Survival of Shot-Hole Inoculum in Association with Dormant Almond Buds

L. M. HIGHBERG, Research Assistant, and J. M. OGAWA, Professor, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

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Viable conidia of *Stigmina carpophila* were found associated with dormant almond buds collected throughout the 1982 dormant season in a commercial almond orchard in Merced County, California. Samples taken from trees where shot-hole disease levels were high during the growing season had significantly more conidia associated with dormant buds than did buds from trees in which disease levels had been significantly lower. In addition, during the 1-mo period between bud swell and bloom, 15- and 10-fold increases in numbers of conidia associated with dormant buds were observed, over previous sampling dates, in samples from trees with high and low disease levels, respectively. Viability of conidia, as determined by germinability, ranged from 65 to 96% for samples throughout the dormant season. In a second study, in which the survival of inoculated conidia on dormant buds was monitored, viability of recovered conidia ranged from 52 to 100% throughout the dormant season. These observations indicate that *S. carpophila* conidia survive the dormant season in association with healthy dormant buds, thereby contributing to the overwintering population of the fungus on the almond tree.

Additional key words: *Coryneum beyerinckii*

Shot-hole disease of stone fruit caused by the fungus *Stigmina carpophila* (Lév.) Ellis (*Coryneum beyerinckii*) has recently been shown to cause significant yield losses in California almond orchards (2). Although bloom-time fungicide applications for shot-hole control are a standard orchard practice in almond production, the number and timing of applications necessary to maintain economically profitable yields have not been related to overwintering population levels of the pathogen.

Unlike other stone fruit hosts where *S. carpophila* overwinters as mycelium in twig infections and blighted buds, on almond, neither type of infection was found in sufficient abundance to constitute the major source of primary inoculum for severe spring leaf and fruit infections (6,7). Although Vuillemin (5) reported the formation of a sexual stage (*Ascospora beyerinckii*) for *S. carpophila* in infected leaf debris, subsequent workers in California, Germany, and Australia failed to find the sexual stage and concluded that only the asexual stage served as an inoculum source (3,4,6).

Despite low frequencies of dormant twig infections and bud blighting on almonds, primary inoculum for spring infection of blossoms and emerging leaves is thought to originate from overwintering sources on the tree, because conidia are disseminated through splashing and windblown rain (6). The occurrence of numerous leaf infections and inoculum buildup in unsprayed almond orchards during fall rains, together with information on the durable nature of the dark, multicelled conidia (3,6), suggest conidia of *S. carpophila* may be deposited on bud scales of healthy dormant buds during fall rains and there survive the subsequent dormant season. If conidia were found to survive the dormant season in association with healthy dormant buds, a control program aimed at preventing fall buildup of inoculum could reduce the amount of primary inoculum the following spring.

The purposes of this study were to determine if viable conidia of *S. carpophila* are associated with healthy dormant almond buds collected from the orchard and to test the survival of *S. carpophila* conidia throughout the dormant season when inoculated onto healthy dormant buds.

MATERIALS AND METHODS

Natural association of *S. carpophila* conidia with dormant buds. Dormant bud samples were collected from 23-yr-old Nonpareil almond trees in a

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commercial almond orchard in Merced County, California, where a study on the effect of shot-hole disease on almond yields had been conducted (2). Plots within the orchard were arranged in a randomized, complete block (RCB) design with eight treatments and three replicates. Treatments consisted of various bloom-time fungicide applications applied over a 4 yr period.

Initial observation of dormant buds collected from lower branches of trees throughout the orchard indicated the presence of *S. carpophila* conidia. To determine whether conidial populations associated with dormant buds parallel disease levels, buds were obtained from trees within the two treatments where disease levels had been the highest (100% infected fruit in group 1) and lowest (11% infected fruit in group 2) when rated earlier in the 1982 season (2). Results from preliminary testing indicated that a sample size of 60 buds was optimal for conidial detection and sample processing.

