# Relationships Between Pepper Weevil and Internal Mold of Sweet Pepper

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**ABSTRACT**

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*Alternaria alternata* was recovered from 37–63% of pepper weevil (*Anthonomus eugenii*) punctures on bell pepper (*Capsicum annuum*), depending on age of the fruit. In artificial inoculation studies, the germ tube of *A. alternata* elongated and entered puncture wounds, which ultimately showed internal mold similar to natural infections. Evidence of direct penetration of epidermal tissue was not observed. Our data suggest a direct relationship between pepper weevil damage and internal mold caused by *A. alternata*.

*Alternaria alternata* (Fr.) Keissler (syn. *A. tenuis*) is generally considered a weak pathogen on injured pepper fruit (*Capsicum annuum* L.). Peppers contaminated with conidia at harvest, however, can have appreciable losses during transit and marketing (6). *A. alternata* can cause various diseases of pepper fruit, including fruit rot and internal mold (1,5,7–11). Leyendecker (5,6) first reported an internal mold in chilies from New Mexico in the early 1950s. He noted that lesions could seldom be detected by external examination of the fruit. Fungi developed internally primarily after the first frost, at which time the pod wall at the calyx end pulled away from the seed mass, providing entry for airborne conidia (5). Researchers (4,11) in Israel noted that *A. alternata* entered the developing fruit at the flowering stage through the stigma and style.

Internal mold of apparently healthy bell peppers has been observed in the Lower Rio Grande Valley of Texas during most years. Preliminary evidence suggested an association between internal mold occurrence and damage inflicted by the pepper weevil (*Anthonomus eugenii* Cano) (L. D. Chandler, *unpublished*). Previous studies also recognized an association between weevil feeding and disease incidence in pepper fruit (2,3, 12,13). The disease-causing organisms were not identified, however, and the relationship was not explored.

The objectives of the present study were to determine the relationship of internal mold in bell peppers to the feeding behavior and incidence of the pepper weevil and to demonstrate that infection by *A. alternata* can follow mechanical injury produced by the pepper weevil.

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## MATERIALS AND METHODS

**Field studies.** Grande Rio 66 bell peppers were directly seeded on 19 February 1985 in a 0.4-ha plot at USDA facilities in Weslaco, TX. Plants were grown by means of normal horticultural practices, with the exception that no fungicides or insecticides were applied. The plot was divided into six equal subplots, each 20 rows wide (102 cm) by 38 m long. Beginning on 14 May and

continuing weekly through plant and fruit maturity (20 June), 10 fruit from each of four size categories were randomly collected from each subplot and brought to the laboratory. The following size scale was used: category 1 = ≤ 1.3 cm in diameter (newly developed), category 2 = 1.31–2.5 cm in diameter, category 3 = 2.51–5.0 cm in diameter, and category 4 = ≥5.0 cm in diameter (mature fruit). Each category represented a developmental period of about 5–7 days (B. Villalon, *personal communication*). Locations for fruit selection within each subplot were derived from a random number table. Fewer than 10 fruit per category were available on 20 June, which resulted in reduced numbers of harvested fruit from all size categories. Only small, newly developed fruit were present on 14 May, and no fruit in category 4 was available for sampling on 21 May. The following information was recorded for each fruit: number of weevil feeding and oviposition punctures on exterior fruit surfaces, location of weevil feeding damage within the fruit (outer walls and/or seed layer), and incidence of internal mold (*A. alternata*) by site location within the fruit (blossom end, stem end, outer wall, and/or seed layer).

A Kramer-Collins spore trap (GR Electric Mfg. Co., Manhattan, KS) was set about 50 m into the plot on 7 May. The orifice of the wind vane was about 0.3 m above the bed, and airflow rate was 3 L/min for 1 min four times each hour until 24 June. Temperature, relative humidity, rainfall, wind speed, and leaf wetness hours were recorded hourly. Temperature and relative humidity were recorded by hygrothermograph, rainfall was recorded with USDA standard gauges, and wind speed was recorded by an anemometer. Leaf wetness was measured with an Ag-Tech A1-101B-7 Dewdynamics System (Ag-Tech Instrument Co., Savannah, GA).

