Effect of Temperature on the Discharge and Germination of Ascospores by Apothecia of *Monilinia fructicola*

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ABSTRACT

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Naturally growing apothecia of *Monilinia fructicola* were collected from two commercial plum orchards near Reedley and Sanger, both in Fresno County, California. Ascospore discharges from 90 (1996) and 86 (1997) apothecia were monitored individually using spore traps at four constant temperatures. The period of discharge decreased as temperature increased from 10 to 25°C. However, daily discharge increased as temperature increased from 10 to 15°C and remained high at 20 and 25°C. The greatest discharge occurred with apothecia at 15°C, followed by those incubated at 20, 10, and 25°C. The germination of ascospores of *M. fructicola* and the length of germ tubes increased as temperature increased from 7 to 15°C; however, increasing temperatures above 15°C did not increase either ascospore germination or length of germ tubes. This information may help in the development of warning systems and management strategies for brown rot blossom blight of stone fruits.

Additional keywords: epidemiology, primary inoculum, *Prunus* spp.

Ascospore maturity and discharge are critical to timing fungicide sprays for the control of many plant diseases (7-11,25,26-29,35-39). Physical factors that influence ascospore maturation and discharge include temperature (8,9,11,12,20,27,28,35-37), rain (9,20,26,27,37,39), moisture (9,12,20,37,39), relative humidity (12,25,35,36), and light (37,39). Definition of ascospore maturity and understanding factors affecting ascospore maturation and discharge by *Venturia inaequalis* has tremendously improved the efficiency of scheduling fungicide application for the control of primary infection by apple scab (7,10,27). Unfortunately, no comparable research has been completed with *Monilinia fructicola*, a principal causal agent of brown rot of stone fruits (*Prunus* spp.). Apothecia of this fungus have been observed in orchards worldwide (1,4,6,14,15,24,31-34,42,43) and ascospores have been shown to serve as a major source of primary inoculum for initiating infection of blossoms in California (19) and South Carolina (23).

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The objectives of this research were to determine the effects of temperature on (i) the pattern of ascospore discharge by apothecia of *M. fructicola*, (ii) production of individual apothecia, and (iii) ascospore germination.

MATERIALS AND METHODS

Sampling apothecia. All the apothecia of *M. fructicola* examined were collected from two commercial plum orchards near Reedley and Sanger, respectively, both in Fresno County, California (Table 1). The plum orchard near Reedley was established in 1980 with two cultivars of Sweet Heart and Royal Diamond, planted every one to three rows from south to north. The orchard trenches were rototilled twice (late November and mid-February) in both the 1995 to 1996 and 1996 to 1997 winters. Only the tops of berms received a mixture of norflurazon (Sandoz Crop Protection, Des Plaines, IL), oxyfluorfen (Rohm & Haas Company, Philadelphia), and glyphosate (Monsanto Agricultural Company, St. Louis) at rates of 882 g, 54 g, and 696 g a.i./ha, respectively, on 14 February 1996, while both the tops and sides of berms received the same mixture on 22 February 1997. The orchard near Sanger was planted with the cultivar Red Beaut in 1973 and then was grafted with Santa Rosa in 1983. In this orchard, the trenches were rototilled in September 1996 and only the berm tops received a mixture of paraquat (Zeneca Inc. Ag Products, Wilmington, DE) and glyphosate at rates of 408 g and 696 g a.i./ha, respectively, in mid-February 1997 to control weeds.

To distinguish the apothecia of *M. fructicola* from those of *Sclerotinia sclerotiorum*, both types of apothecia were collected and positioned to discharge their ascospores onto acidified (with 2.5 ml of 25% lactic acid/liter) potato dextrose agar (APDA) petri dishes. After forcing ascospore discharge and recovering the white fluffy isolates with typical black sclerotia, characteristic of the latter fungus, it was easy to distinguish the apothecia of *M. fructicola* in subsequent collections. Apothecia of *M. fructicola* are characterized by their larger, typical cup-like, brown or dark brown disks. In the present study, only apothecia of *M. fructicola* were examined for their ascospore discharges.

