Production and Dispersal of Ascospores of Eutypa armeniacae in California

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ABSTRACT

Mature perithecial stromata of *Eutypa armeniacae* develop readily on dead, infected apricot wood in the high (> 508 mm) rainfall area around San Francisco Bay. Stromata are sparsely distributed in other apricot districts where the mean annual rainfall exceeds 330 mm, but are not present in the more arid interior valley districts. Grape and *Ceanothus,* two additional hosts of *E. armeniacae,* do not contribute significantly to the spore inoculum in California. A two-year study of the seasonal abundance of airborne ascospores showed a period of low ascospore frequency in late fall and early winter, but the only safe period for pruning apricot trees is during the dry summer months. Evidence is presented for long-distance dispersal of airborne ascospores from areas of high (> 508 mm) rainfall to the interior valley apricot-producing districts. Inoculum levels of *E. armeniacae* were compared for their ability to infect fresh pruning wounds in apricot trees. Infection resulting from 1, 10, or 100 ascospores per wound was not significantly different when wounds were inoculated during tree dormancy, whereas infection by one ascospore during the growing season was significantly lower than that caused by 10 or 100 ascospores.

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Little is known about the source and dispersal of inoculum of the apricot (*Prunus armeniaca* L.) pathogen, *Eutypa armeniacae* Hansf. & Carter, in the interior valley of California. The imperfect stage of the pathogen plays no part in the infection process (2, 6), but airborne ascospores originating in areas with annual rainfall exceeding 330 mm constitute the inoculum (2). Perithecia are exceedingly rare and disease incidence is much lower in irrigated districts where the mean annual rainfall is below 279 mm (2). In the Tracy-Patterson district of the San Joaquin Valley, where most California apricots are produced, the rainfall is less than 254 mm, but the disease incidence is high. This has led to speculation concerning other possible hosts for the perithecial stage and/or long distance dissemination of inoculum from areas with higher rainfall.

Grape (*Vitis vinifera*) (3), tamarisk (*Tamarix* sp.), almond (*Prunus amygdalus*), and apple (*Malus sylvestris*) (4) are additional sources of the perithecial stage (2). Stromata of *E. armeniacae* are widely distributed on deadwood of grape throughout the 330-762 mm rainfall districts of South Australia (3). Later, Moller (9) found perithecia of the pathogen on old deadwood of *Ceanothus thyrsiflorus*.

In California, perithecia of *E. armeniacae* were first detected in 1962 (7) and were present in several apricot areas in 1965 (11). Stromata collected from apricot trees in three coastal counties (Solano, San Benito, and Santa Clara) contained mature perithecia and viable ascospores. Subsequently, a few mature stromata were found on deadwood of grape in Solano County (12) and on California wild lilac (*Ceanothus* sp.) in Sonoma County (13).

This paper reports (i) surveys for sources of ascospore inoculum in the west side of the San Joaquin Valley, the mountains and valleys of the Coast Range, and the San Francisco Bay area, (ii) the seasonal abundance of airborne ascospores in Tracy and the Suisun Valley of Solano County, and (iii) the relationship between rainfall and wind patterns and discharge and dissemination of ascospores. Any hypothesis for long-distance dispersal of a pathogen hinges on the feasibility of minimum inoculum densities for infection and subsequent disease, and thus, (iv) inoculation of fresh wounds with various inoculum densities were studied.

MATERIALS AND METHODS.—In October, 1970, a Burkard 7-day recording volumetric spore trap (Burkard Mfg. Co., Ltd., Rickmansworth, Herts, England) was placed in a commercial apricot orchard in the Suisun Valley (hereafter called "Suisun") (mean annual rainfall 524 mm) of Solano County, California, where perithecia of *E. armeniacae* were abundant. Dead apricot wood bearing mature stromata were positioned around the trap, in a 1.8 m diameter circle, to provide an effective source of inoculum and a more precise estimate of the seasonal variation in ascospore release. The inoculum sources were placed on field boxes, 23 cm above the ground surface. The spore trap was placed within the inoculum circle and was operated continuously during the rainy seasons of 1970-71 and 1971-72. In 1971-72, a second spore trap was placed in a commercial orchard near Tracy (mean annual rainfall 228 mm) approximately 56 km east of any known source of *E. armeniacae* inoculum. Weather data were obtained at both sampling sites using a recording rain gauge, anemometer, and hygrothermograph. Both traps were operated with the intake orifice 0.6 m above ground level and an air flow rate of 10 liters per minute. Techniques used for adhesive preparation, and dissection and mounting of the tape were as described in the instructions for the Burkard sampler.

