Release and Dispersal of Conidia and Ascospores of Valsa leucostoma

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Portion of a Ph.D. thesis submitted to the University of California by the senior author.
Research supported in part by a grant from the California Prune Advisory Board.
Accepted for publication 13 February 1976.

ABSTRACT

BERTRAND, P. F., and H. ENGLISH. 1976. Release and dispersal of conidia and ascospores of Valsa leucostoma. Phytopathology 66: 987-991.

Release and dispersal of conidia and ascospores of *Valsa leucostoma* were studied. Conidia were trapped during rains occurring in all seasons, whereas, ascospores tended to be most common in the spring. Ascospores were water-borne (released *during* rain or other wetness) or air-borne (released *following* rain or other wetness). There was no evidence for any nonwater-borne release of conidia. Increasing time and/or temperature between rains and a high rate of rainfall were correlated with increasing numbers of conidia subsequently caught. Conidia were shown to be dispersed by wind-blown rain. The distance of dispersal was correlated with the mean wind velocity during the rain. Either conidia or ascospores were able to cause infection. Conidia, however, were 10 to over 4,000 times more common than water-borne ascospores. Pycnidia generally form during the first year after infection. The ascostromata do not form until 2 or 3 years later. Since prune orchards generally are pruned on a yearly basis to remove dead or excess wood, conidia probably serve as the major inoculum.

Fungi such as *Cytospora leucostoma* Sacc. that form conidia in a pycnidium generally are assumed to release their spores in response to wetting. Wind-blown rain and localized splashing of rain have been suggested as possible mechanisms of conidial dispersal for *Cytospora* spp. (1, 4) although dispersal gradients have not been determined. The perfect stage of this fungus, *Valsa leucostoma* Fr., also occurs in some prune orchards where pruning is neglected. The centrum development of *Valsa* spp. [Family Diaporthaceae sensu Miller (5)] is such that at maturity the asci become detached from the base of the centrum and swim free in the central cavity of the perithecium (6). Ascospores of *Valsa ceratosperma* have been reported to be discharged from the perithecia by two mechanisms (7). Under continuously wet conditions, the ascospores ooze en masse from the perithecium much the same way that the conidia are released. The oozing of the conidial masses from the pycnidium has been described in some detail by Schreiner (8). Forcible ascospore discharge also was demonstrated to occur. *Calonectria* spp. have been reported to show passive or forcible ascospore discharge depending on environmental conditions (3). *Daldinia concentrica* also is known to liberate ascospores by forcible discharge or by oozing (2). Experiments were initiated to study the release and dispersal of conidia and ascospores of *V. leucostoma* in the field.

MATERIALS AND METHODS

Relative numbers of conidia and ascospores in water-borne spore populations and the ability to infect French prune trees.—The relative proportion of ascospores and conidia in water-borne spore populations (spores released during rain or other wetness) was determined from counts of spores collected at trapping stations set up in orchards of French prune, *Prunus domestica* L. 'French,' and President plum, *P. domestica* 'President,' near Yuba City and Davis, California, respectively. Each trapping station included instruments to record rainfall, temperature, and relative humidity. Spores were trapped in plastic bottles by collecting rain water runoff from cankered branches. Traps were constructed as shown in Fig. 1-A, or modified by cutting part of the funnel to fit the trapping surface. The funnels were held in place below fruiting cankers (Fig. 1-B) with waterproof florist's clay and wire. Water in the traps was collected on a weekly basis during rainy periods. Counts of conidia and ascospores were made with a hemacytometer.

On 19 January 1974, 15 French prune trees were inoculated with suspensions of ascospores and conidia. The ascosporic inoculum was prepared by scooping the centrum contents from mature perithecia and suspending the ascospores in it in sterile distilled water. The perithecia were collected in the field from naturally infected French prune bark. The conidial inoculum was prepared by dispersing spores from tendrils in sterile distilled water. The spore tendrils were collected in the field from a naturally occurring, fruiting Cytospora canker. Both spore suspensions were adjusted to a concentration of 2.5×10^5 spores/ml. Inoculation was accomplished by injecting 0.5 ml of the proper spore suspension into wounds created by placing a 1.7-cm-diameter steel bolt against the bark and striking it with a hammer until the bark was broken. The broken bark was lifted slightly, inoculated, and bound with tape. Check trees received 0.5 ml of sterile water in place of inoculum. Inoculations were made on branches about 6 cm in diameter. After an incubation period of 9 weeks, the resulting cankers were evaluated for longitudinal extension.

