Effects of Maggots and Wounding on Occurrence of Fusarium Basal Rot of Onions in Colorado

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ABSTRACT

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Inoculum densities of *Fusarium oxysporum* f. sp. *cepae* varied from 22 to 3,600 colony-forming units per gram of dried soil in onion production soils in Colorado. The percentage of *F. oxysporum* isolates pathogenic to onion increased from 25–56% at midseason to 62–90% later in the growing season. Seed-corn maggots (*Delia platura*) were commonly recovered from plants showing Fusarium basal rot symptoms. In preference tests, seed-corn flies oviposited eggs 78% of the time on diseased rather than on healthy bulbs. An ottidid fly (*Euxesta notata*) was most commonly recovered from cull onions, whereas no onion maggots (*D. antiqua*) were found in any field or infested bulbs in Weld County. Mechanical wounding significantly increased the incidence of Fusarium basal rot in Fusarium-infested soil, but seed-corn maggots generally appeared to be secondary invaders of diseased bulbs in onion fields.

Colorado ranks third in onion production in the United States. Management of disease and insect pests of onions has received little attention in the state. Soilborne diseases are a major onion production constraint in Colorado, and one of the most important soilborne diseases is Fusarium basal rot of onion caused by *Fusarium oxysporum* Schlect. emend. Snyd. & Hans. f. sp. *cepae*. Yields of susceptible onion cultivars such as Brown Beauty can be reduced more than 50% by basal rot (15). The pathogen exists in nearly every onion-growing area of the world and is a serious problem in some areas of New York and in many midwestern and southern states (15,18).

The incidence and severity of Fusarium basal rot can increase in fields cropped frequently to onions (4). The pathogen survives between onion crops as dormant hyphae or chlamydospores in decaying plant debris (3) and can infect rapidly if susceptible plants become stressed by high soil temperatures (4,22). *F. oxysporum* can be spread in irrigation water, on farm equipment, and in and on onion seed (5,6,20,21). *F. oxysporum* has been isolated frequently from transplants brought into Colorado (10).

Various maggot species are associated with onions as either primary (initiating damage) or secondary pests. Onion maggot (*Delia antiqua* Meigen) is the major insect pest in most onion-growing areas of the northeastern United States (11,12). Onion maggots may kill seedlings and reduce stand. After bulbing, feeding by onion maggots may predispose the bulb to field or storage rot and to subsequent invasion by secondary insect pests. Seed-corn maggot (*D. platura* Meigen) can be a primary or secondary pest on onions in Michigan (19).

Appearance of both rot and maggots causes much grower concern (17), but it has not been readily apparent which is the primary pest. Therefore, implementation of control measures has been variable. A 3-yr, or longer, crop rotation is generally recommended to most Colorado onion growers. However, long survival of the basal rot fungus in soil, inconsistent crop rotation by growers, and continued use of infected transplants limit the success of crop rotation. Disease management with fumigants has been expensive, and commercially acceptable resistant or tolerant cultivars are not widely grown in Colorado. Some onion growers routinely apply a soil insecticide at planting to protect against early-season maggot damage. Applications of other pesticides control adult maggot flies and onion thrips (*Thrips tabaci* Lindeman), as well as foliar diseases, later in the season. Because losses are increasing and existing soil pest control measures are expensive or ineffective, new management strategies must be identified.

This study was designed to survey densities of *F. oxysporum* and *F. oxysporum* f. sp. *cepae* in Colorado soils; survey damage from maggots and identify those maggots occurring in onion bulbs; and study the association between maggot infestation, mechanical wounding, and disease occurrence.

MATERIALS AND METHODS

Survey of *F. oxysporum* in soil. Surveys were made to determine the number of colony-forming units (cfu) of *F. oxysporum* present in Colorado soils during the growing season or in soils where transplants were produced for shipment to Colorado production regions. In 1982, soil samples were submitted by growers and consultants to determine population levels of *F. oxysporum*. Many of these soils were to be used for production of onion transplants and had been recently fumigated. Soil (mixture of 10 random soil probes) from the Colorado State University Experiment Station at Rocky Ford was also collected in the spring and fall of 1982. All soil samples (about 150–200 g fresh weight each) were stored at 3 C until used.

Colony-forming units of *F. oxysporum* were determined by dilution plating. A suspension was made by adding 5 g fresh weight of soil to a 500-ml, 0.1% water agar blank that was then agitated vigorously for 60 sec. One milliliter of the soil suspension (1:100 dilution) was pipetted onto an agar plate of the selective and differential medium developed by Komada (14). These plates were incubated at 23 C under continuous fluorescent lighting for 1 wk or until colonies were large but not coalescing. Colonies were counted, and one colony typical of each Fusarium type from each plate was subcultured on potato-carrot agar (PCA) for species identification. One gram of the same soil was oven-dried for 48 hr, weighed, and mean pathogen counts adjusted to compensate for soil moisture content.

