Attractiveness of Diseased Red Clover Roots to the Clover Root Borer

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ABSTRACT

The ability of clover root borers to discern between diseased and healthy red clover roots was demonstrated in laboratory tests. Adult borers preferred diseased roots more than healthy roots in tests with diseased root pieces on moist filter paper, buried in nonsterile soil, or with aqueous leachates from such pieces. Fungi and bacteria isolated from attractive, diseased roots and cultured on potato-dextrose agar were not attractive to the borers. Root pieces infected by *Colletotrichum trifolii, Fusarium roseum, F. tricinctum*, or *Rhizoctonia solani* were more attractive to borers than were healthy root pieces. These results support the field observation that borers are associated with diseased or dying roots and agree with the preference for dead tissue that is characteristic of bark beetles.

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The clover root borer is a bark beetle in the family, Scolytidae. It differs from other bark beetles in that it spends nearly its entire life in the roots of its host. Rockwood (9) cited evidence that borers were the direct cause of clover stand losses, as well as contrasting evidence that borers attacked only dying plants. Many other associations of borer infestation and disease have been reported but usually the health of the plants at the onset of borer infestation was not known. Severe root rot has also been reported in the absence of root borers (3), but Koehler et al. (4) controlled borers with insecticides and reduced root rot of clover. In another test chemical control of borers did not consistently increase persistence, although total root sugars were less in borer-infested plants (4). They suggested that factors other than the borer may account for the poor persistance of red clover beyond the first harvest year.

Graham & Newton (2) found that root borer injury increased the amount of root rot over that

which occurred in the uninjured control plants in a greenhouse test, but with *Fusarium* added to steamed soil, the borer was no more effective than was mechanical injury.

Adult root borers mechanically transmitted viable spores of *Kabatiella caulivora* and *Colletotrichum trifolii* (8), and Henderson & Newsom [*unpublished data* cited in (6)] cultured fungi, including Fusaria, from the digestive tracts of clover root borers. Newton & Graham (7) determined the chronological development of clover root rot and root borer populations over a 17-month period in the field. They found that root rot development preceded high populations of root borers.

Our repeated observations of higher borer numbers in diseased and dead roots prompted this investigation to determine if adult borers preferred diseased roots and whether they could discern between diseased and healthy roots. A portion of this work has been reported (5).

MATERIALS AND METHODS.—*General.*—Clover roots (*Trifolium pratense* L.) were obtained from the field and the greenhouse. Diseased roots were those with external and internal necrosis; healthy roots were without obvious lesions or discolorations. All borers (*Hylastinus obscurus* Marsh.) were collected from roots of field-grown, 'Pennscott' red clover plants, using a Berlese-funnel technique. Some borers were used immediately in tests; others were held on modified wheat germ medium (1) for varying periods prior to use in preference tests. A minimum of five borers were used per petri dish replicate; all tests included at least three replicates and were conducted at 20 C in the dark. Materials and methods specific to individual tests are given with the results of each test.

RESULTS.—*Leachate preference test.*—A 9-cm-diam, plastic petri dish was fitted with four, triangular, filter-paper wicks (Fig. 1) that radiated out from near the center of the dish to the periphery. The wicks did not touch each other and the narrow tip of each wick protruded through a slot in the dish bottom. Test root pieces were placed on the outer edge of each wick and the borers were caged with access to the wicks just prior to where they protruded near the center of the dish. Glass-distilled water was applied to the root pieces. The portion of the wicks protruding through the dish bottom provided an evaporative surface thus ensuring water movement from the test piece toward the central portion of the wicks where the borers were caged. Seitz filter plugs soaked with water served as controls. The plugs approximated the diameter and volume of a root cross section and kept the wick wet as did the root pieces. The root pieces used were cross sections made near the crown and were approximately 5-mm thick. Test dishes containing root disks were equilibrated at 5 C for 4-16 hr prior to introducing borers.