Ten twigs from each of three Nonpareil almond trees within treatments were randomly collected on 8 December 1982 (leaf fall), 12 January 1983 (bud swell), and 11 February 1983 (early pink bud). For each treatment, 60 apparently healthy flower buds were removed from collected twigs, pooled, and all inner and outer bud scales removed. Scales from each 60-bud sample were placed in glass centrifuge tubes containing 5 ml of sterile distilled water and subsequently mixed on a Vortex mixer for 3 min, centrifuged at 3,000 rpm for 5 min, and remixed for 30 sec to suspend conidia. Washings containing the suspended conidia were removed from the bud scales with a Pasteur pipette and transferred to a clean centrifuge tube. The entire washing procedure was repeated three times for each sample, then suspensions containing conidia were pooled and centrifuged for 5 min at 3,000 rpm. Pelleted materials were resuspended in 0.5 ml of sterile distilled water and spread onto water agar plates. Plates were incubated at room temperature (24 C) for 8 hr, then the total number and viability (determined by germination) of conidia in each sample were recorded (Table 1). Under these conditions, 98% of *S. carpophila* conidia derived from culture germinated when placed on water agar.

Preliminary tests in which a wetting agent was added to bud scale samples before the successive washings did not show an increase in conidia removed from buds previously washed with distilled water. Thus, no wetting agent was used to remove conidia from buds.

Survival of *S. carpophila* conidia inoculated onto dormant buds. Studies on the survival of *S. carpophila* conidia on dormant buds were conducted on 6-yr-old Nonpareil almond trees at the U.C. Davis Armstrong Experimental Field Station. One hundred apparently healthy dormant buds on each of 12 trees were tagged for inoculation.

Conidial suspensions of *S. carpophila* used to inoculate dormant buds were derived from naturally infected almond leaves collected in fall of 1982 and held in cold storage at 32 C. Conidial masses from the centers of sporulating leaf lesions were removed with a sterile dissecting needle, placed in a test tube with 5 ml of distilled water, and mixed on a Vortex mixer to disperse conidia. Conidial suspensions were then adjusted to concentrations of 1.5×10^4 and 1.5×10^5 conidia per milliliter with sterile distilled water.

Inoculations were made on 12 December 1982 by pipetting 20-μl aliquots of the conidial suspension onto the surfaces of tagged dormant buds. Buds on five trees were inoculated with a suspension containing 1.5×10^4 conidia per milliliter (300 conidia per bud) and buds on five trees with a suspension containing 1.5×10^5 conidia per milliliter (3,000 conidia per bud). Buds on two trees were inoculated with sterile distilled water and served as controls.

Beginning 5 January 1983, eight buds per tree were removed at biweekly intervals through pink bud stage of bloom, and the number and viability of conidia present were determined according to the procedure outlined in the previous section.

RESULTS

Viable ungerminated *S. carpophila* conidia were detected in all bud samples collected from December 1982 through February 1983. The average numbers of conidia in 60-bud samples collected from group 1 trees on 8 December, 12 January, and 11 February were 194, 213, and 3,301, respectively. Corresponding figures for group 2 samples were 53, 147, and 1,535 (Fig. 1).

Regression analysis performed on collected data revealed a positive correlation ($P = 0.01$) for the general model between numbers of conidia and time. Regression equations for group 1 and group 2 samples and corresponding coefficent of correlation (r) values were $y = 2.0754 + 0.01826x$, $r = 0.85$, and $y = 1.5759 + 0.0223x$, $r = 0.94$, respectively.

The number of conidia detected in bud samples was significantly correlated ($P = 0.05$) with initial disease severity levels for plots from which samples were obtained. Greater numbers of conidia were consistently detected in samples from trees with higher disease severity levels (group 1) than in those from trees with lower disease severity levels (group 2) (Fig. 1).