Survey of *F. oxysporum* f. sp. *cepae* in soil and organic matter. Soils from fields with onions sustaining severe, moderate, or no Fusarium basal rot in 1983 (as determined on 26 August) were sampled during June, July, and August. Ten

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random soil samples were taken and combined for each field. Colony-forming units of *F. oxysporum* were determined as described before, except 25 g of soil was added to the 250-ml, 0.01% water agar blank and agitated for 5 min. Ten milliliters (1 g of soil) of this suspension was added to a 90-ml, 0.01% water agar blank and agitated 60 sec, then 1 ml was pipetted onto modified Komada's medium (14).

Organic matter pieces (up to 1 cm in diameter) were randomly selected from soil collected during June through September. These pieces were rinsed, wrapped in cheesecloth bags, washed under distilled water for 2 min, and placed on modified Komada's medium.

Plates of soil suspensions and pieces of organic matter were incubated as described before. All colonies of *F. oxysporum* were subcultured onto PCA. After positive identification, pathogenicity tests on onion slices were conducted using Abawi's method (1) to differentiate the *F. oxysporum* f. sp. *cepae* isolates from others. Pathogenic isolates colonized extensively and rotted the onion slices after 5–8 days. Colony-forming units were adjusted for soil moisture content.

Survey of maggots in onions. Commercial onion fields in northern Colorado were monitored weekly throughout the growing season for maggot activity. Fourteen, eight, and seven fields were monitored in 1981, 1982, and 1983, respectively. Early-season damage of maggots was studied in 1981. Eight to 12 rows in each field were left untreated with insecticides at planting. Twenty-five plants from untreated areas were checked weekly beginning on 1 May for signs of maggot infestation (feeding tunnels in bulb) until the onset of bulbing. Fields were monitored for maggot infestation after the onset of bulbing in 1981 and throughout the season in 1982 and 1983. Onions, 25 per field, were sampled randomly each week, and plant appearance was recorded. Other chlorotic plants were also pulled and bulbs examined for maggots. Maggots were collected and reared in the laboratory at room temperature to the adult stage to confirm species identification.

Additionally, flies were reared from damaged onions collected during the growing season and from onion culls left in the field after harvest. These onions were placed in large emergence containers. Presence of adult flies was determined by using cone traps baited with cut onions and placed at the edges of the fields (7).

Seed-corn maggot fly oviposition preference. Seed-corn maggot flies were offered healthy and *Fusarium*-infected bulbs for oviposition in the laboratory. Onion bulbs were collected from commercial fields that had not been sprayed recently with any pesticide. Tests were run on 24 pairs of bulbs. "Infected" bulbs had chlorotic leaves and "healthy" appearing bulbs had no chlorotic leaves. Bulbs with pink or decaying roots caused by factors other than *F. oxysporum* f. sp. *cepae* were discarded. Tops were removed, bulbs were washed, and each bulb was placed separately in 500-ml oviposition cups containing washed medium-coarse sand. Three female and three male seed-corn maggot flies were released in a cage with a nondiseased and a diseased bulb of similar size. After 48 hr, flies were removed. Eggs were counted under a dissecting microscope after separation from the outer onion scale and adjacent sand by repeated washing and straining through cheesecloth. A *t* test was used to determine if there was a significant difference between the population of eggs oviposited on healthy versus diseased bulbs.

Correlation of disease incidence and maggot presence in commercial fields. Onion plants were collected from commercial fields with high disease incidence to determine the relationship between disease and the presence of maggots. In 1982, 272 plants and in 1983, 383 plants were collected from the same field; 203 plants were also collected from a different field in 1983. All collections from a field were made on the same day and rated for disease. There were four disease classes based on percent leaves chlorotic: 0% = none, about 0–20% = light, about 20–50% = moderate, and up to 50–100% = heavy. A fifth class, 100% = severe with all leaves chlorotic, was collected from the first field, where plants were pulled 1 wk before the collection day and dropped between rows. After rating, the foliage was removed and each bulb incubated separately in a 500-ml container with a vented lid at room temperature until adult fly emergence. All flies were identified and percent infested bulbs per disease category was determined.