The number of apothecia of *M. fructicola* was counted under each of 5 trees in both orchards during the first collection of apothecia in 1996 and 1997. Apothecia were collected from the soil surface with a semi-cylindrical (1.5 by 1.5 cm) metallic scoop and placed into rectangular plastic containers (7.0 by 3.5 by 2.0 cm), either individually or in clusters with either whole or fragmented mummified fruit from which apothecia grew. During apothecia collection and transportation to the laboratory at Kearney Agricultural Center, Parlier, the plastic containers were kept on ice in a cooler to reduce or prevent apothecia from discharging ascospores or desiccating. Apothecia collected on different dates were considered to be different apothecia groups when they were monitored for ascospore discharge. Ascospore discharge was monitored immediately after apothecia were transported to the laboratory. Apothecia of Group 96-4 were only examined for ascospore discharge after they were stored at 4°C for 8 days.

Wet and dry spore traps. Two types of spore traps, consisting basically of multiwell tissue culture plates (Falcon 3046, Becton Dickinson Company, Franklin Lakes, NJ) and blocks of water agar (5 by 5 mm), were used. A multiwell plate comprises six sub-plates (or wells), with the inside diameter of each well measuring 35 mm and 18 mm in depth. One of these six wells was considered a spore trap and used to monitor the ascospores discharged by an individual apothecium, and six wells served as six replicate spore traps. In each of these six replicate spore traps, four blocks (5 by 5 mm) of water agar were attached onto the underside of the plate cover just above the well's center to hold

an apothecium. The plate cover with six apothecia facing upward was then inverted over the plate bottom (multiwells) so that the surface of each apothecium faced a well. An aliquot of 2 ml 0.01% (vol/vol) Tween-80 solution was added to each well of the "wet spore traps," but none was added into the "dry spore traps." Apothecia discharged their ascospores into the Tween-80 solution in the wet spore traps or directly onto the well bottom and side walls in the dry spore traps.

The efficiency of the two types of spore traps was compared at 25°C for monitoring ascospore discharge. The test was conducted initially with apothecia of Group 96-3 and repeated with apothecia of Group 96-4. Apothecia were prepared by cutting off their stipules and removing attached soil from them before they were positioned in contact with the four agar blocks onto the multiwell plate cover. Disk diameter of

each apothecium also was measured prior to inversion of the cover over the multiwells. The multiwells were gently replaced daily until the last of 3 successive days during which the ascospore discharge declined to an undetectable level.

Upon replacement, each of the collected multiwells was filled with 2 ml of deionized water and the Tween-80 solution (only for the dry spore trap), then the ascospore layer was scraped off the well bottom and side walls by rubbing 20 times with the flat end of a smooth wooden rod (7 mm in diameter). The ascospore clusters were further broken down by collecting the suspension from the wells and replacing it into the wells 20 to 30 times with a 5-ml pipette. Initial ascospore suspensions were diluted as needed for microscopic examination by adding deionized water. Number of ascospores in 2.5 µl of each diluted ascospore

suspension was counted microscopically at 100× magnification.

Ascospore discharge was characterized by the disk size, the period of discharge (the time from the start of the incubation until the day when the ascospore discharge was not detectable), the number of days in which an apothecium discharged more than 1×10^6 ascospores, the maximum and average daily discharges, the total discharge, and the average discharge (computed by dividing the total discharge by disk area). The data were analyzed using Proc GLM of SAS (Release 6.11, SAS Institute Inc., Cary, NC) and means were separated by Fisher's least significant difference (LSD) test at $P = 0.05$.

Effect of temperature on ascospore discharge. Following the procedures described above, ascospore discharge of three groups (96-1, 96-2, and 96-3) of apothecia were monitored daily using the wet spore

Table 1. Sources of apothecia of *Monilinia fructicola* and spore traps for determining the effect of temperature on ascospore discharge in 1996 and 1997

Group	Date ^w	Number of apothecia examined	Host	Location	Incubation temperature (°C)	Spore trap ^x	Type of data collected
96-1	150396	24	Plum	Reedley	10,15,20,25	Wet	Daily
96-2	200396	24	Plum	Reedley	10,15,20,25	Wet	Daily
96-3	250396	30 ^y	Plum	Reedley	10,15,20,25	Wet/Dry	Daily
96-4	030496	12	Plum	Reedley	25	Dry/Wet	Daily
97-1	270297	24	Plum	Sanger	10,15,20,25	Dry	Weekly
97-2	280297	18	Plum	Reedley	10,20,25 ^z	Dry	Weekly
97-3	070397	24	Plum	Sanger	10,15,20,25	Dry	Weekly
97-4	080397	20	Plum	Sanger	10,15,20,25	Dry	Weekly

^w The date on which the apothecia were collected (ddmmyyrr).