Hourly estimates of ascospore concentration were obtained by scanning short traverses across the slides at 2-mm intervals, which corresponds to the movement of the slide past the intake orifice. Ascospores of *E. armeniacae* were distinguished by their characteristic size, shape, and presence in octads (2). Cotton blue in the mountant stained some ascospores similar to those of the nonstaining *E. armeniacae* and provided a background against which the hyaline ascospores were more easily seen (10). Our observations supported an earlier conclusion that ascospores were common in air only during or soon after rain (2). Therefore, traverses were made at least 1 hour before rain commenced and continued until no *E. armeniacae* ascospores were observed on at least three traverses after the rain ceased. Slides from the Tracy trap were examined in relation to the rainfall pattern at Suisun as well as at Tracy.

To determine the quantity of inoculum required for infection, 1, 10, and 100 ascospores were applied to fresh pruning wounds (14), 1.27 cm in diameter, on 1-year-old Patterson apricot trees. Inoculum was applied in a 5 μliter drop followed by an additional application of 20 μliters of sterile distilled water. Control wounds received 25 μliters of sterile water. This experiment was repeated on 19 January and 11 February 1971, with 50 trees per treatment. All of the trees (three trees per 11.4-liter can) were dormant when placed in a greenhouse for the first series of inoculations. However, after 3 weeks in the greenhouse with a daily mean temperature of 10 C, the trees started to grow before the second series of inoculations. On 19 March, the trees were moved to a lathhouse.

RESULTS.—*Geographic distribution of the perfect*

TABLE 1. Mean annual rainfall and the occurrence of perithecia of *Eutypa armeniacae* in various California apricot-producing districts

District	County	Mean annual rainfall (mm)	Abundance of perithecia[z]
Fairfield	Solano	524	+++
Hayward	Alameda	661	+++
Brentwood	Contra Costa	371	+
San Jose	Santa Clara	333	+
Hollister	San Benito	334	+
Tracy	San Joaquin	243	–
Patterson	Stanislaus	250	–
Los Banos	Merced	222	–

[z]Rating scale: +++ = abundant and well developed; + = limited and poorly developed; – = none found.

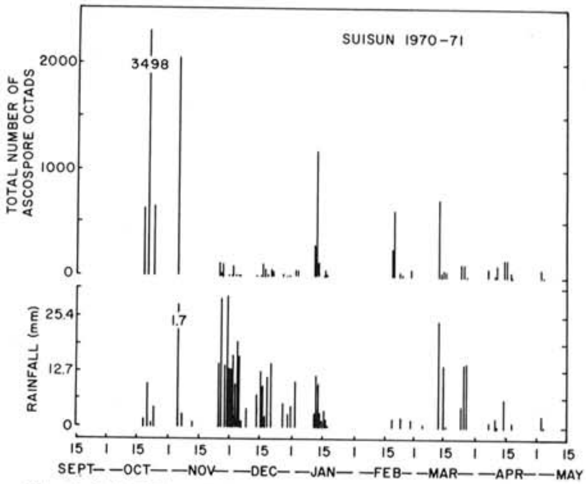

Fig. 1. Rainfall and total number of airborne ascospore octads detected daily by sampling 0.6 m^3 of air each hour with a Burkard spore trap which was placed within a 1.8 m diameter circle of dead apricot branches with mature perithecia of *Eutypa armeniacae*. Suisun Valley, California, 1970-71.

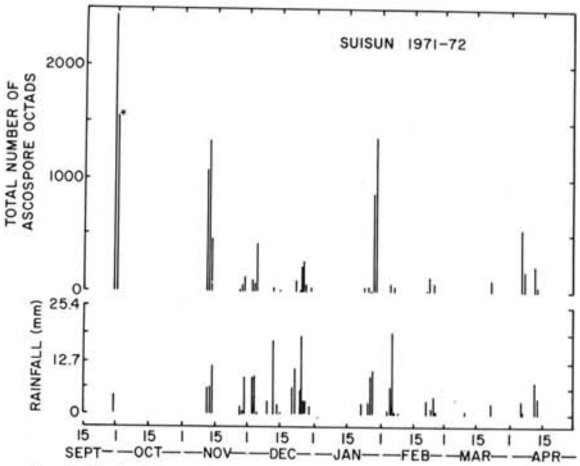

Fig. 2. Rainfall and total number of airborne ascospore octads detected daily by sampling 0.6 m^3 of air each hour with a Burkard spore trap which was placed within a 1.8 m diameter circle of dead apricot branches with mature perithecia of *Eutypa armeniacae*. Suisun Valley, California, 1971-72.(*Denotes incomplete record.)

TABLE 2. Total number of airborne ascospore octads of *Eutypa armeniacae* trapped in a commercial apricot orchard at Tracy, California, when rainfall amounted to 1.27 mm or less, September, 1971-April, 1972

Date	Days since last rain	Amount of rain (mm)	Total number of ascospore octads[z]
29-30 Sept.	>100	1.02	1,264
11-12 Nov.	26	0.25	647
28 Nov.	2	1.02	66
29 Dec.	2	0.51	15
23 Jan.	25	0.25	29
22 Mar.	23	0.25	37
5-6 Apr.	1	1.02	15
12-13 Apr.	1	1.02	44

[z]Each figure represents the daily total of ascospore octads detected by sampling 0.6 m^3 of air each hour with a Burkard spore trap.