A significant correlation ($P = 0.01$) also existed between numbers of conidia and time; increased numbers of conidia were detected in both group 1 and group 2 samples as the dormant season progressed. The greatest increase in numbers of detected conidia occurred during the 1-mo period between bud swell and early pink bud stage of bloom, where 15- and

Table 1. Number and viability of conidia inoculated onto dormant almond buds in 1982–1983

Inoculum density[a] (conidia/ dormant bud)	Sample date[b]								
	5 January			19 January		2 February		16 February	
	Tree no.	No. of conidia[c]	Viability[d] (%)	No. of conidia	Viability (%)	No. of conidia	Viability (%)	No. of conidia	Viability (%)
0 (control)	1	2	100	0	···	3	67	1	100
	7	0	···	0	···	2	100	1	100
300	2	17	76	53	94	7	100	21	95
	3	29	52	20	90	9	100	12	92
	4	59	90	49	86	10	90	28	89
	5	40	72	36	83	26	88	15	100
	6	43	84	51	94	7	100	9	89
3,000	8	>800	82	>800	91	>800	84	>800	90
	9	746	82	>800	86	>800	92	>800	88
	10	>800	75	>800	85	>800	92	>800	87
	11	>800	83	>800	88	>800	93	>800	91
	12	>800	84	>800	84	570	90	>800	95

[a] Conidial suspensions were obtained from sporulating leaf lesions.
[b] Eight tagged inoculated buds were randomly sampled at each sample date during the dormant season.
[c] Total number of conidia present in eight pooled buds.
[d] Percentage of total number of conidia that germinated on water agar after 8 hr.

10-fold increases over previous sampling dates were observed for group 1 and group 2 samples, respectively. There was not a significant correlation between number of conidia and the interaction term, date by disease, for collected samples ($P = 0.05$).

Rainfall data for the 2-mo period when bud samples were collected show that rain occurred on 7 days between 8 December 1982 and 12 January 1983 and on 15 days between 12 January 1983 and 11 February 1983. The total amount of rainfall during these periods was 1.63 and 6.53 in., respectively (1).

Germination percentages for conidia found within the samples ranged from 65 to 96 on water agar after 8 hr, indicating that most of the conidia associated with dormant buds were viable (Table 1).

Survival of *S. carpophila* conidia inoculated onto dormant buds. Viable conidia were recovered from buds inoculated on 12 December 1982 throughout the period from inoculation to early pink bud stage of bloom. More than 800 conidia per eight-bud sample were recovered on each sampling date from buds inoculated with 3,000 conidia per bud with two exceptions: 746 conidia from an eight-bud sample on 5 January and 570 conidia from an eight-bud sample on 2 February (Table 1). Fewer conidia were recovered from buds inoculated with 300 conidia per bud, ranging from seven to 59 per eight-bud sample.

The increase over time in number of conidia per sample observed in naturally infested bud samples from Merced County did not occur with inoculated bud samples in this study. Rain occurred on 41 days from 12 December 1982 to 16 February 1983, for a total of 10.67 in. during this period.

A few blighted buds were observed in trees in which dormant buds had been inoculated with 3,000 conidia per bud. These blighted buds were not evident before the last sampling date, 16 February 1983, when healthy buds were in the pink bud stage of development. Both mycelium and conidia were observed in the blighted buds.

Germination percentages for inoculated conidia recovered from dormant buds ranged from 52 to 100, indicating that most inoculated conidia remained viable throughout the 2-mo dormant season (Table 1).

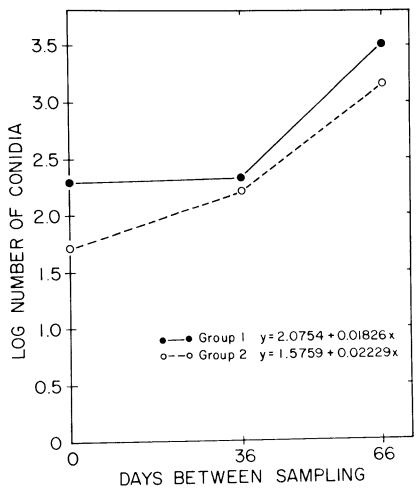

Fig. 1. Number of conidia associated with dormant bud samples collected during the 1982–1983 dormant season from a commercial almond orchard in Merced County, California. Samples were obtained from trees in which disease severity was rated at 100% infected fruit (group 1) and 11% infected fruit (group 2) on 12 May 1982. Each data point represents the mean of three replicates of three 60-bud samples each.