**Laboratory studies.** Isolations from pepper weevil puncture sites were randomly made from fruit collected weekly in each size category. Puncture sites were excised, surface-sterilized with 0.5% sodium hypochlorite for 1 min, plated on potato-dextrose agar (Difco), and incubated for 5–7 days. Isolations were not made from punctures that showed mycelial growth. A total of 385 isolations were made. The incidence and types of organisms were recorded for all isolates by fruit size and date.

Mature bell peppers obtained from a retail grocery store were used for testing the ability of *A. alternata* to infect simulated weevil puncture holes. A sterilized needle was used to make punctures 0.2–0.4 mm in diameter and 1.0–1.5 mm deep in the exterior surface of each fruit. *A. alternata* cultures 15–20 days old were used for inoculation purposes. Conidial preparations were

**Table 1.** Percentage of sweet pepper fruit showing pepper weevil damage on seed layer and outer wall and mean number of feeding/oviposition punctures and adult exit holes by fruit size and date[x]

| Fruit size category | Date | | | | |
|---|---|---|---|---|---|
| | 21 May | 28 May | 4 June | 11 June | 20 June |
| | | | **Seed layer** | | |
| 1 | 0.0 b[y] | 8.3 b | 25.0 b | 11.7 c | ...[z] |
| 2 | 6.7 a | 43.3 a | 70.0 a | 81.7 a | 100.0 a |
| 3 | 3.3 ab | 40.0 a | 60.0 a | 75.0 a | 100.0 a |
| 4 | ... | 23.3 b | 36.7 b | 36.7 b | 62.5 b |
| | | | **Outer wall** | | |
| 1 | 0.0 a | 0.0 b | 3.3 b | 3.3 b | ... |
| 2 | 1.7 a | 8.3 ab | 21.7 a | 30.0 a | 100.0 a |
| 3 | 5.0 a | 16.7 a | 33.3 a | 41.7 a | 71.4 a |
| 4 | ... | 15.0 a | 23.3 a | 40.0 a | 75.0 a |
| | | | **Punctures** | | |
| 1 | 0.0 c | 0.5 c | 1.0 b | 1.1 c | ... |
| 2 | 0.5 a | 2.0 a | 3.6 a | 6.4 a | 14.0 a |
| 3 | 0.3 b | 1.5 a | 3.0 a | 5.3 a | 10.9 a |
| 4 | ... | 1.0 b | 1.6 b | 2.5 b | 2.3 b |
| | | | **Emergence holes** | | |
| 1 | 0.0 | 0.0 a | 0.0 b | 0.0 b | ... |
| 2 | 0.0 | 0.0 a | <0.1 b | 0.2 b | 0.8 a |
| 3 | 0.0 | 0.0 a | 0.1 a | 0.5 a | 0.4 a |
| 4 | ... | 0.5 a | <0.1 b | 0.1 b | 0.1 a |

[x]Ten fruit sampled per category per date.
[y]Means in a column followed by the same letter are not significantly different ($P>0.05$, LSD test).
[z]No fruit of that size available.

**Table 2.** Percentage of sweet pepper fruit showing internal mold (*Alternaria alternata*) growth by site and size and by date[x]