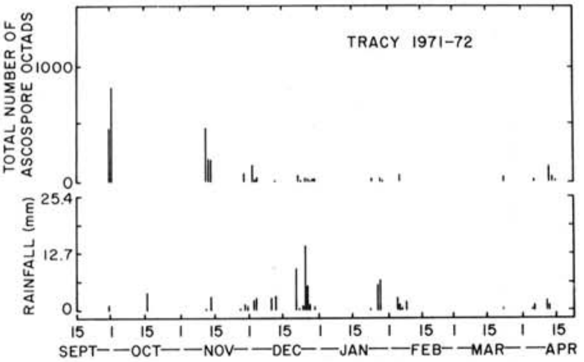

Fig. 3. Rainfall and total number of airborne ascospore octads of *Eutypa armeniacae* detected daily by sampling 0.6 m^3 of air each hour with a Burkard spore trap which was placed in a commercial apricot orchard. Tracy, California, 1971-72.

stage of Eutypa armeniacae on apricot.—Abundant stromata bearing mature and immature perithecia of *E. armeniacae* were observed on apricot trees near Suisun Bay in Solano County and along the east side of San Francisco Bay near Hayward, California. Despite prevalence of the disease on the west side of the San Joaquin Valley, not a single perithecial stage of the pathogen was found. In some cases, a thin stroma appeared on the decorticated wood of diseased trees, but these stromata failed to mature. Limited quantities of mature perithecia were found in areas of intermediate rainfall such as Santa Clara and San Benito counties as well as in the Brentwood district of Contra Costa County, but the stromata were poorly developed in contrast to those observed near Suisun or Hayward. Table 1 summarizes annual rainfall and perithecium occurrence in apricot-producing areas of California.

Contribution of infected vineyards to the supply of inoculum for infection of apricot.—The survey of vineyards in the Livermore Valley (mean annual rainfall 366 mm) in 1971 failed to detect perithecia of the pathogen. Although grape isolates are pathogenic on apricot, it appears that significant inoculum from this source is unlikely.

Contribution of Ceanothus to the supply of inoculum.—Discovery of *E. armeniacae* perithecia on *Ceanothus* in California suggested a possible role of native *Ceanothus* spp. as a source of inoculum for infection of apricot trees. Surveys were conducted in the Mt. Oso and Mt. Diablo regions of the interior Coast Range and in the mountainous area around Watsonville and Sebastopol along the coast. No stromata of *E. armeniacae* were observed, except for a few in the high rainfall area near Sebastopol. Native *Ceanothus* spp. are unpruned, except near roadsides, which makes them unlikely hosts for the production of ascosporic inoculum in significant quantity.

Seasonal abundance of airborne ascospores.—Seasonal changes in ascospore output from concentrated inoculum sources at Suisun during 1970-71 and 1971-72 are shown in Fig. 1 and 2, and Fig. 3 shows the frequency of airborne ascospores in Tracy during 1971-72. The daily total of airborne ascospore octads was obtained by summation of the hourly estimates of octads per cubic meter of air. The daily totals were plotted together with daily rainfall; totals for June, July, and August are not shown since this is a rain-free period with no ascospore discharge.

A significant feature of the data is the relatively low concentration of airborne ascospores during late fall and early winter. In 1970-71, a decrease in numbers of ascospores occurred after 4 November followed by a rise on 10 January. This amounted to a 66-day period of low numbers of airborne ascospores. In 1971-72, the period of low ascospore frequency was from 14 November to 27 January, a total of 74 days. Thus, there is a period of 2 to 2.5 months in the winter in which ascospore discharge is significantly reduced, despite frequent rains sufficient for ascospore release (2). This period of low frequency of airborne ascospores is similar to that found in Australia (10), but the duration and magnitude of the reduction in California appears to be less.

A second significant feature is the high ascospore concentrations obtained with fall rains as compared to those in the spring. This might be the result of a prolonged dry period in the summer and an accumulation of mature asci.

Also noteworthy is the reduction in ascospore concentrations in the spring after a large release in January (Fig. 1 and 2), and the detection of significant numbers of ascospores at Tracy (Fig. 3), where no known source of inoculum is present.

The incidence of rain in the two areas is similar, although the total rainfall of Suisun is substantially higher than at Tracy. In addition, storms typically come into California from the northwest, so that precipitation normally begins in the Suisun area in advance of that in Tracy.

