Factors Affecting Apple Leaf Scar Infection by Nectria galligena Conidia

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ABSTRACT

Five percent of the leaf scars of Red Delicious, Golden Delicious, and Rome Beauty apples were still susceptible to conidial infection 10 days after leaf abscission. Red Delicious was the most susceptible 1 day after leaf fall. A minimum of 6 h of free water was needed to infect Gravenstein with conidia, and increased infection occurred with longer moisture periods. Five conidia were insufficient to infect a leaf scar of Red Delicious but 50 to 5,000 conidia did so. Infection percentage increased directly with spore dose.

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Several climatological and biological factors which affect infection and canker production by *Nectria galligena* Bres. were studied as a background to understanding the epidemiology of European apple canker in California. Reports on duration of susceptibility of leaf scars to infection range from 48 h (2) to 28 days (6). Wilson (6) noted a need for a lengthy free-moisture period for infection of unnatural wounds made by cutting across healed leaf scars before inoculation. Crowdy (2) makes no mention of moisture conditions in his inoculation procedure. Extremely high concn of conidia were found in rain drip water in a heavily infected orchard during the month of November 1970, the most important month for infection (3).

Studies reported herein were initiated to determine how long leaf scars are susceptible to conidial infection under natural conditions, the length of the free-moisture period needed for infection, and the inoculum concn needed to infect leaf scars.

MATERIALS AND METHODS.—*Preparation of inoculum.*—Inoculum of *N. galligena* used for all studies was obtained from a single ascospore culture (SA3). The isolate was obtained from a canker of Red Delicious apple near Sebastopol, California. The inoculum was prepared by streaking a dense suspension of conidia onto Matsushima's (4) medium modified by adjustment to pH 5.2 with 1.0 M KOH and omission of vitamins. On this medium *N. galligena* produced abundant, normal, multiseptate conidia. The cultures were grown 8-10 days at 20 C under Sylvania Gro-Lux fluorescent lights with intensity ca. 1076 lux. Spores were suspended in sterile distilled water as needed for all studies.

Duration of leaf scar susceptibility.—Leaf scars were inoculated 1, 10, 20, and 30 days after removal of abscising leaves from mature Red Delicious, Golden Delicious, and Rome Beauty apple trees in Davis, California, on 6 December 1970. The twigs were moistened, 2×10^3 conidia were placed at each leaf scar, and each twig was covered for 1 wk with a small wetted polyethylene bag. Twenty inoculations on four trees per cultivar were made. Canker incidence was observed at weekly intervals from mid-January 1971, to mid-May 1971. Controls consisted of five twigs per cultivar moistened for 1 wk without inoculation.

Number of hours of moisture needed for infection.—Two-year-old potted Gravenstein apple trees were inoculated with 2×10^3 conidia/leaf scar in November 1971. Leaves approaching abscission were removed by hand, and twenty inoculations were made per tree and two trees used per moisture period. Immediately after inoculation, the trees were moistened by misting with water, covered with wet cheesecloth, and enclosed in a large, moist, polyethylene bag for 6, 12, 24, 48, and 96 h. Zero time received misting without cheesecloth or

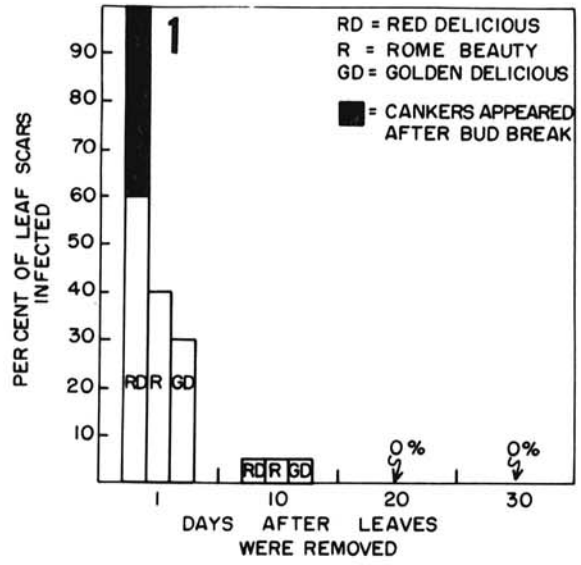

Fig. 1. Duration of leaf scar susceptibility of Red Delicious, Rome Beauty, and Golden Delicious apple trees to *Nectria galligena* conidia. Inoculations were done 1, 10, 20, and 30 days after leaf fall.