Relationship of disease incidence, mechanical wounding, and maggot infestation. The effect of mechanical wounding or maggot damage on disease incidence was examined in 1982 and 1983. A randomized block design was used with five replicates, each separated by an untreated border row. Chlorthal dimethyl (Dacthal) herbicide was applied preemergence at a rate of 12.3 kg a.i./ha. Plots were also hand-weeded as necessary throughout the growing season. One row of onions was planted per 56-cm-wide bed. The planting rate in 1982 varied from 10 to 30 plants per meter row because of variability in planting and emergence. The planting rate and stand in 1983 was 30/m of row. The crop was irrigated every 10–14 days throughout 1982 and early 1983. After 20 July 1983, the field was irrigated every 7–10 days.

Treatments were 1) untreated control, 2) injury to roots by mechanical wounding, 3) infestation with onion maggots, 4) inoculation of roots with *F. oxysporum* f. sp. *cepae*, 5) inoculation of roots with *F. oxysporum* f. sp. *cepae* and infestation with onion maggots, and 6) inoculation of wounded roots with *F. oxysporum* f. sp. *cepae*.

Inoculum of *F. oxysporum* f. sp. *cepae* was prepared from three pathogenic isolates collected from various onion-growing regions of Colorado. Isolates were grown on acidified potato-dextrose agar for 1–2 wk at 23 C under supplemental fluorescent light. Mycelia and spores (macroconidia and microconidia) were then scraped from plates into sterile distilled water and comminuted 15 sec in a Waring Blendor. A hemacytometer was used to adjust inocula concentrations to 8×10^4 and 5×10^6 spores per milliliter in 1982 and 1983, respectively. The higher concentration was used in 1983 since we thought it might favor infection under improved moisture conditions and reduced plant stress. Onions were inoculated by pouring 15 ml of inoculum into shallow (2.5-cm) trenches alongside each bulb.

Onions were wounded mechanically in situ by nicking the basal plate with a sterile knife. Bulbs were infested with either 10 eggs or five early-instar larvae of onion maggots obtained from a laboratory colony. Onion maggots, rather than seed-corn maggots, were used because of culture problems raising seed-corn maggots. Since both types of maggots induce similar damage to onions after the seedling stage, we used onion maggots exclusively. The first 10 bulbs in each treatment row were inoculated with *F. oxysporum* f. sp. *cepae*, infested with maggots, or wounded on 20 July each year.

Plots were monitored periodically during the season for occurrence of Fusarium basal rot. Final evaluations were made on 25 September 1982 (66 days after treatment) and on 28 September 1983 (69 days after treatment). All plants were removed from the soil and examined for symptoms of Fusarium basal rot.

RESULTS

Survey of *F. oxysporum* in soil. Soils surveyed had a wide range of levels of *F. oxysporum*, which varied from 22 to 3,600 cfu/g of dried soil (Table 1). Average levels of *F. oxysporum* were much lower in a fumigated (232 cfu/g) than in a nonfumigated (1,416 cfu/g) soil. Soils collected from the southern Colorado site (field L) in the spring of 1983 had the highest level.

Survey of *F. oxysporum* f. sp. *cepae* in soil and organic matter. The percentage of soil isolates that were pathogenic to onion increased in all three fields from June to August; however, *F. oxysporum* in field 1 had the highest proportion of pathogenic isolates, increasing from 56% on 8 July to 90% on 17 August. The percentage of isolates from organic matter that were pathogenic to onion were similar in all three fields and ranged from 45 to 81%.

In field 3 (where no Fusarium basal rot was observed near the end of the season), colony-forming units of *F. oxysporum* per gram of soil were stable and low (258–596 cfu/g of soil) throughout the summer (Table 2). Field 2 (4% disease incidence) had a higher level of *F. oxysporum* (1,064–1,942 cfu/g) during the summer. Field 1 (17% disease incidence) had low levels of *F. oxysporum* on 8 July (301 cfu/g of soil) and 17 August (571 cfu/g of soil) but a high level (3,000 cfu/g of soil) on 27 July. Organic matter collected on that date also had a higher population of pathogenic *F. oxysporum* than at any other collection time or location.

Survey of maggots in onions. Five types of flies were recovered from eggs or larvae in diseased onion bulbs: one anthomyiid, *D. platura*; two ottidids, *Euxesta notata* Wiedemann and *Meleria* sp.; one syrphid, *Eumeris strigatus* Fallen; two muscids, *Fannia canicularis* L. and an unidentified species. *D. patura*, *Meleria* sp., and an additional anthomyiid, *D. neomexicana* Malloch were collected in cone traps baited with cut onion. Early-season feeding was infrequent, and seed-corn maggot larvae generally were associated with plants displaying chlorotic leaves or some evidence of bulb disease. In general, *D. platura* was the most common maggot in diseased bulbs during the growing season, and *E. notata* was the most common maggot in culls. Onion maggots were not found in any commercial fields.