Pattern of discharge of ascospores as related to rainfall and wind.—Data collected from the experimental source of ascospores in Suisun over a 2-year period showed that a minimum rainfall of 1.27 mm was necessary for ascospore discharge. In no case was discharge noted when rainfall was less, and this agrees with results obtained in Australia (10). In contrast, airborne ascospores were trapped at Tracy with increments of rain < 1.27 mm (Table 2), although in each case rainfall in excess of 1.27 mm had been recorded to the northwest in Suisun several

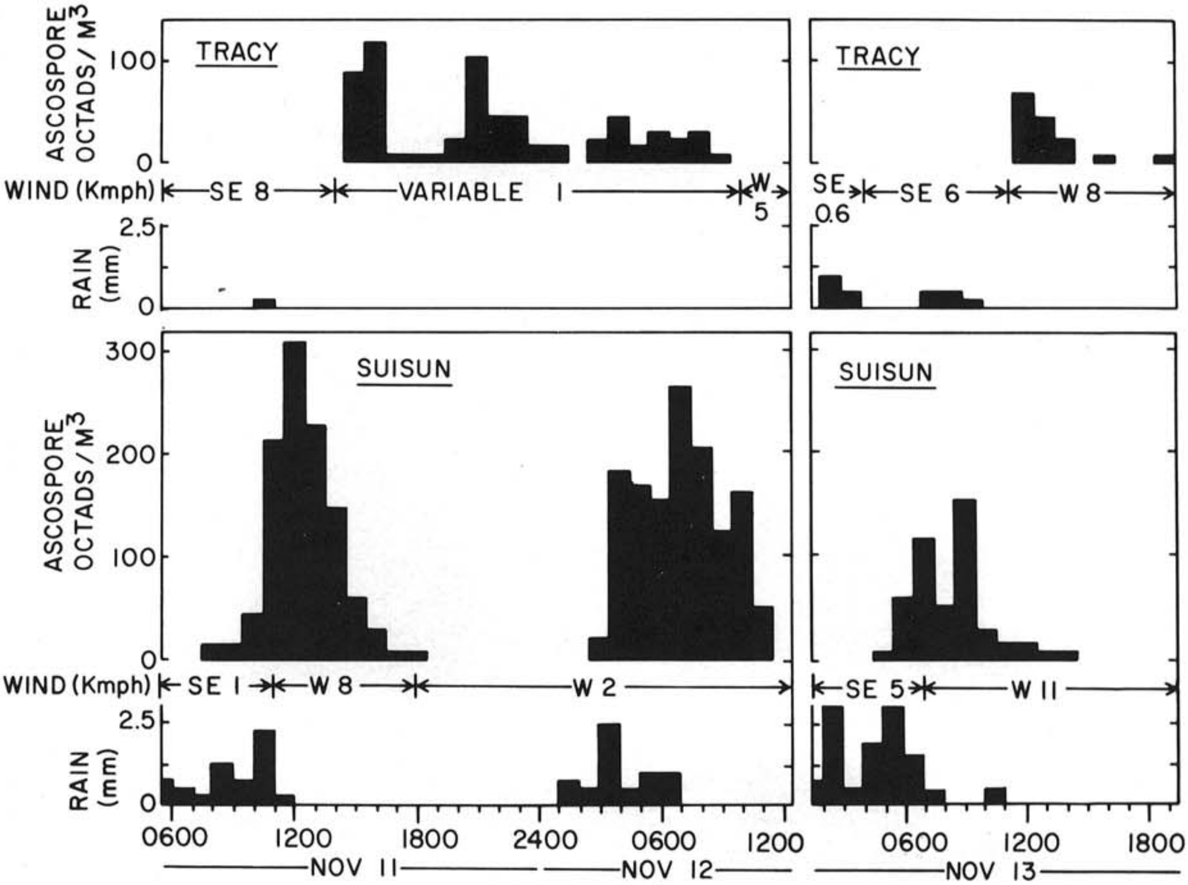

Fig. 4. The effects of rainfall and speed and direction of wind on concentrations of airborne ascospore octads of *Eutypa armeniacae* at Suisun Valley and Tracy, California, 11-13 November 1971.

hours earlier. In periods of frontal rain, the wind is initially from the southeast, but it normally shifts at about the time precipitation ceases and then comes from the west. Thus, airborne inoculum could move eastward with the front from the higher rainfall area into the more arid interior valley.

The possible association between airborne ascospore concentrations and weather condition was studied at Suisun and Tracy. The study periods chosen represent rainy periods in the fall (Fig. 4), winter (Fig. 5), and spring (Fig. 6) in which there were large releases of ascospores at Suisun. In each example, the concentration of ascospore octads, rainfall, and wind direction and speed were recorded on an hourly basis.

The rainfall on 11 November was sufficient to discharge ascospores in Suisun, but not in Tracy where only 0.25 mm was recorded (Fig. 4). At Suisun, the rain was accompanied by wind from the southeast which shifted to a west wind (8 kmph) immediately following the rain and continued through the period of maximum ascospore discharge. High concentrations of ascospores were detected in Tracy beginning at 1500 hours, 4 hours after the shift in wind direction at Suisun. Ascospores were not collected in Tracy following the large discharge at Suisun on 12 November, but this could be explained by the light (2 kmph) wind condition. Rainfall was in excess of 1.27 mm in both areas on 13 November, but ascospores were not detected in Tracy until 8 hours after the onset of rain. The ascospore concentrations at Tracy appeared more related to the ascospore release and strong (11 kmph) westerly wind at Suisun which began 5 hours before the initial detection of ascospores at Tracy.