| | Percentage of infected fruit | | | | |
|---|---|---|---|---|---|
| | 21 May | 28 May | 4 June | 11 June | 20 June |
| **Site** | | | | | |
| Blossom end | 4.4 a[y] | 0.8 ab | 0.0 a | 1.3 bc | 0.0 b |
| Stem end | 0.0 b | 0.4 ab | 0.0 b | 0.0 c | 0.0 b |
| Outer wall | 0.0 b | 0.0 b | 0.4 b | 3.3 b | 20.0 a |
| Seed layer | ...[z] | 1.7 a | 3.3 b | 8.8 a | 30.0 a |
| **Fruit size** | | | | | |
| Category 1 | 0.0 b | 1.7 a | 0.0 b | 3.3 b | ... |
| Category 2 | 1.7 b | 6.7 a | 6.7 ab | 11.7 a | 80.0 a |
| Category 3 | 11.7 a | 1.7 a | 8.3 a | 16.7 a | 28.6 b |
| Category 4 | ... | 0.0 a | 0.0 b | 6.7 ab | 12.5 b |

[x]Ten fruit sampled per category per date.
[y]Means in a column followed by the same letter are not significantly different ($P>0.05$, LSD test).
[z]No fruit of that site or size available.

**Table 3.** Incidence of pathogens isolated from pepper weevil puncture sites by sweet pepper fruit size and by date[x]

| | Pathogens (%) | | | | | |
|---|---|---|---|---|---|---|
| | *Alternaria alternata* | Bacterial spp. | *Cladosporium* sp. | *Fusarium* spp. | None | Other |
| **Fruit size** | | | | | | |
| Category 1 | 63.2 a[y] | 10.5 b | 5.3 a | 21.1 a | 0.0 b | 5.3 a |
| Category 2 | 57.7 a | 21.6 b | 8.3 a | 6.2 b | 7.2 a | 3.1 a |
| Category 3 | 53.2 a | 18.6 b | 16.3 a | 7.1 b | 7.7 a | 5.8 a |
| Category 4 | 37.2 b | 35.4 a | 9.7 a | 9.7 ab | 5.3 a | 3.5 a |
| **Date** | | | | | | |
| 21 May | 44.4 a[z] | 5.6 b | 22.2 ab | 11.1 b | 16.7 b | 5.6 b |
| 28 May | 28.9 ab | 40.4 a | 3.8 cd | 15.4 bc | 15.4 bc | 0.0 d |
| 4 June | 48.3 a | 24.2 b | 6.7 cd | 12.5 c | 8.3 cd | 2.5 d |
| 11 June | 58.3 a | 20.4 b | 9.3 c | 3.7 c | 1.9 c | 8.3 c |
| 20 June | 56.3 a | 21.8 b | 13.8 bc | 3.4 d | 2.3 d | 4.6 c |

[x]Ten fruit sampled per category per date.
[y]Means in a column followed by the same letter are not significantly different ($P>0.05$, LSD test).
[z]Means in a row followed by the same letter are not significantly different ($P>0.05$, LSD test).

made by removing aerial mycelium from the agar surface with a rubber spatula. Cultures were incubated an additional 48 hr to permit formation of conidia. Before puncture and inoculation, petri dishes were opened and placed in a laminar flow hood and allowed to dry for 1 hr. Cultures were inverted over the punctured area of the fruit and vibrated vigorously, allowing the deposition of conidia without mycelial trash. Inoculated peppers were subsequently placed in a Percival model I-35 dew chamber (Percival Mfg. Co., Boone, IA) at 21 C and 100% RH. Fruit were removed after 24, 48, 72, and 96 hr and prepared for examination with a scanning electron microscope (SEM). Samples were prepared by immersing 0.7-$cm^2$ sections of fruit in a fixative solution of 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH adjusted to 7.2) and postfixed in 2% osmium tetroxide. Subsequently, specimens were washed with distilled $H_2O$ and dehydrated in a graded ethanol series. Samples were critically point-dried, mounted on metal stubs with silver adhesive paint, sputter-coated with gold, and examined with a Hitachi model H-300 scanning electron microscope.

**Statistical analyses.** Means and standard errors were calculated for all field-collected data as well as data on puncture isolations to determine disease incidence. Analysis of variance procedures were conducted and least significant difference (LSD) tests used to separate means ($P = 0.05$). Percentage data were transformed to arc sine. Regression and correlation procedures were conducted to evaluate relationships between environmental factors and field disease incidence.