DISCUSSION

Results from these studies show viable conidia of *S. carpophila* were associated with healthy dormant blossom buds throughout the 1982–1983 dormant season in a commercial almond orchard. This observation, together with results from a study in which conidia inoculated onto dormant buds at leaf fall remained viable throughout the dormant season, supports the hypothesis that *S. carpophila* conidia contribute to the overwintering of the fungus on almond trees.

The significant increase in number of conidia detected in bud samples collected in the Merced County orchard over time can be explained in terms of both climatic and disease conditions that existed within the orchard during the dormant season. Rainfall data collected during the study show the frequency of rain increased twofold and total rainfall increased fourfold during the study.

Because *S. carpophila* conidia are easily dislodged and disseminated by rain (6), conidia associated with dormant buds on limbs higher in the trees were perhaps being washed downward by rain onto dormant buds lower in the tree. Limited sampling of twigs during the dormant season showed conidia present on twig surfaces as well as on buds. Thus conidia on aerial portions of the tree, in addition to buds, may have contributed to the number of conidia associated with bud samples. Because bud samples were collected from lower limbs, the occurrence of increased numbers of conidia over time would have been expected because significant amounts of rain fell as the dormant season progressed.

The infrequency and low amount of rainfall during the interval between the first and second sampling dates would account for the relatively small increase in numbers of conidia detected in samples from both group 1 and group 2 collected on 12 January compared with samples collected on 11 February. The significantly greater numbers of conidia consistently detected in group 1 samples compared with group 2 samples (evidenced by the significant difference in the midpoints of the two regression lines) would also have been expected because trees within group 1 had higher initial disease severity levels.

Although dormant bud blighting by *S. carpophila* commonly occurs on other hosts, fewer than 1% of the almond buds observed in either of our two studies were blighted despite the presence of viable conidia on buds throughout the dormant season. Although mechanism(s) that inhibit germination of conidia and bud infection are unknown, this situation does not appear unique to almond. On peach, dormant bud blighting results predominantly from early winter infection of twigs at the bases of buds with subsequent bud kill and invasion of the dead tissue rather than from direct infection of the bud itself (6). The fact that blighted buds found in the bud-inoculation study were not detected until time of bloom suggested that infection had occurred after bud swell.

Despite the low frequency of twig infections on almond and the apparent lack of sexual state formation by *S. carpophila*, results from this study suggest that the buildup of leaf infections and inoculum levels that occur during fall rains, previously considered to be of little consequence (6), is an important component in the shot-hole disease cycle on almond. These conidia, formed within leaf infections during fall rains, not only contribute to the overwintering population of the fungus but also constitute a ready source of primary inoculum for spring infections. In California, seasonal rains normally begin in November and continue through the first of May (1).

Unlike the situation found for other multiple-cycle diseases, the amount of primary inoculum present for initial infections appears to be an important factor in shot-hole disease development on almond. Because almonds begin blooming around mid-February, there is a period of about 2.5 mo in which spring infections can occur. If viable conidia are present on the bud surfaces and blossoms as the young nutlets and leaves emerge, infections and inoculum buildup can readily occur. Thus, if the amount of conidia that survive the dormant season could be reduced by preventing or decreasing the amount of fall leaf infection, perhaps spring infections and inoculum buildup could be delayed long enough to significantly reduce disease levels. Evidence for the effect of reduced amounts of fall inoculum on primary inoculum levels the following spring can be seen in results from our Merced County field plot, where both initial and overall inoculum levels were significantly lower from trees that had significantly lower disease severity levels the previous year. Control strategies such as fall applications of protectant or eradicant fungicides, antisporulants, or zinc sprays to induce defoliation should be investigated in terms of their potential for reducing fall and early winter inoculum buildup and inoculum survival.

ACKNOWLEDGMENTS

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