Factors Determining the Relative Number of Ascospores Released by *Eutypa armeniacae* in California

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ABSTRACT

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Based on data taken from a Hirst-Burkard spore trap surrounded by a ring source of inoculum, two parameters have been identified as important for determining the number of ascospores discharged in a single release period of *Eutypa armeniacae*. These are 1) duration of surface free moisture and 2) days from previous release. An "exhaustion" phenomenon was observed during discharge episodes of long duration. Release was triggered either by rainfall or irrigation and ascospores were not released in significant numbers in summer in California despite wetness conditions that caused release at other times of the year.

Additional key words: apricots, grapevines

Eutypa armeniacae (Hansf. & Carter) is a wound pathogen of grapevine and apricot and has been reported as a pathogen or saprophyte of several ornamental and natural species of woody plants (2–4,6,7,9,11). Ascospores serve as the only inoculum and infect the agriculturally important hosts largely through fresh pruning wounds made during the dormant season (2,9). The disease on grapevine called Eutypa dieback is characterized by symptoms that include stunting and yellowing of shoots, leaf size reduction, and leaf cupping and necrosis (8). Symptoms of Eutypa dieback on apricot include gumming, canker formation, and death of infected branches (1).

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Ascospores are discharged from perithecia in octads after sufficient moistening. It has been reported that a minimum of about 1.3 mm of rain is necessary to initiate release (2). Several studies have established a yearly pattern of ascospore release by *E. armeniacae* in various parts of the United States and Australia (7,10,12,13). The pattern is characterized by the discharge of large numbers of ascospores by fall rains. Winter rains cause the release of fewer ascospores but spring rains result in discharges similar in magnitude to those of fall. Except in Australia (7), few if any releases have been observed during summer rains. Although studies report the number of spores trapped and the amount of rain triggering spore release, no quantitative relationship between amount of rain and spore discharge has been formulated.

This paper presents evidence that 1) factors other than amount of rain are important in determining the relative magnitude of release, 2) either rain or irrigation may trigger ascospore release, 3) release of ascospores does not occur to any great extent in California in summer despite wetness conditions that would be favorable to ascospore release at other times of the year, and 4) many release episodes, regardless of season, exhibit an "exhaustion" phenomenon in which additional increments of key factors do not result in a proportional release of ascospores.

MATERIALS AND METHODS

A Hirst-Burkard 7-day volumetric spore trap (Burkard Mfg. Co., Ltd., Rickmansworth, Herts., England) was operated for 28 mo (January 1978 through April 1980) with a source of *E. armeniacae* ascospores at Davis, CA. The inoculum used throughout the study consisted of perithecial stroma on apricot wood collected from the Napa Valley in January 1978. Spore trap tapes were prepared using a Gelvatol tape-base medium containing 35 g Gelvatol, 35 ml distilled water, 50 ml glycerol, and 2 g phenol. This was coated onto the spore tape and allowed to dry for 24 hr. A sticker coating of 90% Vaseline and 10% paraffin and toluene was painted over the Gelvatol mixture. The trap was operated at an airflow rate of 10 L/min. Tapes were brought back to the laboratory, mounted on glass microscope slides measuring 25 × 76 mm (1 day per slide),