Seed-corn fly oviposition preference. Seed-corn maggot oviposition was recorded for 15 tests, with 78.2% of the eggs occurring on infected bulbs and 21.8% on healthy bulbs ($P < 0.05$). A similar preference has been observed in onions infested with bacterial soft rot (8,16).

Correlation of disease incidence and maggot presence in commercial fields. An association was found between increased disease rating and maggot infestation (Table 3). No maggots were found in healthy bulbs, whereas percent infestation by seed-corn maggot increased from 0% in bulbs with light to 9–50% in bulbs with heavy disease ratings. *E. notata* infestation was less than 10% in light and moderate ratings but was most common (20–60% infestation) in bulbs with severe disease. Muscids appeared sporadically with infestation levels of 3–20% in all damage ratings except healthy.

Relationship of disease incidence, mechanical wounding, and maggot infestation. Onion plants with advanced Fusarium basal rot showed chlorotic dieback of leaves. Dead and necrotic leaves often remained upright as a result of rapid desiccation. When plants with these symptoms were pulled, they had a mealy decay of the basal plate. The basal plate was often covered with mycelium. A few bulbs developed a watery soft rot caused by secondary bacterial invaders. In 1982, only a 2% incidence of basal rot occurred in injured and uninjured bulbs that were not inoculated. In symptomless bulbs, maggot feeding resulted in shallow tunnels that were walled off with callus tissue. Tunnels could not be detected in diseased bulbs because of extensive rotting, and maggots were commonly found feeding on this rotting tissue.

The highest incidence of Fusarium basal rot during both years occurred in Fusarium-infested soil in which onion bulbs had been wounded mechanically (Table 4). Disease incidence in wounded bulbs in Fusarium-infested soil was 20% higher in 1982 and 28% higher in 1983 than in uninjured bulbs.

Disease incidence was slightly higher, although not significantly, in infested or uninfested soil when maggots were present. Less incidence of Fusarium basal rot occurred in all treatments in 1983 than in 1982. The higher inoculum concentration did not increase disease incidence in 1983, apparently because plants were irrigated more frequently after inoculation and were more vigorous than in 1982. No disease was observed in uninfested plots.

Table 1. Colony-forming units (cfu) of *Fusarium oxysporum* in onion production soils in 1982 and 1983[a]

Field designation	Soil treatment	Collection date	cfu/g Dried soil
A	Chloropicrin	Fall 1982	83
F	None	Fall 1982	22
G	Metam-sodium	Spring 1983	163
H	Unknown fumigant	Spring 1983	22
I	Unknown fumigant	Spring 1983	659
J	None	Spring 1983	225
L	None	Spring 1983	3,600
M	None	Spring 1983	1,818

[a] Soils from L and M collected by mixing 10 random samples each per field. Other soils collected by growers and consultants (sampling methods unknown).

Table 2. Incidence (colony-forming units [cfu] per gram) of *Fusarium oxysporum* and *Fusarium oxysporum* f. sp. *cepae* in organic matter and soil in three Colorado onion fields during 1983

Sampling date	Total *Fusarium* spp.	Organic matter isolates (% of 20 pieces): *F. oxysporum*[a]	Organic matter isolates (% of 20 pieces): Percent pathogenic to onion	Soil isolates (cfu/g soil): *F. oxysporum*	Soil isolates (cfu/g soil): Percent pathogenic to onion[b]	Disease incidence (%)
			Field 1			
8 July	1,505	9	67	301	56	
27 July	4,200	28	79	3,000	73	
17 August	2,265	14	79	571	90	17[c]
			Field 2			
8 July	3,511	9	56	1,064	30	
12 August	8,859	17	71	1,086	60	
26 August	9,577	16	81	1,942	76	
19 September	···	17	76	···	···	4
			Field 3			
8 July	2,043	6	67	258	25	
27 July	2,979	11	45	596	57	
17 August	2,242	12	58	593	62	
9 September	···	5	60	···	···	0

[a] Incidence of *F. oxysporum* originating from 20 pieces of organic matter. Plant origin of pieces was not determined.
[b] Between 10 and 38 isolates per field and date were tested for pathogenicity on onion slices (1).
[c] Percentage of plants per 8-m bed with Fusarium basal rot symptoms on 26 August 1983.