^x Wet spore traps containing 2 ml of 0.01% Tween-80 solution/well; in contrast, dry spore traps were empty.

^y There were six apothecia per temperature with the wet spore traps. At 25°C, an extra set of six apothecia were also included using the dry spore trap to compare the difference of ascospore discharge between two types of spore traps.

^z Incubator of 15°C malfunctioned, so no data were collected.

Table 2. Comparison of the wet and dry spore traps for the efficiency of monitoring ascospore discharge by apothecia of *Monilinia fructicola* at 25°C

Variable	Apothecia of Group 96-3			Apothecia of Group 96-4		
	Wet	Dry	P^z	Wet	Dry	P^z
Disk size (mm ²)	94.2 a	207.5 a	0.2039	120.1 a	89.6 b	0.0388
Period of discharge (days)	3.8 a	3.5 a	0.5301	3.1 a	3.0 a	0.6109
Number of days with a discharge of more than 1×10^6 ascospores/apothecium	0.8 a	1.3 a	0.4896	0.7 a	0.7 a	...
Maximum daily discharge ($\times 10^6$ ascospores/apothecium/day)	0.6 a	1.6 a	0.2172	0.7 a	0.6 a	0.5023
Average daily discharge ($\times 10^3$ ascospores/mm ² apothecium/day)	7.4 a	8.2 a	0.8282	5.2 a	5.1 a	0.8860
Total discharge ($\times 10^6$ ascospores/apothecium)	1.8 a	5.5 a	0.2000	2.5 a	2.3 a	0.7590
Average discharge ($\times 10^3$ ascospores/mm ² apothecium)	20.3 a	26.6 a	0.3636	1.7 a	1.7 a	0.9638

^z Level of significance of the difference between the two types of spore traps for collecting ascospores from apothecia by Proc GLM of SAS. Each value is the mean of six replicate apothecia, and the numbers followed by the same letter within a row and under the same apothecia group did not differ significantly according to Fisher's least significance test at $P = 0.05$.

Table 3. Effect of temperature on the pattern of ascospore discharge by apothecia of *Monilinia fructicola*^y

Variable	Incubation temperature (°C)				P^z
	10	15	20	25	
Average daily discharge ($\times 10^5$ ascospores/apothecium/day)	1.6 b	5.2 a	4.4 ab	5.6 a	0.0442
Maximum daily discharge ($\times 10^6$ ascospores/apothecium/day)	0.5 b	1.4 ab	1.3 a	1.4 a	0.0962
Number of days with a discharge of more than 1×10^6 ascospores/apothecium	0.2 b	1.3 a	0.7 ab	0.6 ab	0.0353
Period of discharge (days)	12.9 a	7.8 b	5.7 c	3.9 d	0.0001

^y Data of the groups (96-1, 96-2, and 96-3) of apothecia at constant temperatures were pooled and analyzed using Proc GLM of SAS; no differences were observed among groups, and among apothecia in each group, in all variables of the productivity, except for the period of discharge (first to last days).

^z Level of significance of the difference among incubation temperatures. Each value is the mean of 18 replicate apothecia; the numbers followed by the same letter within a row did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

trap in 1996, and of four groups (97-1, 97-2, 97-3, and 97-4) of apothecia weekly in 1997 with the dry spore trap, at 10, 15, 20, or 25°C, respectively. Based on the number of ascospores in 2.5 µl of dilution, the period of discharge, the number of days with a discharge of more than 1×10^6 ascospores, the maximum and average daily discharges and the total and average discharges were calculated for individual apothecia examined in 1996, while only the total and average discharges were computed for those examined in 1997. In 1996, a few apothecia with water agar blocks fell into the Tween-80 solution or disintegrated before the end of the experiment. Subsequently, data from these apothecia were excluded from the statistical analyses.

Homogeneity of the data of each characterizing variable from different groups of apothecia was tested by analysis of variance using Proc GLM of SAS. Data for each group of apothecia also were analyzed with Proc GLM, and treatment means of each variable were separated by Duncan's multiple range test at $P = 0.05$. The combined data of different groups of apothecia were further analyzed with Proc NLIN after they were normalized by dividing treatment means by their group means. The relationship between total discharge and disk size of the apothecia also was investigated using Proc REG of SAS.