The same technique as previously described was used to demonstrate the activity of clover root leachates in the soil. Approximately 0.5 ml of soil was placed on the wicks, and the borers caged in the center had access to the various wicks. The leachates tested were from soil in which red clover had grown

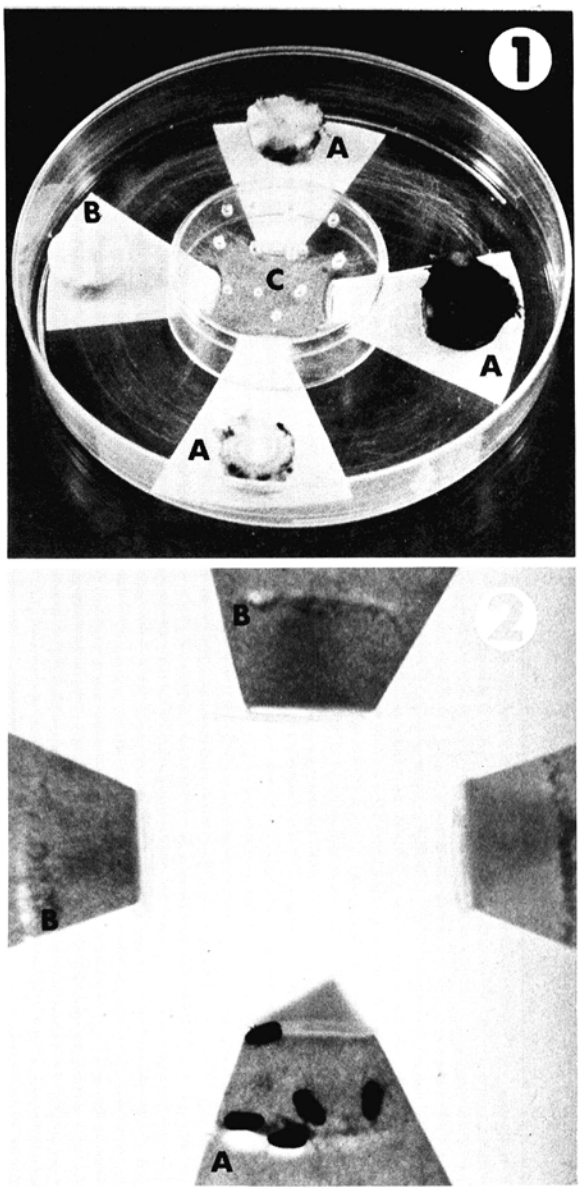

Fig. 1-2. 1) Petri dish test chamber with cover removed and clover root pieces (A) and control filter disk (B) in place on filter paper wicks. Borers (*Hylastinus obscurus*) were restricted to central caged area (C). **2)** Tip portions of wicks with cage removed. Feeding evidence on wick (A) where borers are gathered was stimulated by leachate from diseased root piece. Markings (B) on wicks are from borers walking around inside cage rim.

in the field, soil in which alfalfa had grown in the greenhouse, and potting soil with an unknown crop history. The control was distilled water. All soil was sifted to remove plant debris, and moistened at the start of the test.

Evaluation of borer response was made by counting the number of borers on a given wick. Evidence of feeding in the wicks was another

TABLE 1. Number of borers (*Hylastinus obscurus*) selecting leachates from diseased and healthy roots of red clover

Treatment	Borers on wick
Distilled water	0[a]
Healthy-root leachate	18
Diseased-root leachate[b]	129

[a]Data are the total from nine tests.

[b]Naturally diseased roots from field or greenhouse.

indication of borer response.

Leachates from the diseased root pieces were much more attractive to the borers than were those from healthy root pieces or the water control (Table 1). Borer feeding in the wick (Fig. 2) was stimulated more frequently and more extensively by the diseased-root leachate than by the healthy-root leachate. No feeding occurred on the water control wick.

Borers preferred any soil leachate over the distilled water, and twice as many borers selected the leachate from soil in which red clover had grown over either of the other soil leachates (Table 2).

A test was also done to determine if fungi and bacteria isolated from a root with demonstrated attractiveness to borers, would still be attractive when grown on PDA. Six fungi and two bacteria were isolated and cultured. Plugs from these cultures were transferred to wicks in test chambers with noninoculated PDA plugs as controls. No preference was ever shown by the borers for any organism.

Root-piece preference test.—Cross sections from diseased and healthy roots, 7-mm thick, were placed in a petri dish on moist filter paper so that a central piece of healthy tissue was surrounded by pieces of diseased roots placed in a ring about 2-3 cm from the healthy root. A reciprocal test was also done with the diseased piece in the center surrounded by healthy root pieces. Borers were placed on the central piece. After 24 hr, pieces were dissected and the number of borers that tunneled into each was determined.