On 26-28 January 1972, there was ample rainfall in both locations for ascospore release, but high concentrations of airborne spores were detected only in Suisun (Fig. 5). Ascospore release was initiated in Suisun by steady rain which began on 26 January 1700 hour, but discharge during and immediately following this rain was relatively light. However, the concentrations of airborne ascospores increased substantially between 1800 hours on 27 January and 0800 hours on 28 January, 7 hours after the last rain. The abnormally long delay between the end of rain and detection of maximum ascospore concentrations can be explained by the time required to thoroughly wet the perithecial stroma and underlying wood before ascospores can be released in abundance (2). This "conditioning" of the perithecia requires longer rain period when it follows a long, dry period (10). Prior to the rain on 26 January, significant rain had not fallen since 25 December 1971. In addition, late January is the beginning

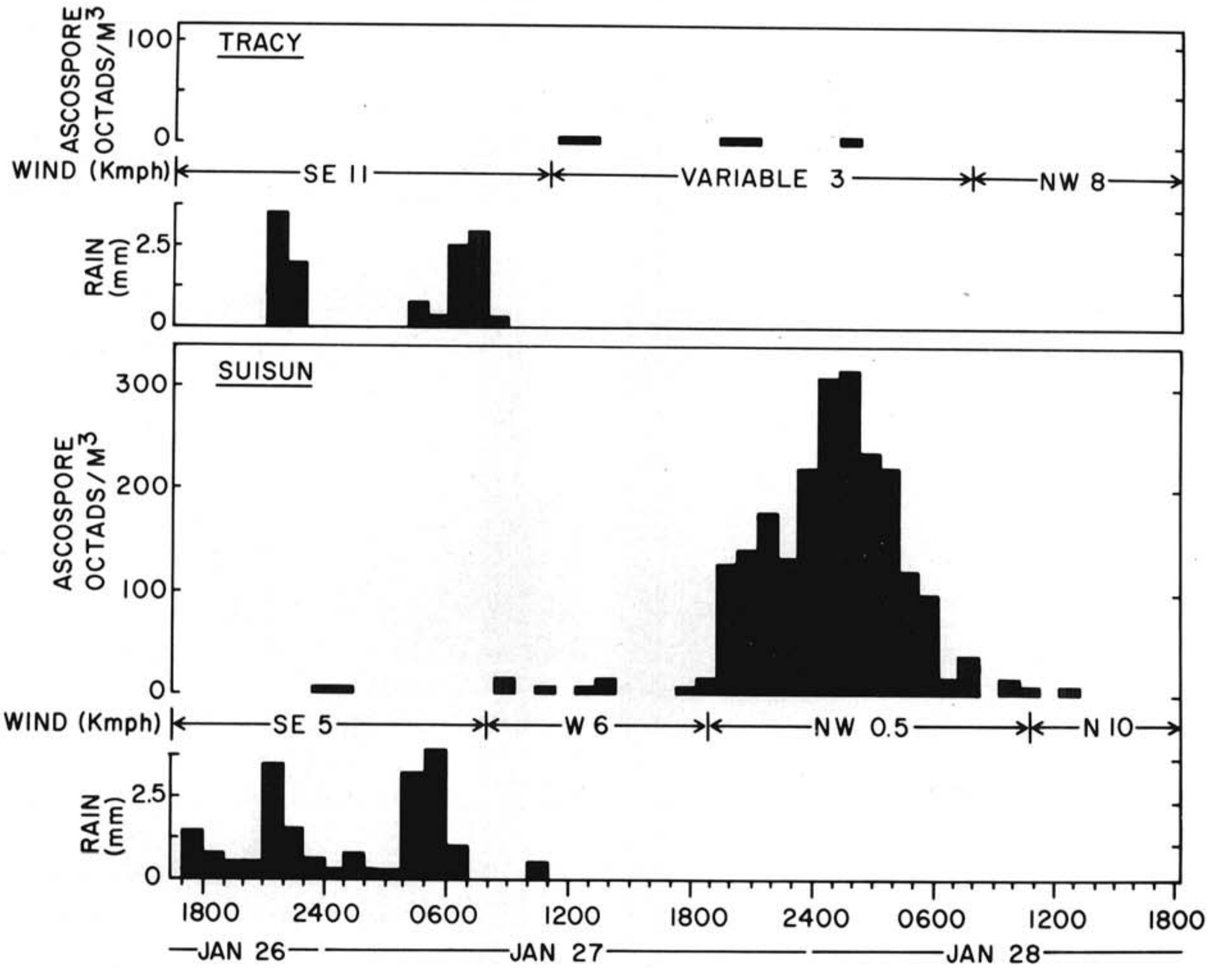

Fig. 5. The effects of rainfall and speed and direction of wind on concentrations of airborne ascospore octads of *Eutypa armeniacae* at Suisun Valley and Tracy, California, 26-28 January 1972.

of increased ascospore release after the low discharge characteristic of the early winter period. Between 27 January 1900 hours and 28 January 0800 hours the maximum ascospore concentrations in Suisun was accompanied by light (< 0.5 kmph) wind from the northwest which might explain the lack of movement of airborne inoculum into the Tracy area.