Environmental factors affecting conidial numbers.—During 1972-1973, conidia were trapped from cankers on four President plum trees near Davis, California. Trapping was done in the same manner as described above, except that the water collections were made at the end of separate rain showers rather than weekly. The environmental parameters considered in the development of a regression equation to account for the variability in spore numbers were: (i) hours between rains, (ii) hours during rains, (iii) mean temperature between rains, (iv) mean temperature during rains, (v) total rainfall, and (vi) mean rate of rainfall.

Wind-blown rain as an agent of spore dispersal.—To test the hypothesis that wind-blown rain is an effective agent of dispersal of *Cytospora* conidia, two parallel series (35 m apart) of vertical trapping surfaces (9.5×180 cm) were set up at 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, and 76.8 m from an inoculum source. The conidial numbers at the source were monitored by placing traps in an infected tree nearest the beginning of each trap series. One trap series is shown in Fig. 2. The traps employed were the funnel traps noted previously, and water collections were made on a weekly basis. Wind velocity and rainfall were recorded on a continuous basis.

The occurrence of air-borne ascospores of Valsa leucostoma.—The seasonal occurrence of air-borne ascospores (spores released other than during rain or other wetness) was determined in the field. A ring source of French prune branches bearing the ascigerous stage of *V. leucostoma* was constructed around a Burkard 7-day recording volumetric spore trap (Burkard Mfg. Co., Ltd., Rickmansworth, Herts, England). The vacuum pump that served the trap drew air through the trap orifice at a rate of 10 liters/minute. The *Valsa*-bearing material and the orifice of the trap were set on the same level, about 40-60 cm apart. A continuous record of rainfall, temperature, and relative humidity was maintained near the ring source. Trapping was begun on 28 November 1972 and continued until 17 April 1973. The tapes collected from the traps were examined for ascospores on an hourly basis.

RESULTS AND DISCUSSION

Relative numbers of conidia and ascospores in water-borne spore populations and their ability to infect French prune trees.—The relative numbers of ascospores and conidia in rain-water runoff collected from cankered branches in the 1972-73 season are shown in Table 1. The results of these counts clearly showed that conidia were the dominant spore type throughout the year. They were detected 10 to over 4,000 times more commonly than ascospores. Conidia caught at various times of the year were germinated on slides coated with potato-dextrose

Fig. 1-(A, B). A) A funnel trap attached to a President plum tree; **B)** typical fruiting Cytospora (Valsa) canker.

agar (PDA) (27 C for 24 hours) and always found to be 98-100% viable. Ascospores were never numerous enough in the traps to allow determination of their viability in this manner. However, at various times of the year perithecia containing mature-size ascospores were scooped out and a spore suspension was prepared. Drops of this suspension were placed on PDA-coated slides and incubated at 27 C for 24 hours. Germination always was greater than 90%.

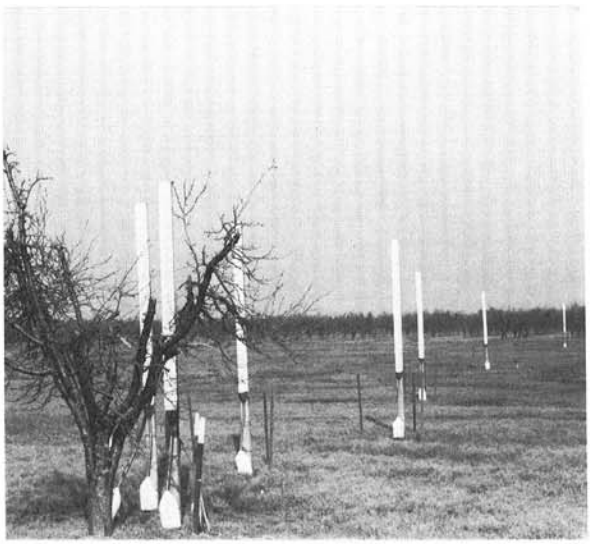

Fig. 2. Series of traps set up to monitor spore dispersal by wind-blown rain.