Table 3. Incidence (%) of maggot infestation and Fusarium basal rot in onion plants

Disease rating[a]	No. of bulbs	Bulb infestation (%)			
		Delia platura	*Euxesta notata*	Muscidae	Total[b]
Field A		1982			
None	88	0	0	0	0
Light	22	0	0	4	4
Moderate	67	12	0	6	16
Heavy	5	40	0	0	40
Severe	90	4	29	3	31
Field A		1983			
None	98	0	0	0	0
Light	18	0	5	0	5
Moderate	154	1	4	0	4
Heavy	6	50	0	0	50
Severe	107	1	60	9	63
Field B					
None	44	0	0	0	0
Light	63	0	0	0	0
Moderate	50	2	8	0	10
Heavy	46	9	0	20	26

[a] Rating based on percent leaves chlorotic (none = 0%; light = 0–20%; moderate = 20–50%; heavy = 50–100%; severe = 100%, bulb pulled and left in furrow for 1 wk).
[b] Total incidence of infestation not cumulative since some bulbs became infested by more than one species.

Table 4. Effect of onion injury by mechanical wounding or onion maggot feeding damage on incidence of Fusarium basal rot (*Fusarium oxysporum* f. sp. *cepae*)

Treatment	Disease incidence (%)[z]	
	1982	1983
Inoculated, wounded	50 a	42 a
Inoculated, maggot-infested	40 ab	20 b
Inoculated	30 b	14 b
Maggot-infested	10 c	0 c
Wounded	2 c	0 c
Control	2 c	0 c

[z] Means followed by the same letter in a column are not significantly different ($P = 0.05$) from other means according to Fisher's LSD.

DISCUSSION

Many factors, including inoculum level of the pathogen, affect the incidence of Fusarium basal rot of onion. The inoculum level of *F. oxysporum* f. sp. *cepae* can be affected by temperature (4,22) and previous disease history (2,4). Soil fumigation apparently reduced the average level of *F. oxysporum* (22–659 cfu/g of dried soil); however, more sampling and research is warranted.

In three fields sampled throughout the growing season, there was an increase over time in the portion of the population of *F. oxysporum* pathogenic to onion (Table 2). Field 1, which had severe disease incidence on 26 August near the end of the season, had high levels of *F. oxysporum* f. sp. *cepae* in soil and in soil organic matter earlier on 27 July. This date may have been preceded by environmental conditions favoring pathogen increase in soil or plant stress conducive to establishment of Fusarium basal rot. The size of the total populations of *Fusarium* sp. or *F. oxysporum* in soil had little influence on the pathogenic portion. Field 1 had low levels of *F. oxysporum* in soil on 8 July; however, a high percentage of isolates was pathogenic and able to increase rapidly in soil under favorable conditions.

Mechanical wounding significantly increased incidence of Fusarium basal rot. Wounding can result from cultivation, from hand weeding if weed and onion roots are intertwined, and from clipping plant roots before transplanting. Wounding may also expose onion plants to new populations of *Fusarium* spp. that can cause disease. The isolates used in these studies and those of Walker and Tims (22) caused disease on wounded and unwounded bulbs; however, other isolates apparently existed that infected wounded bulbs only (17). These types of pathogens are probably unimportant unless conditions exist that stress or wound onion bulbs or roots.

In Colorado, Fusarium basal rot is often associated with maggot infestation. Some farmers therefore blame maggots for introducing and spreading the disease. The maggots used in this study came from a laboratory colony, so introduction of *Fusarium* by the maggots into the field was eliminated. Both onion maggot and seed-corn maggot can harbor and transmit bacterial soft rot (8,16), but there are no reports of either maggot species carrying *Fusarium* spp. Maggot infestation did not significantly increase disease incidence in either *Fusarium*-infested or uninfested soil compared with maggot-free onions. A slight increase in disease probably was due to wounding of the bulb by the onion maggot. Naturally occurring onion maggots were not found, however, in commercial fields studied in northern Colorado; but southern and western production areas were not sampled for maggots. Seed-corn maggots were commonly found in rotted bulbs along with other less frequently occurring maggots but rarely in disease-free bulbs. The presence of seed-corn maggots was associated with disease incidence, so we suggest that the seed-corn maggots were secondary invaders attracted by already diseased bulbs. Additionally, seed-corn maggot flies were found to oviposit preferentially on rotting bulbs. Hough et al (13) reported that onion maggot oviposition was affected by the presence of nonpathogenic bacteria but not fungi. Microorganisms associated with germinating seeds attract oviposition by seed-corn maggot flies (9). Maggots, then, may be attracted to bulbs showing fungal infection with secondary bacterial development.

Disease management strategies should be based on the relationship between onion maggots, seed-corn maggots, and Fusarium basal rot and emphasize the importance of minimizing stress and injury to bulbs. The effects of different cultivation regimes and environmental factors on pathogen increase in soil and basal rot incidence should be examined.

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