At the onset of rain on 5 April there was sufficient (> 1.27 mm) precipitation for ascospore release at Suisun but not at Tracy (Fig. 6). There was a large discharge at Suisun but a light west (3 kmph) wind and few ascospores detected in Tracy several hours later. In the morning of 11 April, there was over 1.27 mm of rain at both locations, but ascospores were detected only at Suisun. However, relatively large concentrations were collected after 1800 hours in Tracy which may have occurred because of a strong west wind (13 kmph) at Suisun earlier in the day.

The three periods studied are typical of other periods (shown in less detail in Fig. 2 and 3) in which it appears that ascospore concentrations at Tracy were related to the discharge pattern and wind conditions recorded in the Suisun area. A comparison of climatological data from Oakland International Airport (near Hayward) with that obtained in Suisun showed that the pattern of rainfall and changes in wind direction were similar. However, the speed of the westerly wind following a rain was consistently 10-13 kmph stronger in Oakland. The greater wind velocity in the Hayward area is more compatible with a theory of spore transport into the Tracy area. This suggests that the Hayward area, where *Eutypa* also is abundant, might be more important than the Suisun area as a source of inoculum for infection of apricot trees in the west side of the San Joaquin Valley.

Quantity of inoculum required for infection.—There was no significant difference in the level of infection resulting from 10- and 100-ascospore inoculations, regardless of inoculation date (Table 3). However, the incidence of infection produced by one ascospore per wound varied significantly with the date of inoculation; e.g., infection in wounds inoculated on 19 January was not significantly different from that obtained with 10 or 100 ascospores per wound. By contrast, wounds inoculated 11 February (after trees had commenced active growth) had significantly fewer infections than trees inoculated with 10 and 100 spores on this date. The