The results of the inoculation experiment demonstrated that both ascospores and conidia could induce cankers. The mean length of the cankers resulting from inoculation with ascospores (4.9 cm) was not significantly different ($P = 0.01$) from the mean canker length produced by the conidial inoculations (6.4 cm). Throughout the course of this work, any rain which resulted in measurable amounts of water in the traps stimulated the release of conidia, whereas, several rains occurred during which no ascospores were detected in the traps. The ascospores counted in this study are believed to have been released mainly by oozing during the rain.

Environmental factors affecting conidial numbers.—The mean conidial counts from four traps and the environmental data were tabulated for 46 rain periods and a multiple regression analysis was run on the data. The coefficient of determination (R^2) showed that the regression equation produced accounted for 47.4% of the variation in conidial numbers. The inefficiency of the trapping and counting methods, as well as the lack of synchrony in spore production and liberation by the numerous pycnidia, could contribute to a low R^2 value. Time and temperature between rains and rate of rainfall made a significant ($P = 0.01$) contribution to the regression equation. Duration of rainfall, total rainfall, and mean temperature during the rain had no significant ($P = 0.1$) effect on conidial counts. A long period and/or warm temperatures between rains as well as a high rate of rainfall tended to result in higher conidial counts in the traps. Increasing time and temperature between rains

TABLE 1. Mean numbers of ascospores and conidia caught at Yuba City and Davis, California, in rain-water runoff from French prune and President plum, respectively[a]

	President plum		French prune	
Collection date	Conidia × 10^6	Ascospores × 10^6	Conidia × 10^6	Ascospores × 10^6
1972:				
12 Dec	910.00	0.00	110.00	0.00
20 Dec	290.00	0.00	28.00	2.50
27 Dec	560.00	10.00	2.60	0.03
1973:				
2 Jan	78.00	0.47	. . .	. . .
9 Jan	66.00	0.30	370.00	4.50
16 Jan	33.00	0.83	190.00	9.40
23 Jan	460.00	1.10	0.98	0.08
30 Jan	130.00	0.87	0.47	0.09
6 Feb	100.00	0.87	100.00	0.00
13 Feb	70.00	1.20	33.00	1.60
20 Feb	43.00	0.12	0.27	0.02
27 Feb	450.00	0.46	410.00	1.40
6 Mar	1,100.00	1.30	160.00	1.10
13 Mar	100.00	0.11	4.40	0.01
20 Mar	330.00	0.54	430.00	0.48
27 Mar	200.00	0.05	12.00	0.00
3 Apr	140.00	0.14	180.00	0.04
17 Apr	340.00	0.30	280.00	0.00
24 May	4.60	0.00	. . .	. . .
8 Oct	61,000.00	13.00	. . .	. . .
23 Oct	22,000.00	8.90	. . .	. . .
6 Nov	11,000.00	12.00	. . .	. . .
13 Nov	1,800.00	1.70	. . .	. . .
20 Nov	2,300.00	5.60	. . .	. . .

[a]Mean catches of conidia and ascospores in four funnel traps at each location.

should be favorable to production and accumulation of a greater spore load. Increasing rates of rainfall would result in a more rapid wetting to stimulate spore release and, more importantly, would provide more efficient washing of cankered areas.

Wind-blown rain as an agent of spore dispersal.—Wind-blown rain proved to be an effective means by which conidia are dispersed. The dispersal gradients for selected storms are shown in Fig. 3. Following two storms not shown in Fig. 3 conidia were detected in traps 76.8 m from the source. The distance of dispersal and the numbers caught at each distance were highly correlated with the mean wind velocity during the rain periods. Ascospores were so seldom encountered in this study that they were not considered in the counts. It seems reasonable to conclude, however, that water-borne ascospores would have dispersal gradients quite similar to those determined for conidia. Infection gradients, which reflect spore dispersal gradients, quite similar to the dispersal gradients in Fig. 3 were observed by Wilson (9) for the dry spored, brown rot fungus, *Sclerotinia laxa*.