## RESULTS AND DISCUSSION

**Field studies.** Pepper weevil damage was first noted in sampled fruit on 21 May and increased throughout the remainder of the study period (Table 1). Most weevil-induced damage occurred on the seed layer of the fruit (Fig. 1A), although entire fruits were heavily damaged on 20 June. In most instances, significantly more ($P \leq 0.05$) fruit in categories 2, 3, and 4 were damaged in both the outer wall and the seed layer than fruit in category 1. The number of weevil-feeding/oviposition punctures and adult emergence holes per fruit also increased as the season progressed (Table 1).

Fewer ($P \leq 0.05$) fruit in category 4 were punctured than fruit in categories 2 and 3. Mature (category 4) fruit may not be the preferential site for *A. eugenii* feeding or egg deposition. Elmore et al (3) suggested that mature fruit are resistant to the pepper weevil. Our results indicate that mature (category 4) fruit are less susceptible but are not immune to pepper weevil damage.

Visual observations of internal mold (*A. alternata*) were made from 21 May until the end of the study in all size categories of fruit. On 21 May, mold growth was noted only on the blossom end of the fruit and only on categories 2 and 3 fruit (Table 2). Infection has been documented to occur during the flower stage as the fungus enters through a tiny opening on the blossom end of the fruit (4,11). In the present study, infection by *A. alternata* was not associated with weevil damage at this early stage. As the season progressed, however, incidence of internal mold (Table 2) and weevil damage (Table 1) increased at other locations within the fruit (Fig. 1B). After 21 May, each pepper infected with *A. alternata* was also infested with *A. eugenii*. Fungal growth occurred on the part of the fruit damaged by the weevil, which in most instances was the outer wall and seed layer. Occasionally, fungal growth was also noted on the blossom end; in these instances, fungal infection may have originated without the aid of weevil damage. In cases where infection occurred without corresponding blossom-end infection, weevil damage provided the avenue for fungal invasion. Categories 1 and 4 fruit generally had the least amount of disease on each date (Table 2).

Conidia of *A. alternata* were trapped throughout the sampling period (Fig. 2). Peak densities occurred 9, 15, and 45 days after initiation of the study. These periods corresponded to periods of high relative humidity (>90%) and increased leaf wetness hours. On days when more than 90 conidia/$m^3$ were collected, the increase in conidial numbers could be related to the number of hours of relative humidity ≥92% during the previous 24 hr ($r^2 = 0.82$, $f = 43.93$, $P = 0.0001$). Leaf wetness, rainfall, and wind, however, were not as useful in explaining periods of increased conidial densities. These data demonstrated that conidia of *A. alternata* are ubiquitous during pepper fruiting, thus increasing the likelihood of infection in fruit damaged by *A. eugenii*.

**Laboratory studies.** *A. alternata* was recovered from 37–63% of the weevil punctures, depending on fruit size (Table 3). Because isolations were made from weevil punctures on fruit not showing evidence of infection and fungal colonization (aerial mycelium), the percentage of

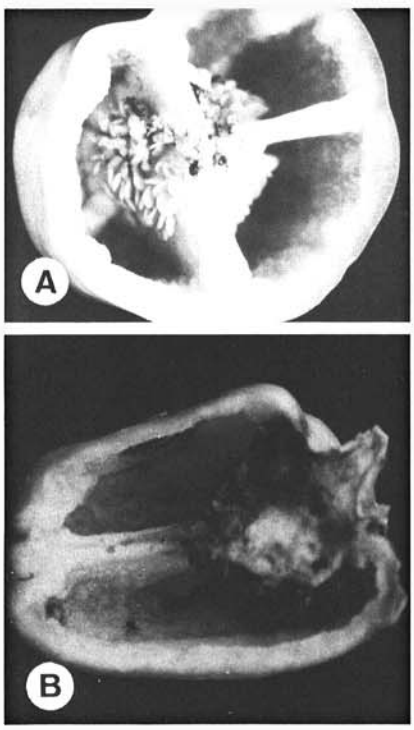


**Fig. 1.** Weevil-induced damage to bell pepper: **(A)** Damage to seed layer and presence of adult weevils and **(B)** colonization of seed layer and outer wall by *Alternaria alternata*.