Effect of temperature on the germination of ascospores. The effect of temperature on ascospore germination was tested using ascospores collected in dry spore traps. Ascospores discharged by individual apothecia were considered as subgroups and were suspended separately in deionized water. An aliquot of 1 ml of each suspension was then pipetted into each of 15 test tubes, and 3 each were incubated at 7, 10, 15, 20, and 25°C. An ascospore was considered germinated when the length of its germ tube was no shorter than the width of the ascospore. A total of 100 ascospores were examined from each test tube at 100× magnification, and the number of ascospores germinated was recorded after 24 h incubation; the length of germ tubes of 10 arbitrarily selected germinated ascospores also was measured after 24 h incubation. The data of germination rate and length of germ tubes were analyzed using Proc ANOVA of SAS, and treatment means were separated by Duncan's multiple range test at $P = 0.05$. The test was run four times, but only the data of ascospores discharged at 10°C by three apothecia (Group 97-1) are presented.

RESULTS

Sampling apothecia of *M. fructicola*.

Apothecia of *M. fructicola* were commonly observed in the orchards during samplings. Isolations made from these apothecia produced conidial sporulation typical of *M. fructicola*, but did not produce sclerotia on APDA plates.

The plum orchards near Reedley and Sanger were selected as the best sources for collecting apothecia because they had the most apothecia among plum orchards surveyed during the spring of 1996 and 1997. The plum orchard near Reedley had an average of 16.8 apothecia/tree (maximum of 54) in 1996, and 36.4 apothecia/tree (maximum of 175) in 1997. The orchard near Sanger averaged 1.8 apothecia/tree (maximum of 5) in 1996 and 4.4 apothecia/tree (maximum of 11) in 1997. All apothecia collected were found

on the north side of the berms in both orchards.

Wet and dry spore traps. Apothecia of *M. fructicola* used for examining ascospore discharge in both wet and dry spore traps had similar sizes of disks ($P = 0.20$ for the first run and $P = 0.04$ for the second run). No differences were observed in the period of discharge, the number of days with a discharge of more than 1×10^6 ascospores per apothecium, the maximum and average daily discharges, the total and average discharges between the two types of spore

Fig. 1. Daily discharge of ascospore by *Monilinia fructicola* with apothecia of three groups at four constant temperatures of 10, 15, 20, and 25°C.

traps in either of the two apothecia groups of the experiment (Table 2).

Effect of temperature on the pattern of ascospore discharge. The daily discharge of ascospores by *M. fruticola* is shown in Figure 1. At 25°C, individual apothecia discharged the most ascospores during the first 24-h incubation; then the daily discharge decreased gradually as the incubation time progressed. In contrast, at 10, 15, and 20°C, the daily discharge fluctuated from day to day at the beginning of incubation. Comparatively, the fluctuation of daily ascospore discharge diminished the earliest at 20°C and the latest at 10°C. Daily variability in discharge was greatest at 15°C and least at 10°C.

The period of discharge increased from 3.9 to 12.9 days as incubation temperature

decreased from 25 to 10°C (Table 3). Apothecia discharged fewer ascospores at 10°C (1.6×10^5 ascospores/apothecium/day) and for a shorter period (0.2 day), with a daily discharge of more than 1×10^6 ascospores/apothecium, than at the other temperatures (Table 3).

Effect of temperature on the production of ascospores. Total discharge differed ($P < 0.001$) among the seven groups of apothecia. In general, the total discharge by apothecia from the Sanger orchard in 1997 was greater than that by apothecia from the Reedley orchard in 1996 and 1997 (Table 4). Apothecia of Groups 96-1, 96-2, and 96-3 collected from the Reedley orchard in 1996 discharged discernibly more ascospores than those (Group 97-2) from the same orchard in 1997.