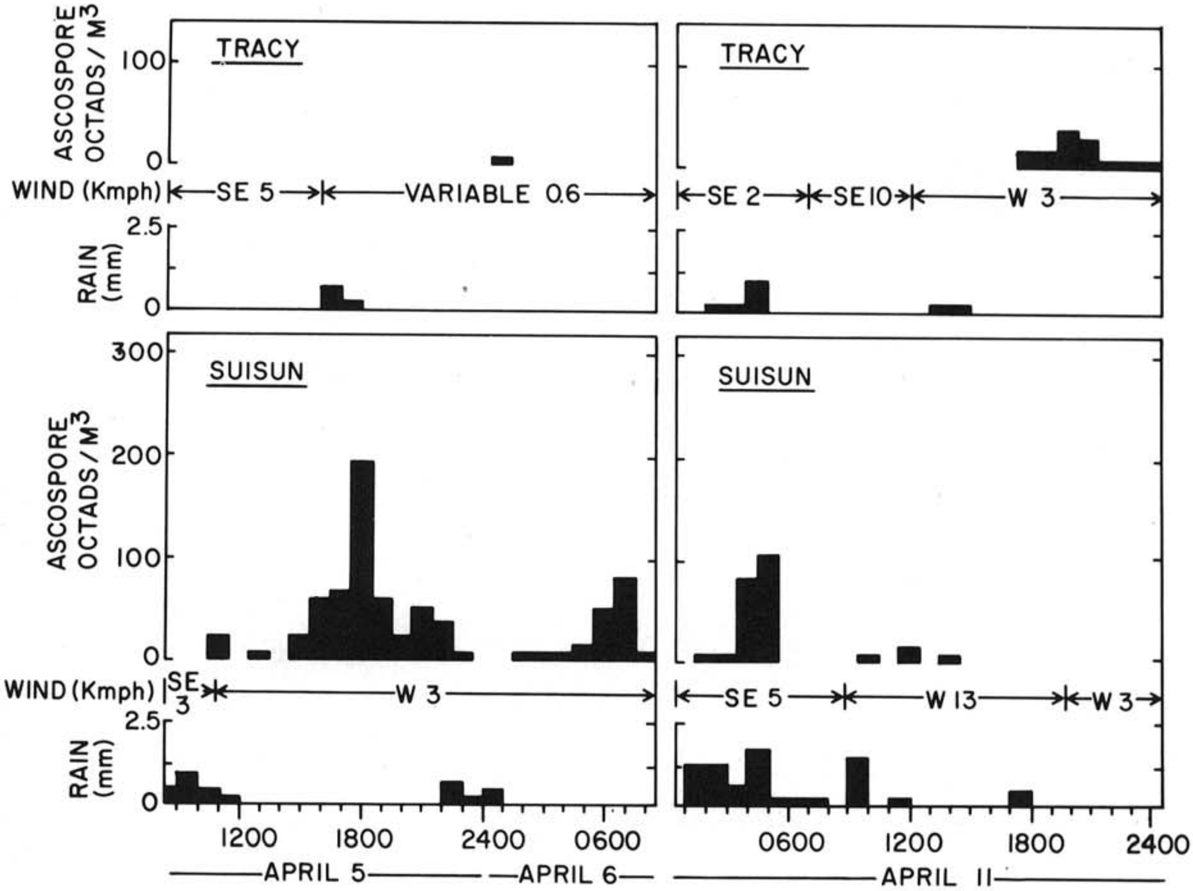

Fig. 6. The effects of rainfall and speed and direction of wind on concentrations of airborne ascospore octads of *Eutypa armeniacae* at Suisun Valley and Tracy, California, 5-11 April 1972.

only apparent difference between these two inoculation dates was an increase in physiological activity of the trees in February, accelerated by the modified environment in the greenhouse.

The infections in control trees (uninoculated) are attributed to natural infection during rainfall beginning on 23 March, 4 days after the trees were moved from the greenhouse into the lathhouse. The proportion of natural infection in the younger wounds made on 11 February was significantly greater ($P < 0.05$) than in the wounds made 3 weeks earlier on 19 January. This can be attributed to the differential susceptibility of pruning wounds of different age. However, the relatively high incidence of natural infection observed in the control trees may have occurred because of prolonged susceptibility when the wounds remain in a sheltered, dry environment (14).

DISCUSSION.—Diseased apricot trees in the higher (> 508 mm) rainfall areas near the San Francisco and Suisun bays appear to be the only major source of ascospores of *E. armeniacae* for infection of apricot trees in the San Joaquin Valley. Distribution and occurrence of the perithecial stage in California is related to mean annual rainfall (> 330 mm) just as in Australia (2). *E. armeniacae* on grape or native *Ceanothus* sp. is insufficient to supply significant amounts of inoculum for infection of apricot trees.

Ascospore production by the pathogen is maximum in the fall, probably due to the almost total absence of rain

TABLE 3. Incidence of infection of fresh pruning wounds of apricot trees artificially inoculated with 1, 10, or 100 ascospores of *Eutypa armeniacae* per wound[w]

Inoculation date	Ascospores (no.)	Cankers[x] (%)	Total wounds infected[y] (%)
19 January 1971	100	88 a	98 ab
	10	70 bc	98 ab
	1	56 c	88 b
	control[z]	0 e	10 d
11 February 1971	100	78 ab	100 a
	10	70 bc	98 ab
	1	22 d	48 c
	control[z]	8 de	30 c

[w]Total of 50 wounds per treatment. Figures followed by different letters in the same column are significantly different ($P = 0.05$) as determined by χ^2 tests.

[x]Based on observation of cankers 5 to 8 months after inoculation.

[y]Based on reisolation of the pathogen after 8 months.

[z]Natural infection from exposure to rain beginning on 23 March 1971.

during the summer. This, coupled with the greater susceptibility of pruning wounds in the fall (14), suggests the inadvisability of pruning apricot trees during the early fall. There is a period of relatively low ascospore release in late fall and early winter, but the frequency of airborne ascospores during this period is sufficient to produce a significant level of natural infection. Late December and January pruning can be particularly risky because of the