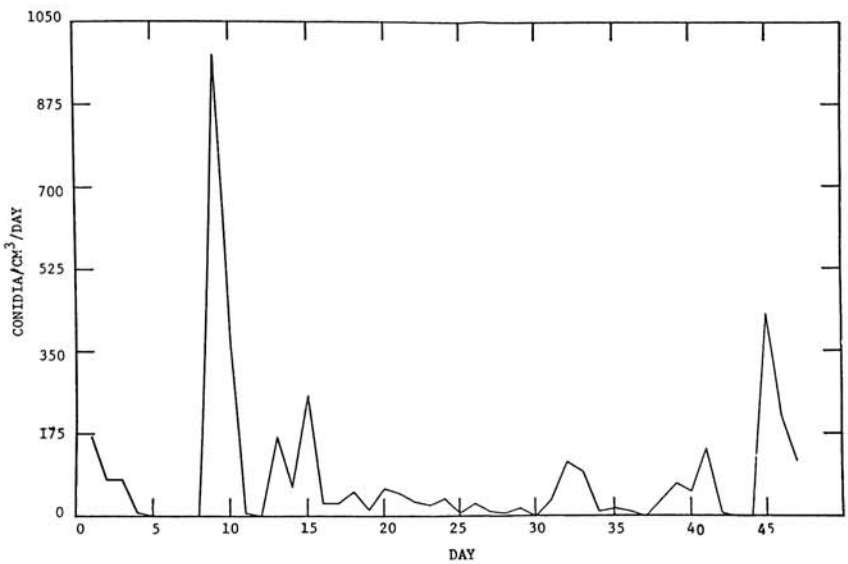


**Fig. 2.** Density of conidia collected per cubic centimeter per day using a Kramer-Collins 7-day spore trap at Weslaco, TX, 1985. Air was sampled for 1 min four times each hour with an airflow rate of 3 L/min.

weevil punctures infected by *A. alternata* is underestimated. Bacterial spp., *Cladosporium* sp., and *Fusarium* spp. were isolated but were not involved in visible internal mold growth. The total incidence of *A. alternata* in puncture sites generally increased over time.

Pepper fruit artificially inoculated with conidia of *A. alternata* showed internal mold similar to that of natural infections. Necrosis was observed in surrounding cells 7 days after inoculation, indicating the fungus was colonizing uninjured tissue. Quebral (10) noted that colonization of pepper fruit by *A. alternata* was both intercellular and intracellular. Subsequent isolations from artificially inoculated fruit yielded *A. alternata*.

SEM demonstrated fungal colonization in and around weevil puncture holes (Fig. 3A). In inoculation studies, the germ tube of *A. alternata* elongated in a haphazard manner and entered puncture wounds by chance (Fig. 3B). With few exceptions, the germ tube entered into the nearby wounded epidermis. Evidence of direct penetration of epidermal tissue was never observed. Fungal colonization was evident in cross sections of puncture holes (Fig. 3C). Numerous pepper weevils were observed with the SEM to determine if the insects were contaminated with conidia of *A. alternata*. On only one occasion did we observe a conidium on the surface of a weevil. There is no evidence the pepper weevil is a vector of *A. alternata* during feeding/oviposition. Infection by *A. alternata* subsequent to weevil damage appears to be a fortuitous event.