Borers consistently left healthy root pieces to relocate in diseased root pieces. In two tests, no borers remained on the central, healthy pieces, 31 relocated to peripheral diseased pieces, and five were not associated with any root piece. In the reciprocal tests, 35 borers remained on the central, diseased pieces, and one borer relocated in a peripheral healthy piece.

Root-piece preference test in soil.—Two pieces of healthy and two of diseased roots were alternately placed near the edges of a petri dish and completely covered with unsterilized Hagerstown silt loam. A 3-cm diam area in the center of the dish was kept free of soil. Borers were introduced into the clear area in the center and allowed to roam freely within the covered dish. After 24 hr the pieces were removed from the soil and the borers located.

A clear preference was demonstrated by the borers for diseased root pieces buried in soil. The borers walked around on top of the soil surface until they sensed the buried root piece, and then they bored straight down into the piece. In three tests, a total of four borers were found in healthy root pieces, whereas 53 borers entered diseased root pieces. Nine borers remained on or in the soil.

Comparison of specific fungi.—Root pieces were surface-disinfected with 2% sodium hypochlorite, rinsed in sterile distilled water, and placed on 1.5% water agar. Four pieces were inoculated with one of the following pathogens: *Colletotrichum trifolii* Bain & Essary, *Fusarium roseum* Lk. emend. Snyd. & Hans., *F. tricinctum* (Cda.) Snyd. & Hans., and *Rhizoctonia solani* Kuehn. Pieces were incubated for 7 days at 22 C in the light; noninoculated control pieces were kept on water agar at 5 C for this period. One piece from each treatment was placed on moist filter paper in a petri dish. Borers were placed centrally in the dish, equidistant from the pieces, with free access to all pieces. The test was evaluated five times with the same or new borers started in the central area after each evaluation. Preference was determined by counting the number of borers choosing the various pieces. If a root piece was unusually attractive, it was removed from the dish. Therefore, not all pieces were included in all five tests. The total number of borers associated with a treatment was divided by the number of tests in which it was included.

Not only were more borers associated with diseased roots, regardless of the pathogens involved, but the incidence of entry into the root pieces was also higher for the diseased than for the healthy roots (Table 3). The attractiveness of leachates from root pieces infected with *C. trifolii* was compared against healthy root leachate in another test. When given only this choice, the borers showed an almost complete preference for the leachate from the diseased root pieces.

DISCUSSION.—The preference by borers for diseased roots that was suggested by earlier field observations is supported by results of our laboratory tests. The borers were able to select diseased over healthy roots on moist filter paper and in nonsterile soil. Borers apparently sensed healthy roots and fed upon them when not given access to diseased roots but preferred diseased roots when given a choice. The specific fungus involved did not seem to be critical;

TABLE 2. Number of borers (*Hylastinus obscurus*) selecting leachates from various soils

Treatment	Borers on wick
Distilled water	1[a]
Soil from alfalfa	10
Soil from red clover	25
Soil (unknown crop history)	11

[a]Data are the total from two tests.

TABLE 3. Comparison of root borer (*Hylastinus obscurus*) preference for clover root pieces inoculated with different fungi

Fungi	Mean no. borers per piece per test
None-control	1.0[a]
Colletotrichum trifolii	1.5
Fusarium roseum	2.3
F. tricinctum	2.3
Rhizoctonia solani	2.2

[a]Derived from eight or more replications.

varying amounts of decay could have been responsible for the greater attractiveness of one piece over another.

When our results are considered in addition to the report by Newton & Graham (7) that root borer population build up in the field followed root rot development, there is reason to question just how much the borers contribute to the root rot complex. Surely, when borers enter a relatively young stand they create additional infection courts for the root rot pathogens, but roots that have been subjected to winter conditions of freezing and thawing have many surface cracks through which fungi can enter. Borer movement within a root should increase the rate of development of disease within that root, but borers are probably not responsible for the initial inoculation of clover roots with pathogenic fungi.

The physiological stress of borer feeding on the plant may contribute as much or more to root rot development than does the vector-predisposition aspect. Borer feeding lowers carbohydrates (4) as does frequent clipping (11) which has been shown to favor root rot development.

The preference for diseased and decayed roots expressed here and the constant association of borers in the field with diseased or dead roots is similar to behavioral characteristics of other bark beetles that are closely related to the clover root borer (10).

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