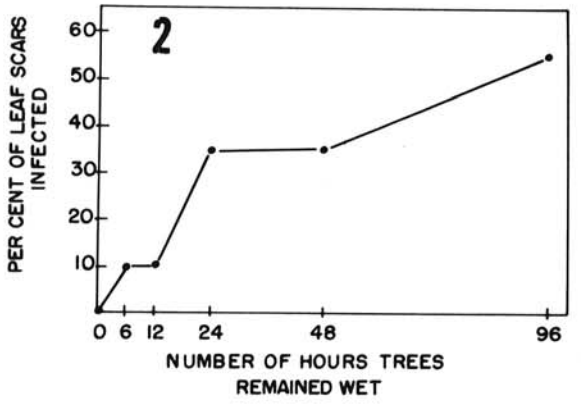

Fig. 2. Effect of different periods of free moisture on *Nectria galligena* infection of Gravenstein apple.

polyethylene cover. Two uninoculated trees were similarly held for 96 h to serve as controls. The trees were kept at 13 C for 1 mo, chilled at 5 C for 1 mo in darkness, then held at 15.5 C for 1 mo with 12 h/day of ca. 5,400 lux light intensity. After this treatment, the trees were moved outdoors in February 1972 and canker production observed until after bud break in April.

Number of conidia necessary to infect leaf scars of Red Delicious apple.—Conidial concn was adjusted to the appropriate level with a hemacytometer, and 1-5 μliter of aqueous spore suspension was applied by a syringe to moistened leaf scars immediately after hand removal of abscising leaves. Counts during inoculations were made to assure proper spore concn. The inoculated trees were covered with large, moistened, polyethylene bags for 9 days. Fifty inoculations were made on two 4-yr-old potted Red Delicious trees per spore concn in August 1970. The trees had previously been subjected to 4 C for 2 mo, with two applications of 100 μg/ml Ethrel (Amchem Co., Ambler, Pa.) to cause leaf abscission. After inoculation, the trees were kept at 13 C for 1 mo, at which time the number of cankers was ascertained. The trees were kept for two additional mo so that any late-appearing cankers could be recorded.

RESULTS AND DISCUSSION.—Only 5% of the leaf scars of the three apple cultivars were susceptible to infection after 10 days and none was susceptible thereafter (Fig. 1). Initial inoculations were made 1 day after abscission rather than at zero time because it started to rain and satisfactory inoculations could not be made. Nevertheless, it is interesting to note that 1 day after abscission Rome Beauty and Golden Delicious seem to be much less susceptible to leaf scar infection than Red Delicious. Crowdy (2) noted that field resistance of Bramley's Seedling was due to its ability to form an adequate morphological barrier. The mechanism in Rome Beauty and Golden Delicious may be similar. First symptoms showed on Red Delicious 3 mo after inoculation and on Rome Beauty and Golden Delicious about 1 wk later. Forty percent of the cankers of Red Delicious were manifested after bud break, indicating that these were "confined lesions" as described by Crowdy (2).

Why Crowdy (2) found that leaf scars became highly resistant within 48 h, whereas Wilson (6) showed that scars were highly susceptible for 28 days, is not clear. Wilson worked with a cultivar different from the one used by Crowdy, and from those used in the present study; however, Wilson (6) kept his trees wet for 72 h before placing them at optimum temp for growth of the fungus. Since the present results could have been influenced by ambient temp at inoculation time, the study was repeated (in 1971) on 4-yr-old potted Red Delicious trees inoculated 1, 10, and 20 days after removal of senescing leaves by hand. Two trees per treatment and 10 inoculations per tree were used. Trees were given the same temp and light regime as noted for the study on hours of moisture needed for infection. Results were almost identical to the previous year's field inoculations on Red Delicious.