The most important aspect of pepper weevil damage is the destruction of blossom buds and immature pods (2,3, 12,13). In many instances, however, fresh market fruits that appear to be sound have internal mold growth. Previous reports have documented fungal invasion of pepper through the blossom end (4,14). In addition, several reports note that injury is a prerequisite to infection by *A. alternata* (2,3,5,7,10). Our data suggest a strong relationship between pepper weevil injury and internal mold growth in bell peppers grown in South Texas.

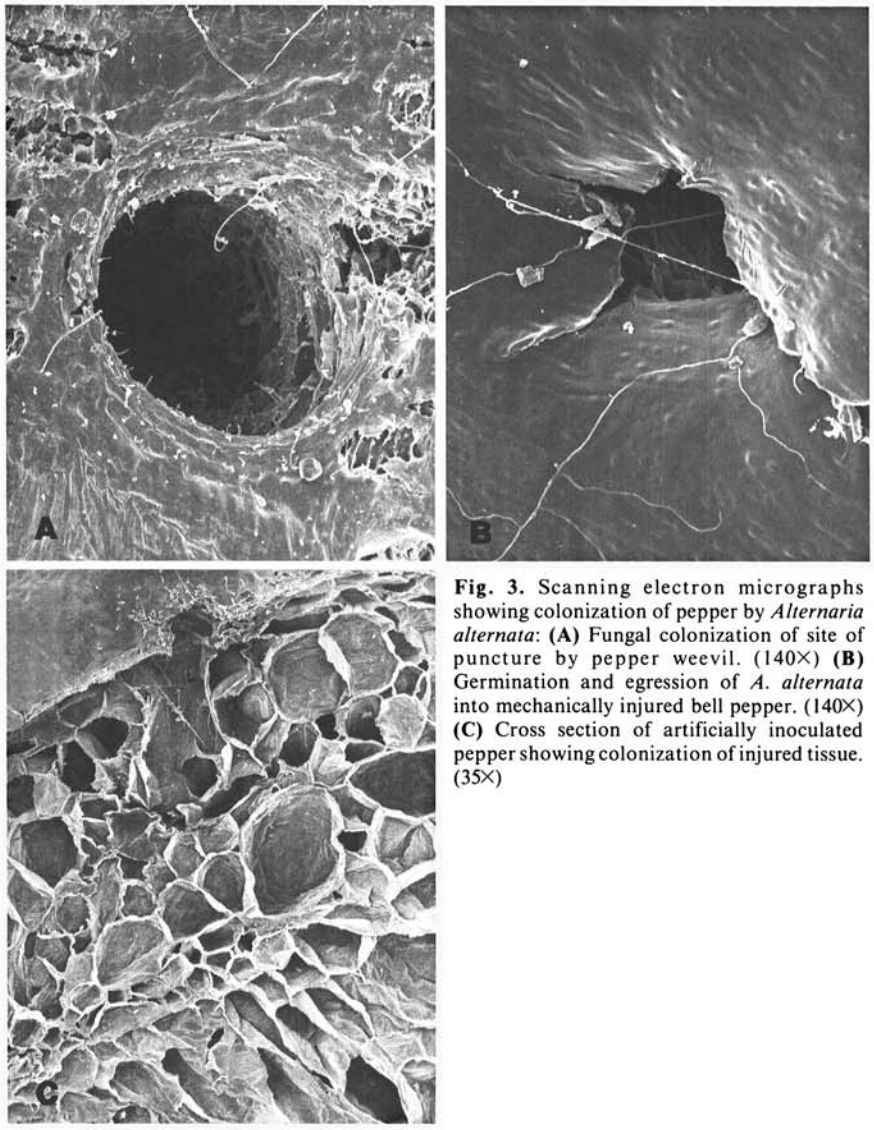


**Fig. 3.** Scanning electron micrographs showing colonization of pepper by *Alternaria alternata*: **(A)** Fungal colonization of site of puncture by pepper weevil. (140×) **(B)** Germination and egression of *A. alternata* into mechanically injured bell pepper. (140×) **(C)** Cross section of artificially inoculated pepper showing colonization of injured tissue. (35×)

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