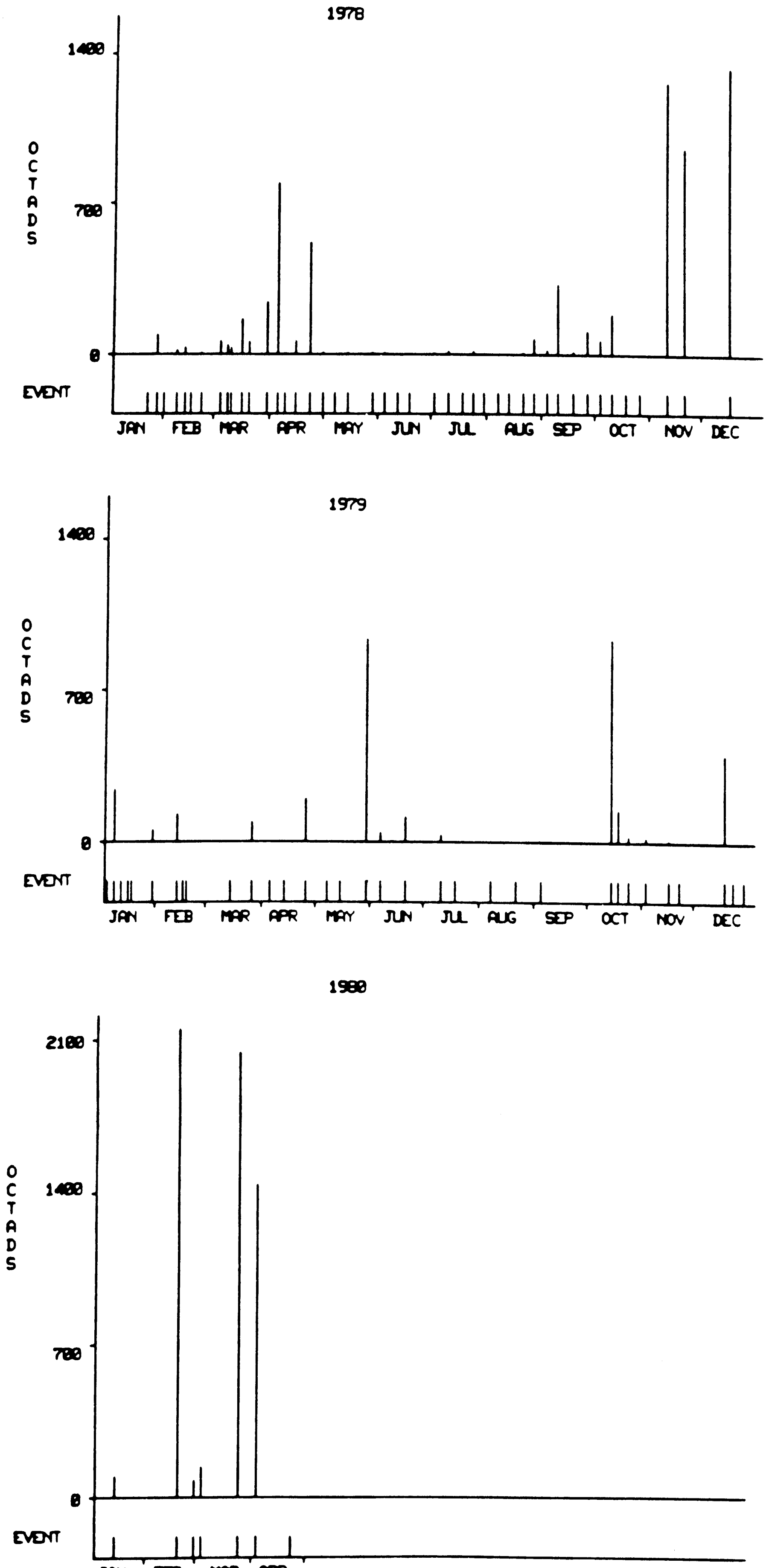

Fig. 1. Number of ascospore octads of *E. armeniacae* trapped during each of 84 possible release events (triggered either by rainfall or irrigation) over a 28-mo period (January 1978 through April 1980).

stained with lactophenol and cotton blue, and examined with a compound microscope. Cotton blue stains allantoid ascospores other than those of *E. armeniacae*, thus aiding in identification. Identification was based on 1) lack of cotton blue uptake, 2) appearance of ascospores in an "octad," 3) ascospore size (7–11 × 1.5–2 μm), and 4) the characteristic allantoid shape. An hourly transverse sampling method that has been shown to be a good indication of catch on a Hirst-Burkard trap was used for counting (5).

Wetness was measured with a Cassella-London leaf wetness recorder (Gluck Barograph and Recorder Co., Ltd., England). Rainfall amounts and temperature data were obtained from the Climatological Benchmark Station at Davis and maintained by the Department of Land, Air, and Water Resources, University of California at Davis. Irrigation amounts were recorded with a Taylor ClearVu Rain Gauge (Taylor Instrument Co., Asheville, NC 28801). Day length was taken from daily reports of sunrise and sunset from the U.S. Naval Observatory. The spore trap and inoculum were covered by lath to provide shading. Irrigation releases were triggered by a manually timed lawn sprinkler mounted over the spore trap.

