irrigation in late July. The majority of the ascospores were

TABLE 2. Effect of fungicide and acaricide applications on leaf loss, greasy spot incidence, and damage by citrus rust mite on orchard and container-grown grapefruit trees in Texas

Year and situation	Spray program[v]	Leaf loss (%)[w]	Leaf area affected (%)[w] or greasy spot severity[x]	Fruit russet (%)[y]
1976 orchard	Acaricide	41 b[z]	5.8 b[w]	14 b
	Fungicide	63 a	1.5 c	83 a
	Acaricide + fungicide	42 b	0.6 c	10 b
	No spray	59 a	13.3 a	90 a
1977 orchard	Acaricide	61 a	2.6 a[w]	3 bc
	Fungicide	65 a	1.3 a	18 a
	Acaricide + fungicide	59 a	0.8 a	1 c
	No spray	66 a	1.6 a	8 b
1978 orchard	Acaricide	64 a	1.8 b[x]	8 c
	Fungicide	66 a	0.0 c	65 a
	Acaricide + fungicide	53 a	0.0 c	1 c
	No spray	57 a	3.8 a	65 a
1978 container trees	Acaricide	74 a	9.0 b[w]	...
	Fungicide	80 a	1.7 c	...
	Acaricide + fungicide	88 a	3.4 c	...
	No spray	83 a	19.7 a	...

[v] Chlorobenzilate (acaricide) and/or cupric hydroxide (fungicide) were applied to the orchard in late April and late June each year. Dicofol (acaricide) was applied in September 1976; September 1977; July and August 1978 to the acaricide plots in the orchard for late-season mite control. Chlorobenzilate and/or cupric hydroxide were applied to the container stock in April, June, and September.

[w] Percentage of leaves lost and percentage of the area on remaining leaves affected by greasy spot on tagged shoots of the previous year's spring flush determined in September 1977; February 1978 and April 1979.

[x] Foliar greasy spot symptoms rated in November 1978 on a scale of 0 = none to 5 = severe.

[y] Percentage of the fruit with moderate to severe damage by the citrus rust mite.

[z] Mean separation in columns and groups for orchard data by Duncan's multiple range test and for container-stock data by Students' t-test ($P = 0.05$).

TABLE 3. Comparison of greasy spot incidence on spring and summer growth flushes on orchard and container-grown grapefruit trees in Texas in 1978

Situation[x]	Leaf area affected by greasy spot (%)[y] on remaining leaves from flushes in: April	early July	late August
Orchard, insects and mites controlled	0.7 (30)[z]	0.4 (8)	1.0 (12)
Orchard, no sprays applied	1.2 (27)	10.0 (4)	4.9 (12)
Container-stock, no sprays applied	19.7 (32)	11.6 (15)	9.7 (73)

[x] Data from control plots receiving no fungicide from Exp. 1, 2, and 3, respectively.

[y] Percentages determined in April, 1979 on tagged growth flushes from the previous year.

[z] Numbers of shoots examined appear in parentheses.

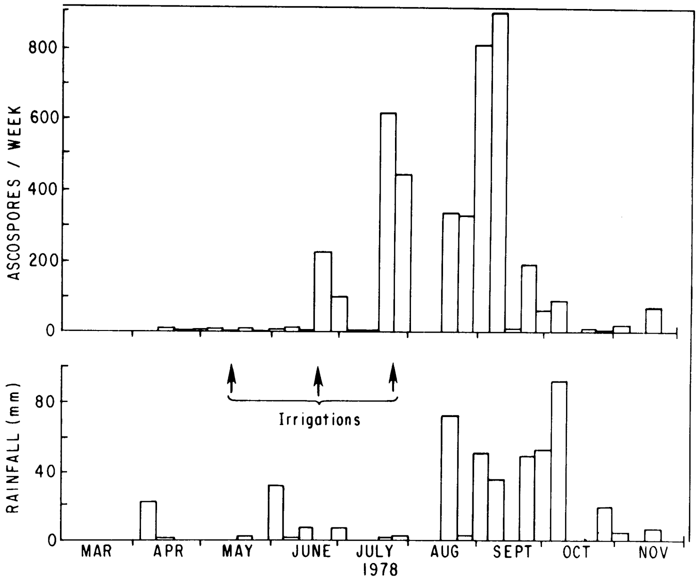

Fig. 1. Relative numbers of *Mycosphaerella citri* ascospores produced per week and weekly rainfall totals in a Texas grapefruit orchard. Arrows indicate dates of flood irrigations.

released during rains in late August and early September. Rainfall in late September and early October resulted in release of comparatively fewer ascospores. Conidia were trapped from spring through late summer, but only sporadically and always in very low numbers.