No differences were observed in the disk sizes of apothecia among incubation temperatures. However, at 15°C, apothecia of the Groups 96-1 and 96-2 discharged significantly ($P < 0.02$) more ascospores than those at the other temperatures; apothecia of Groups 96-3, 97-3, and 97-4 also discharged slightly more ascospores at 15°C than did those incubated at the other temperatures. The analysis over the combined data from apothecia of all groups indicated that the total discharge was significantly different among four incubation temperatures, with apothecia discharged the most (7.5×10^6 ascospores/apothecium) at 15°C and the least (2.3×10^6 ascospores/apothecium) at 25°C (Table 4). Similarly, the average discharge was the greatest at 15 and 20°C (about 5.0×10^4 asco-

Table 4. Effect of temperature on the total discharge of ascospores by apothecia of *Monilinia fruticola*

Location, group	Number of apothecia examined	Total discharge ($\times 10^6$ ascospores/apothecium) at temperature (°C)				<i>P</i> ^w	Group mean
		10	15	20	25		
Reedley							
96-1	24	2.14 b	5.49 a	1.58 b	1.21 b	0.0165	2.6 c
96-2	24	1.41 b	4.16 a	1.62 b	1.41 b	0.0184	2.2 c
96-3	24	0.90 a	7.01 a	3.65 a	1.76 a	0.4142	3.3 bc
97-2	18	1.83 a	... ^x	2.05 a	0.33 a	0.3650	1.4 c
Sanger							
97-1	24	8.02 a	6.63 a	12.60 a	4.03 a	0.3076	7.8 a
97-3	24	2.90 b	12.30 a	11.20 ab	5.24 ab	0.0992	9.7 a
97-4	20	3.22 a	9.42 a	8.36 a	1.92 a	0.2456	6.5 ab
Grand mean ($\times 10^6$ ascospores/apothecium)		2.92 c	7.50 a	5.87 b	2.27 c	0.0004 ^y	0.0097 ^z

^w Level of significance of the difference of the total ascospore discharge among incubation temperatures using Proc GLM of SAS. Each value is the mean of six replicate apothecia and the numbers followed by the same letter within a row (group) did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

^x No data were collected because of incubator failure.

^y Level of significance of the difference among grand means by analysis of variance on combined data after they were normalized by dividing treatment means by their group means. Grand means followed by the same letter did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

^z Level of significance of the difference among group means by Proc GLM. Group means followed by the same letter within the last column did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Table 5. Effect of temperature on the average discharge of ascospores by apothecia of *Monilinia fruticola*

Location, group	Number of apothecia examined	Average discharge ($\times 10^3$ ascospores/mm ² apothecium) at temperature (°C)				<i>P</i> ^w	Group mean
		10	15	20	25		
Reedley							
96-1	24	15.4 b	32.4 a	12.2 b	8.7 b	0.0315	17.2 dc
96-2	24	13.8 b	33.2 a	20.5 b	17.6 b	0.0202	21.3 dc
96-3	24	11.3 b	28.5 ab	38.4 a	20.3 ab	0.1026	24.6 dc
97-2	18	17.6 a	... ^x	14.3 a	5.6 a	0.4872	12.8 d
Sanger							
97-1	24	120.1 a	54.3 a	102.3 a	56.5 a	0.3963	83.3 a
97-3	24	29.2 b	72.7 ab	117.6 a	42.3 b	0.0298	65.5 ab
97-4	20	32.3 a	80.7 a	37.9 a	5.2 a	0.2659	45.8 bc
Grand mean ($\times 10^3$ ascospores/mm ² apothecium)		34.2 ab	50.3 a	49.0 a	22.3 b	0.0224 ^y	0.0001 ^z

^w Level of significance of testing the difference of ascospore discharge between incubation temperatures using Proc GLM of SAS. Each value is the mean of six replicate apothecia and the numbers followed by the same letter within a row (group) did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

^x No data were collected because of incubator failure.

^y Level of significance of the difference among grand means by analysis of variance on combined data after they were normalized by dividing treatment means by their group means. Grand means followed by the same letter did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

^z Level of significance of the difference between group means by Proc GLM. Group means followed by the same letter within the last column did not differ significantly according to Duncan's multiple range test at $P = 0.05$.

spores/mm² apothecium) and the least at 25°C (2.2×10^4 ascospores/mm² apothecium; Table 5). The effect of temperature (t) on ascospore discharge (y) was best described as follows:

$$y = a + b_1x + b_2x^2 + b_3x^3; x = t - 8.56$$

Parameters for describing the relation of temperature to the total discharge are seemingly different from those to the average discharge (Table 6), but temperatures for the maximum total and average ascospore discharges are the same (15 to 16°C).