RESULTS

Results of our trapping study show a yearly pattern of ascospore release similar to the patterns observed in New York, Michigan, and California but slightly different from that in Australia. The numbers of octads trapped during each of 84 spore discharge episodes, ie, periods when spores were continuously observed in spore trap scans, in the 28-mo period are shown in Figure 1. Because of the release of many octads per episode in fall and spring relative to the few released in winter and summer, one would not expect a strong relationship between any factor and octad discharge over an entire year. Therefore, the year was divided into four seasons so that within each season, the numbers of ascospores released per episode were similar.

Release seasons were defined as follows: 1) summer: very few or no ascospores released with each wetness period, approximately May through September, 2) fall: many ascospores released with each wetness period, approximately September through December, 3) winter: fewer ascospores released than fall or spring but more than in summer with each wetness period, approximately December through January, and 4) spring: many ascospores released with each wetness period, approximately February through May.

The number of octads trapped per episode within each season were correlated with six different parameters: recorded hours of surface free moisture (considered the episode duration), days from previous release episode, magnitude of previous release (number of octads trapped during previous release episode), and average daily temperature, day length, and amount of water. Simple linear correlations were made to assist in identifying the factor(s) that show a strong relationship with the magnitude of ascospore discharge and therefore are likely to be useful in more complex models.

The correlation coefficients and levels of significance for each of these parameters in each season are listed in Table 1. As expected, magnitude of ascospore release is not significantly related in a linear fashion to any parameter in the summer, presumably because of the small number of octads discharged. In spring, winter, and fall, the factors most strongly and consistently correlated with magnitude of ascospore release are duration of surface free moisture and days from the previous release period. In no case is the amount of water significantly correlated with magnitude of ascospore release.

In a comparison of rain- versus irrigation-triggered releases, the data from the summer episodes are omitted (Table 2). Although the mean number of octads trapped after rain was much greater than the mean number trapped after irrigation, this difference may be due largely to the much longer duration of surface free moisture associated with rain.

Throughout the 28-mo trapping period, very few ascospores were caught during summer (Fig. 1). In Davis, rain rarely occurs in the summer months; thus, all releases but one in the summer were triggered by irrigation. In spite of periods of surface free moisture in excess of 24 hr, very few ascospores were trapped during these episodes. Twenty-four hours of irrigation was sufficient to release ascospores at other times of the year.

Episodes of long duration often show a characteristic pattern of ascospore release (Fig. 2). When the cumulative number of octads trapped is plotted against the trapping time, a curve that approaches an asymptote is observed. This pattern was noted for many episodes throughout the 28-mo period. It seems to indicate that perithecia may be "exhausted" of available octads for release if the episode duration is long.

DISCUSSION

Ascospore release data for *E. armeniacae* are often reported by plotting the number of octads released in a given episode and showing the amount of water necessary to trigger the release (7,10,12,13). Although rainfall, irrigation, or snow melt have been shown necessary to trigger ascospore release in *E. armeniacae*, no correlation between amounts of precipitation or other water and numbers of octads released has ever been established. Results of this study indicate that no such correlation exists. When octad release data are grouped into seasons in which releases of similar size occur, strong linear correlations emerge for at least two factors: 1) duration of surface free moisture and 2) days from previous release period (in spring, winter, and fall). In summer, no factors could be found that were strongly correlated with magnitude of octad release. This was presumably because of the extremely low releases (near zero) that occurred throughout the summer season in Davis. Perhaps, some other as yet unrecognized factor is important in limiting summer release because ascospores were not discharged during periods of wetness that caused release at other times of the year.