DISCUSSION

Foliar greasy spot symptoms observed in Texas for many years apparently are caused by *M. citri*. A rind blemish and greening of mature grapefruit was described previously in Texas (5) and attributed to *Leptothyrium pomi* (Mont. Fr.) Sacc., which causes flyspeck. However, the symptoms described are those of GSRB and since we have frequently isolated *M. citri* from fruit with such symptoms, this blemish should be designated GSRB and not flyspeck.

In our studies, acaricide applications consistently reduced the severity of greasy spot symptoms, but the mechanism by which this occurs remains uncertain. Van Brussel (8) suggested several possible mechanisms: rust mite feeding punctures could provide entry points for *M. citri* infection, exudates from feeding punctures and cast skins could stimulate epiphytic growth, and rust mite bodies and debris may trap spores on the surface of the leaf. McCoy and Albrigo (3) have shown that citrus rust mites make many punctures per cell and that these punctures are too small to allow entry of *M. citri*, which makes the first possibility unlikely. The second possibility seems more attractive since Whiteside (14) has shown that sugars produced by honeydew-secreting insects increase greasy spot severity by increasing epiphytic growth. The results of the first 2 yr of data collection seemed to be consistent with the hypothesis that high rust mite populations favored greasy spot development. Control of high mite populations the first year reduced greasy spot incidence and, in the second year, when mite populations were low, acaricide applications did not reduce greasy spot severity. However, in the third year of the orchard study and in the experiment on the container-grown trees, there were very low mite populations on leaves throughout the year. In spite of the low populations, greasy spot severity was significantly reduced by acaricides. Therefore, the acaricides may have a direct effect on the fungus rather than affecting greasy spot indirectly by controlling mites.

Copper fungicides have detrimental effects on the rust mite parasite, *Hirsutella thompsonii* Fisher (4) and may adversely affect rust mite control. In this study, applications of copper fungicides resulted in increased mite damage to fruit only in 1977 (Table 2). In the semi-arid climate of the Texas citrus area, *H. thompsonii* may be less important as a biological control agent than in Florida; hence, copper sprays may be less detrimental to mite control.

In the orchard where mites and insects were controlled, greasy spot did not affect yield or fruit size in the 4 yr of the study. Epidemiological studies suggested a reason for the lack of response to greasy spot control in Texas. In Florida, infection occurs primarily in June and July; symptoms frequently appear by November or December; defoliation occurs prior to the spring growth flush and fruit set; and yield losses result in the following crop year (15). In Texas, some ascospores apparently matured by June, but with comparatively low rainfall in early summer, major spore releases resulted only from irrigation. Spores released following flood irrigations probably were deposited on dry leaves, and because there was no free water or high humidity to support germination and development, little or no infection resulted. Dry weather may have delayed leaf decomposition and, hence, perithecial development until late summer. Ascospores were released with high rainfall in late summer and the spring, early and later summer flushes became infected. In contrast, Whiteside (9) reported no disease on August and September growth flushes in Florida. In Texas, infection was delayed under field conditions until late summer, and symptoms did not appear until the following spring. Defoliation which occurred as a result of greasy spot infection was delayed until after fruit set and no yield reductions resulted. Most of the leaf loss in Texas is not specifically attributable to greasy spot as it is in Florida (15). Considering the epidemiological data, our spray applications probably should have been applied later in the summer for optimal control. However, timing of applications for greasy spot control is not critical (10) and even early summer sprays resulted in complete disease control, but no yield or size increases (Table 1).

In most cases pest management programs in Texas do not have to consider greasy spot control. If mites and honeydew-secreting insects are controlled and, especially if citrus spray oil is used for armored scale control, greasy spot should not be a problem. The use of fungicides might be considered to control GSRB on orchards designated for specialty fruit where exceptional external quality is desired, or where cultural practices or climatic factors have resulted in heavy leaf litter with a high potential for ascospore production.

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