At least 6 h of moisture were needed to obtain a low percent of infection and the percent infection increased as the free-moisture period was increased (Fig. 2). After 24 h, 36% of the leaf scars became infected. Crowdy (2) noted that conidia can be sucked into terminal vessel elements of the leaf scar for a period of ca. 48 h. If this is so, it is doubtful that long free-moisture periods would be needed for infection. Yet, field infection occurs only where rainfall is abundant for long periods of time (2, 3, 6). Under rainy conditions in late fall, at least in California, it also seems doubtful that apple trees would be under sufficient negative tension to suck conidia into the vessel elements as senescing leaves fall. Field data on infection obtained in California indicated a definite need of several days free moisture to obtain high levels of infection (3). These results are supported by the present data.

Five conidia placed at a leaf scar were insufficient to cause infection (Fig. 3). Log increases in spore dosages above this number resulted in nearly linear infection

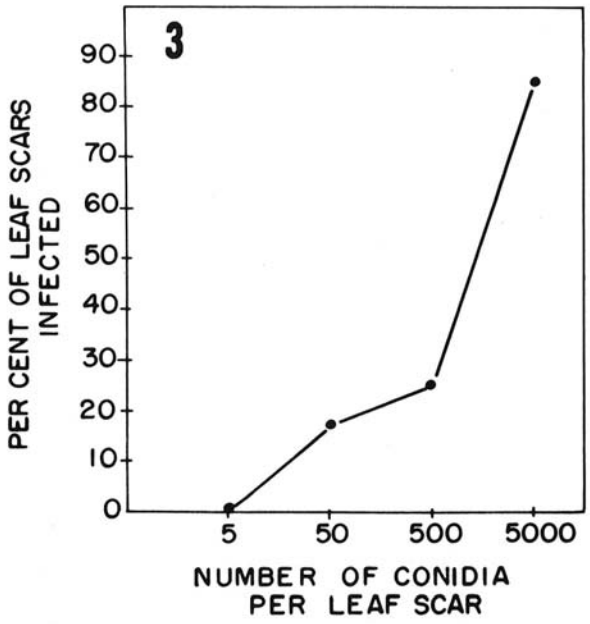

Fig. 3. Percent of Red Delicious apple leaf scars infected with different concn of conidia of *Nectria galligena*.

percentages. Similar inoculation studies on 2-yr-old Red Delicious trees treated the same as the 4-yr-old potted trees in the previous study on concn of conidia needed for infection gave similar results. However, inoculation of Rome Beauty and Golden Delicious gave erratic results, with cankers appearing only at a concn of 5×10^3 conidia per scar. These two cultivars seemed to need a longer chilling period than Red Delicious and did not fully break dormancy under the experimental conditions, thus some positive infections may have remained as "confined lesions" and were not observed. Why so many conidia per leaf scar are necessary to obtain high infection levels is not clear. Extremely high inoculum levels can be observed in the field during the infection period (3). Since penetration is based on a series of successful events it is possible that high spore concn are needed simply to get one or a few germ tubes into the minute vessel element openings. Due to their large size, macroconidia may have trouble entering the vessel segment. Zeller (7) observed consistently higher infection percentages with ascospores than with conidia. This could be due to the comparatively smaller size of the ascospores. Studies related to the inoculum concn of fungal spores capable of causing infection of natural or artifical wounds of trees are sparse. D. E. Ramos and W. J. Moller (*unpublished*) have shown that a single ascospore of *Eutypa armeniacae* Hansf. & Carter is capable of infecting pruning wounds of apricot trees, but the infection percent was significantly less than with 10 spores. The infection locus, in this case, of many open vessel elements is extremely large in comparison to that of leaf scars. Also, ascospores of *E. armeniacae* are considerably smaller than conidia of *N. galligena*. Swinburne (5) has shown that infection of apple fruit with 10 to 100 conidia of *N. galligena* often produced only arrested lesions, whereas higher numbers of spores produced progressive rots. It may be that a "mass action" effect is operating and many spores are needed to overcome some possible resistance mechanism, but no evidence is present to support this. Recent work by Carter and Moller (1) with *E. armeniacae* has shown that the proportion of pruning wounds of *Prunus* species that become infected is approximately doubled when the ascospore dosage per wound is increased from 10 to 100.

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