Ascospore discharge and disk size of apothecia. Total ascospore discharge for the various disk sizes of all apothecia were observed over season and incubation temperature (Figs. 2 and 3). No clear relationship was observed between the total ascospore discharge and apothecium disk size for all the groups of apothecia at all temperatures, except for those at 10 and 15°C in 1996 (Table 7). The total discharge increased as disk size increased at these two temperatures in 1996.

Effect of temperature on the germination of ascospores. The germination of ascospores of *M. fructicola* varied with groups of apothecia and incubation temperatures at which ascospores were discharged. In general, the germination of ascospores increased as incubation temperatures increased from 7 to 15°C (Fig. 4A). Essentially all ascospores discharged at 10°C by apothecia of Group 97-1 germinated after 24 h incubation at temperatures of 15, 20, and 25°C. Similarly, the length of germ tubes of ascospores increased as temperature increased from 7 to 15°C (Fig. 4B). No significant differences were observed in the germ tube length among incubation temperatures of 15, 20, and 25°C.

DISCUSSION

The optimal temperature for discharge and germination of ascospores by *M. fructicola* was 15 to 16°C. This temperature is similar to the optimum for development of the apothecia (5,13,15) and for germination of the conidia (41), but slightly lower than the optimum for infection of peach blossoms (41) and peach and sweet cherry fruits by the conidia of the same species (2). Higher temperatures (up to 25°C) did not affect ascospore germination but did reduce ascospore discharge because apothecia disintegrated faster. High temperatures also expe-

ditioned drying of apothecia in orchards when air humidity is low, particularly for those growing between (not underneath) grasses. These findings can help in monitoring and

counting of apothecia in stone fruit orchards, developing ascospore monitoring programs based on orchard ambient temperatures, and scheduling fungicide applications more effi-

Fig. 2. The total discharge and disk size of apothecia of *Monilinia fructicola* from a plum orchard near Reedley, California, in 1996.

Table 6. Regression analysis of the total and average discharges (y) against temperature (t °C) under $y = a + b_1(t - 8.56) + b_2(t - 8.56)^2 + b_3(t - 8.56)^3$

Parameter	Total discharge			Average discharge		
	Estimate	P ^z	R ²	Estimate	P	R ²
a	0.0016	0.9956	0.62	0.5434	0.0862	0.41
b ₁	0.5630	0.0034		0.2439	0.1883	
b ₂	-0.0588	0.0195		-0.0207	0.4057	
b ₃	0.0016	0.0766		0.0004	0.6909	

^y Combined data of ascospore discharge analyzed with Proc NLIN of SAS after they were normalized by dividing the treatment means by their group means.

^z The level of significance of each estimate.

ciently, particularly in areas where apothecia serve as a major source of primary inoculum, such as California (15,17,19) and South Carolina (23,43).

This is the first attempt to quantify ascospore discharge by apothecia of *M. fructicola*. The use of the dry spore traps eliminated the problems associated with the wet

traps. The period required for the air in the traps to reach saturation may slightly differ between the two types of spore traps and among the incubation temperatures of the same types of spore traps. But under both wet and dry spore trap conditions, apothecia of *M. fructicola* discharged ascospores for the same length of time with the same daily discharge, resulting in the same total discharge of ascospores.

Rain, moisture, and relative humidity (RH) have profound effects on ascospore discharge by pseudothecia (8,10,27–29,37), perithecia (20,25,35,39), and cleistothecia (9,12) of other ascomycetous fungi, because these ascocarps are initially closed and free water can help trigger ascospore discharge. We observed that apothecia of *M. fructicola* discharged ascospores to the outside of the traps during the process of replacing the multiwells daily (1996) or weekly (1997). This unexpected ascospore discharge apparently occurred because the apothecia were subjected to abrupt changes of RH from saturated air within the spore traps to $\leq 50\%$ RH in the laboratory air. These discharges may have resulted in the differences of the total and average ascospore discharges between the 2 years of the tests. Apothecia examined in 1996 in spore traps, when multiwells were replaced daily, were subjected to these abrupt changes of RH more frequently than those studied in 1997. The effects of rain, moisture, and RH on ascospore discharge by *M. fructicola* requires additional investigation.