Duration of surface free moisture is most strongly correlated with magnitude of octad release in seasons of very high octad release, whereas in winter, a period of lower release, episode duration is not significantly correlated. Days from previous release period, on the other hand, is most strongly correlated with magnitude of release in winter, indicating that perhaps in winter, only a small reservoir of octads is available to be released and that they are rapidly exhausted during each episode. Therefore, extending the episode duration would not cause more octads to be released. A period of time may be required to replenish the reservoir and this may be the reason such a strong correlation exists between days from previous release and numbers of octads trapped. In spring and fall, however, a large reservoir of octads may exist so stronger correlations are observed between episode duration and magnitude of release. At the same time, the period required to replenish the reservoir may be less important in

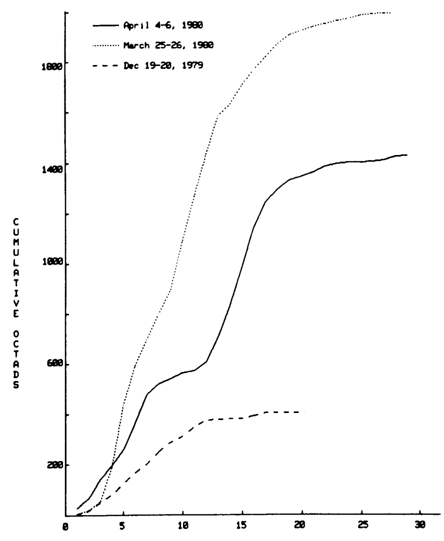

Fig. 2. Accumulated ascospore octads of *E. armeniacae* trapped during three individual release episodes.

Table 1. Correlation coefficients for various parameters and the number of ascospore octads of *Eutypa armeniacae* trapped per release episode using a Hirst-Burkard 7-day volumetric spore trap operated at Davis, CA (January 1978 through April 1980)

Parameter	Fall ($n = 20$)	Winter ($n = 20$)	Spring ($n = 25$)	Summer ($n = 17$)
Episode duration[a]	0.527*[b]	0.227	0.635***	0.350
Days from previous release period[c]	0.594***	0.733***	0.632***	−0.266
Magnitude from previous release period[d]	0.265	−0.136	0.185	−0.188
Average temperature[e]	−0.572**	−0.170	−0.227	0.236
Day length[e]	−0.476*	−0.248	−0.266	0.273
Amount of water[f]	0.066	0.088	0.314	0.165

[a] Number of hours of surface free moisture recorded.
[b] * = Significant at $\alpha = 0.05$, ** = significant at $\alpha = 0.01$, and *** = significant at $\alpha = 0.005$.
[c] Number of days from the last potential release episode.
[d] Number of octads trapped during the previous release period.
[e] Average during the release period.
[f] Total amount of rain or irrigation water recorded during the release period (mm).

Table 2. Comparison of numbers of ascospore octads of *Eutypa armeniacae* trapped per release episode using a Hirst-Burkard 7-day volumetric spore trap operated at Davis, CA (January 1978 through April 1980)

	Release trigger	
	Irrigation ($n = 24$)	Rain ($n = 42$)
Mean number octads trapped	106.5 (±238.8)[a]	307.3 (±553.4)
Mean episode duration	12.7 hr (±15.5)	49.7 (±48.8)
Mean days from previous release	7.1 days (±3.8)	8.6 days (±7.07)

[a] Standard deviation.

determining the number of octads to be released. This concept is supported by the "exhaustion" phenomenon observed in many of the episodes of long duration.

Rainfall and irrigation both appear to be capable of triggering release, but on the average, many more octads were trapped during rainfall-triggered releases than during irrigation-triggered release. It appears that the duration of surface free moisture was proportionately increased during rainfall-triggered releases. Presumably, this is because of the higher relative humidity present on rainy days than on the nonrainy days when irrigation was performed. On rainy days, release episodes lasted well beyond the end of rain because of the slowness of drying, whereas on nonrainy days, episodes ended shortly after cessation of irrigation.

In conclusion, the magnitude of release of ascospores of *E. armeniacae* during a single episode is apparently linearly related to at least the factors mentioned earlier: 1) duration of surface free moisture and 2) days from previous release in seasons of high ascospore discharge. Further work is needed to specifically identify the relationship between these factors and release.

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