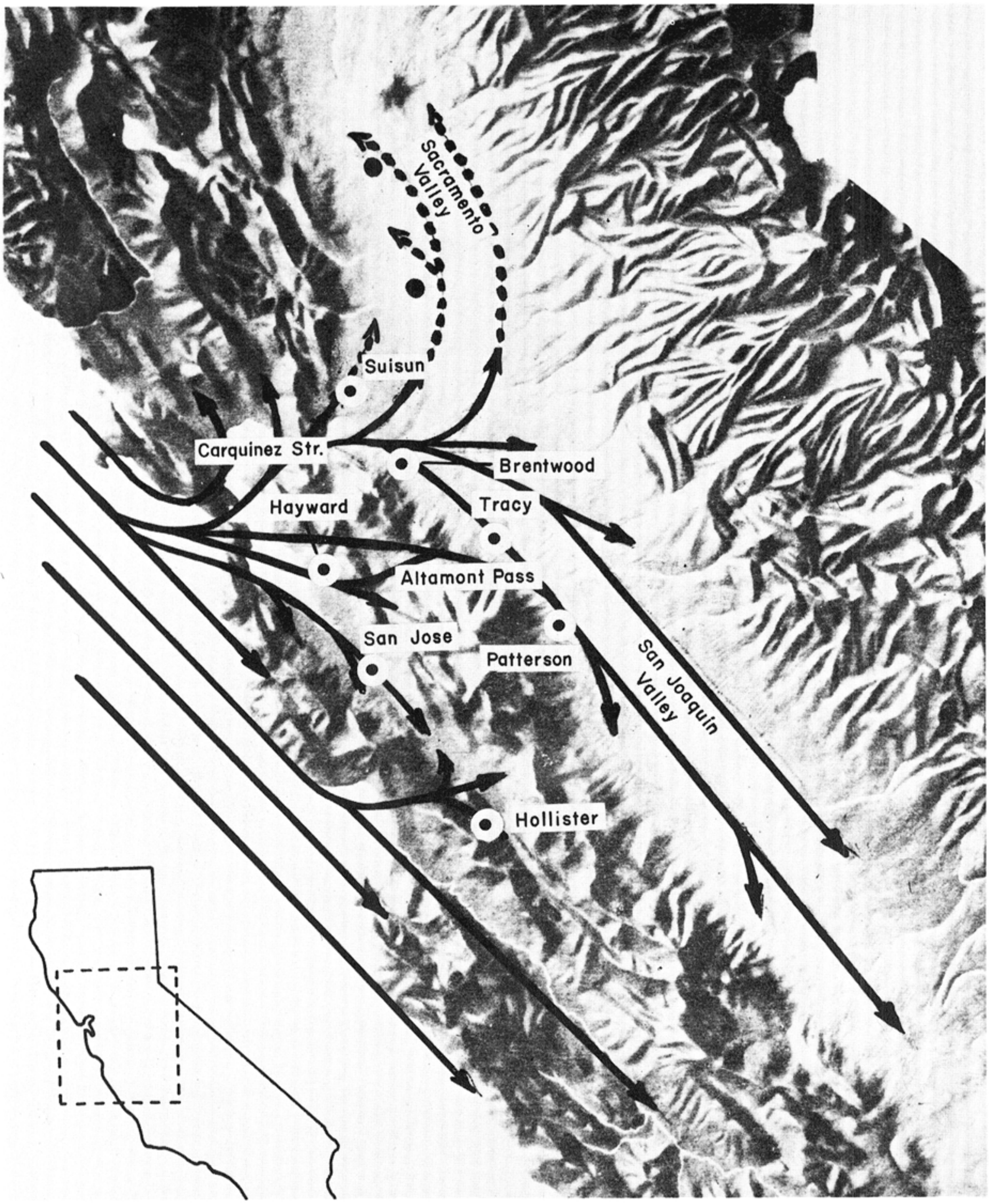

Fig. 7. Topographical view of central California showing prevailing air movement from the sea into the San Joaquin Valley through the Carquinez Straight and Altamont Pass (from Byers 1930) (1).

high concentrations of airborne ascospores in January. The only safe period for pruning in California is during the dry summer months when airborne inoculum is essentially nonexistent. This is particularly important in the training of young apricot trees where pruning wounds are made on limbs which are to become the major scaffold branches of the tree. Infections at this stage can result in serious structural damage later.

Ten ascospores of *E. armeniacae* are as effective as 100 ascospores in causing infection of fresh apricot pruning wounds. This is in marked contrast to reports from Australia where less infection was obtained with 10 ascospores (5). Reduced effectiveness of the inoculum in Australia may be due to differences in virulence of the pathogen (14) and/or a difference in host resistance.

The ability of a single ascospore to produce infection appears to vary with the physiological activity of the host. In dormant trees, one ascospore was as effective as 10 or 100 ascospores per wound in causing infection. When trees near bud break were similarly inoculated, however, a much lower number of the wounds became infected when a single ascospore was used as inoculum. Airborne ascospores almost always appear on spore trap slides in groups of eight, and it is presumed that many of them also arrive at the pruning site as octads. The ability of one spore to cause infection supports the possibility and our contention that infection of trees in the Tracy-Patterson district could arise from airborne inoculum from distant areas of higher rainfall.

Our investigations provide further evidence to support the hypothesis of long-distance transport of airborne inoculum from the San Francisco Bay area into the dry San Joaquin Valley apricot districts. The path of west winds has been well documented (1) and they provide an ideal vehicle for movement of ascospores from the San Francisco Bay area through the Carquinez Straight and Altamont Pass or from Suisun to the west side of the San Joaquin Valley (Fig. 7). The nozzle effect created by the wind entering the valley through these two narrow passes could produce a substantial increase in ascospore concentration. Zogg, as described by Gregory (8) has shown this effect with *Puccinia sorghi* uredospores in Switzerland. The fact that airborne ascospores are detectable in Tracy following rainfall insufficient to produce ascospore discharge strongly supports this hypothesis. Ejected ascospores of *E. armeniacae* have been shown to remain viable for several weeks in laboratory tests (2). Furthermore, ascospore dispersal occurs during overcast weather when solar radiation is minimal, thus enhancing their possible survival. The dilution of airborne ascospore concentration with increasing distance from the source does not detract from this hypothesis since *E. armeniacae* propagules are disseminated in octads and only one ascospore is necessary for wound infection.

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