The occurrence of air-borne ascospores of Valsa leucostoma.—The first ascospores were caught during the rain on 17 December 1972. Throughout the remainder of the trapping period, small numbers of ascospores were occasionally caught during the rain. One cannot make a positive conclusion about the mode of liberation of ascospores caught during the rain. They may be water-borne (i.e., ooze from the perithecium and then be splashed up into wind currents) or air-borne (i.e., forcibly shot from the perithecium into the wind currents). Either means could result in their being caught in the Burkard trap. The first conclusive evidence for forcible discharge, as determined by spore catch continuing after the rain had ceased, was in mid-February 1973. From this time until the end of the trapping period following the final spring rain, spore catches that continued after cessation of the rain were common. Numbers of ascospores caught also rose sharply during late February. This was somewhat surprising since on French prune trees most water-borne ascospores were caught in January (Table 1). It is possible that weather conditions during January were more conducive to the oozing of ascospores from perithecia than to their forcible ejection. It also is possible that the *Valsa* material used in the air-borne study matured more of its perithecia in February than earlier in the winter.

The hourly relation of air-borne ascospore dispersal to rainfall, temperature, and relative humidity for one spore release period is shown in Fig. 4. There was no evidence of any diurnal effect on ascospore discharge. Discharge of ascospores was related closely to the occurrence of rain regardless of the time of day. There was no evidence of any nonwater-borne dispersal of conidia.

It has been observed consistently in this study that only the *Cytospora* (conidial) stage occurs on cankers during the first 2 years after infection. A year or more later, and often only after a branch has been girdled and killed, the *Valsa* (ascospore) stage develops. Where careful pruning practices are employed, as in most commercial orchards, dead or badly cankered branches generally are removed from the trees at least biennially. Under these conditions, conidia probably serve as the major inoculum and possibly as the only inoculum. In cases where dead branches are not removed or a neglected orchard is nearby, infections probably result from both ascospores and conidia.

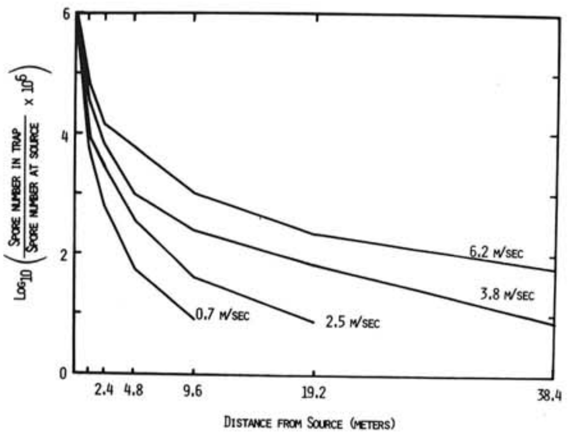

Fig. 3. Dispersal gradients for conidia of *Cytospora leucostoma* as a function of average wind velocity during rain periods.

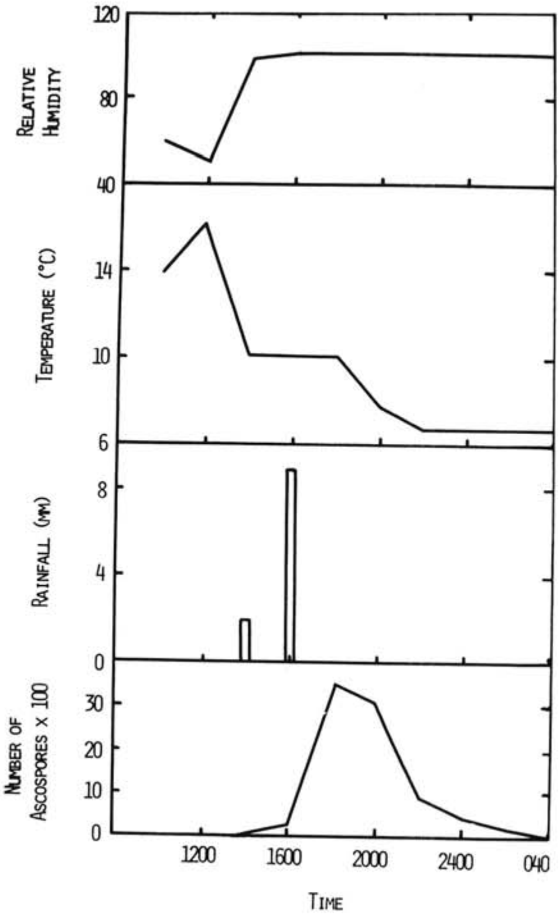

Fig. 4. Air-borne dispersal of ascospores of *Valsa leucostoma* as related to rainfall, temperature, and relative humidity for 13 April 1973.

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