Despite the unexpected ascospore discharges during the process of changing the spore traps (multiwells), the total discharge measured was still as high as 7.5×10^6 ascospores/apothecium at 15°C , and 2.3×10^6 ascospores/apothecium at 25°C . Furthermore, a maximum of more than 3.6×10^7 ascospores discharged by an apothecium was monitored in 1996 (at 15°C) and more than 3.7×10^7 ascospores by an apothecium in 1997 (at 20°C) with this system of ascospore trapping. Ascospores in pseudothecia apparently do not mature all at the same time, and the time required for maturation of the first to last ascospores may take weeks or months with *V. inaequalis*; however, ascospore discharges follow the law of first matured first dis-

Fig. 3. The total discharge and disk size of apothecia of *Monilinia fructicola* from plum orchards near Sanger and Reedley, both in Fresno County, California, in 1997.

Table 7. Regression analyses of the total discharge (y) against disk size (x) of apothecia of *Monilinia fructicola* under $y = a + bx$ at different temperatures in 1996 and 1997^y

Temperature ($^\circ\text{C}$)	Parameter	1996			1997		
		Parameter estimate	P ^z	R ²	Parameter estimate	P	R ²
10	a	-314395	0.4123	0.67	1057469	0.6602	0.08
	b	18222	0.0001		29647	0.1778	
15	a	-4765428	0.0007	0.89	5157191	0.0516	0.12
	b	74111	0.0001		17572	0.1586	
20	a	2015815	0.0750	0.01	6507162	0.0305	0.04
	b	2486	0.7589		12911	0.3625	
25	a	993477	0.0465	0.07	2837135	0.0370	0.00
	b	4206	0.2867		1817	0.8139	

^y Regression analyses were done with Proc REG of SAS.

^z The level of significance of each estimate.

charged (7). The apothecia studied in these tests measured 5 to 28 mm in diameter and all were able to discharge ascospores, suggesting that ascospore discharge occurs when apothecia are as small as 5 mm in diameter. The time required for growth of apothecia from 5 to 28 mm in diameter was not the subject of this research. However, some ascospores might have been discharged before the apothecia were collected from the orchards, since ascospore discharge was observed during the collection process. Variations in the numbers of ascospores discharged by apothecia of the same sizes at the same temperatures in the same seasons could be an indirect evidence of pre-collection discharge. Apparently, some apothecia from the Sanger orchard in 1997 appeared aged and started to wilt when they were collected. Therefore, it is certain that ascospore production of individual apothecia was underestimated with this system of spore trapping. Despite these problems, these quantitative data can help estimate the amounts of ascospores as a source of inoculum for initiating primary infection of stone fruit blossoms, and assess further the relative importance of different sources of primary inoculum at different locations and seasons (3,17-19, 21,22,32,40), as well as between primary and secondary inoculum (16).

With the dry spore trap (replacing multiwells twice), the apothecia collected from the Reedley orchard in 1997 discharged

fewer ascospores than those from the Sanger orchard in the same year, and even discernibly fewer than those from the same orchard in 1996. These differences could be related to exposure to herbicides. Apothecia examined in 1996 appeared vigorous and tender when they were collected from the Reedley orchard. Apothecia of Group 97-2 appeared wilting and soft when they were collected from underneath and among grasses, which had just been killed by herbicides applied on 22 February 1997. It is very common to apply herbicides on the irrigation berms before the season starts in California stone fruit orchards. Interestingly, we never observed any apothecia growing on the top of berms which were sprayed with herbicides. In 1996, when herbicides were applied only on the tops of the berms but not the berm sides, we observed many apothecia on the side of the berms but none on the tops. In 1997, the apothecia collected on 28 February apparently had grown before the application of herbicides; no fresh apothecia were observed on 28 February and afterwards. Herbicides can reduce the germination of conidia and the growth of some fungi (30). Therefore, it is possible that the herbicides applied in these plum orchards either suppressed the development and growth of apothecia, or killed them directly or indirectly by killing grasses above and around the apothecia and expediting wilting and drying of apothecia. The effect of

herbicides and other cultural practices in stone fruit orchards on apothecial development is the subject of further investigation.

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Fig. 4. Effect of temperature on the germination of ascospores discharged at 10°C by three apothecia (A, B, and C, all from the Group 97-1) of *Monilinia fructicola*.

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