

# Prevalence, Severity, and Association of Fungal Crown and Root Rots with Injury by the Clover Root Curculio in New York Alfalfa

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## ABSTRACT

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A survey of 61 randomly selected alfalfa fields in four physiographic regions of New York was utilized to assess the incidence, severity, and fungal flora associated with crown and root rots, as well as the incidence and severity of injury by the clover root curculio (*Sitona hispidulus*) and its possible association with root diseases. Five stratified random subsamples, each comprising four to nine alfalfa plants, were evaluated from each field. Crown and root rot occurred in every field surveyed, with average tissue necrosis estimated at 21, 27, and 37% in plants from 1-, 2-, and 3-yr-old stands, respectively. *Fusarium oxysporum*, *F. solani*, *Phoma* sp., and *F. avenaceum* were the pathogenic fungi predominantly associated with necrotic roots, accounting for 25, 21, 12, and 4% of isolations, respectively. Inoculation of alfalfa plants with each of 28 randomly selected isolates of *F. oxysporum* and 18 of *F. solani* resulted in root and crown necrosis similar to that observed in the field, but no isolate induced rapid wilting and shoot death as did reference isolates of *F. oxysporum* f. sp. *medicaginis*, causal fungus of Fusarium wilt. Every field and 92% of all individual plants exhibited injury from clover root curculio. The number of wounds that breached the root cortex ranged from 0 to 45 per plant and averaged 2.6, 5.5, and 6.1 for 1-, 2-, and 3-yr-old plants, respectively. Epidermal scarring and deep feeding wounds were highly correlated as measures of clover root curculio activity. There were highly significant, positive linear correlations between average number of deep wounds and average tissue necrosis of plants for fields of each age, suggesting that clover root curculio injury had predisposed alfalfa plants to more severe crown and root rot. Although injury levels were generally lower in the two northern counties sampled, plants from individual fields in each physiographic region showed pest injury levels that potentially could result in economically significant reductions in yield. Reduction of pest-induced losses in New York alfalfa will rely on concomitant control of clover root curculio and fungi that cause crown and root rot.

Additional keywords: *Medicago sativa*

Alfalfa (*Medicago sativa* L.) is the most important forage crop in the United States and is the primary hay crop supporting dairy production (3). In New York, it is harvested from just under 400,000 ha annually at a value of over \$150 million (2). Although production costs are relatively low, the initial costs to establish an alfalfa stand have been estimated at over \$740/ha (22). Profitable production depends on maintaining adequate forage harvests for 3 yr or longer. Diseases and other stress factors can severely reduce the productive life of an alfalfa stand (8,10,13,17,20). A pest complex, comprising the clover root curculio (*Sitona hispidulus* Fabricius) and *Fusarium* spp. that cause crown and root rot as well as Fusarium wilt, has been implicated as the cause of alfalfa plant death and decreased yield and quality in large areas of the northeastern United States (8,10,18-20). Prior to this study, the etiology, distribution, and severity of this problem in New York were unknown.

Crown and root rot has been recognized as a disease complex of alfalfa for many decades and occurs wherever alfalfa is grown. Infections may be initiated in the crowns and progress into the roots, and vice versa. Causal agents are numerous and vary with geographic area and

environmental conditions (17,18,20). In the northeastern United States, *Fusarium oxysporum* Schlechtend.:Fr., *F. solani* (Mart.) Sacc., and *F. avenaceum* (Fr.:Fr.) Sacc. (the latter also included in the taxon *F. roseum* Link:Fr.) are the species most frequently associated with cortical rots of roots and crowns (20). These fungi can maintain a parasitic relationship with their host without apparent injury, as evidenced by their frequent isolation from nonnecrotic tissue even as early as 4 wk after germination (20). Stress factors such as poor cutting management, inadequate fertility, suboptimum pH, water stress, adverse winter conditions, and insect pests are known to increase damage by crown- and root-rotting organisms (17,20). Although many *Fusarium* spp. penetrate roots directly, wounding often results in increased penetration of root cortical tissue adjacent to the wound and increased disease severity (30).

The clover root curculio (CRC), a small weevil, is a widespread pest of alfalfa that causes severe root injury. Although discovered in the United States in 1876 (15), its complete developmental cycle and feeding behavior have only recently been determined (5,31). Eggs hatch in the spring and the larvae burrow into the soil and begin feeding on *Rhizobium*-induced root nodules, a step essential to their survival. As the larvae develop, their feeding progresses from the fibrous roots to the fleshy taproot,

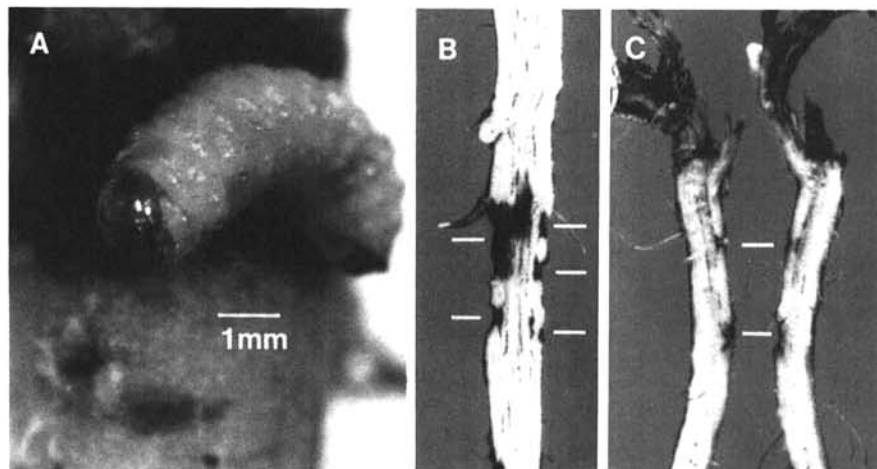


Fig. 1. Development of *Fusarium* crown and root rot in alfalfa in association with injury by the clover root curculio: (A) A fourth-instar larva feeding on an alfalfa taproot. (B) Initiation of root rot at sites of wounds (white lines) produced by clover root curculio. (C) Systemic development of crown and root rot.

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first scarring the epidermis and eventually wounding the taproot deeply and exposing the cortical and vascular tissues to invasion by fungi (Fig. 1). We have observed in New York that adult weevils migrate into alfalfa fields to lay eggs at the base of plants during warm days of August through November and March through May. From June to July, larvae pupate and adults emerge to spend part of the summer in aestivation.

Root feeding by CRC larvae has been shown to reduce nitrogen fixation significantly (13) and to contribute to overwinter death of alfalfa (32). Insecticide control of CRC has been shown to increase yield of alfalfa (7,23) and to improve some quality components (9). In Oregon, CRC increased root necrosis of alfalfa by soilborne fungi (4). In Vermont, the interacting stresses of CRC and *Fusarium* spp. were thought to reduce alfalfa stand longevity primarily by reducing the plant's cold tolerance (10). CRC can also serve as a vector of pathogenic fungi. In New Hampshire, isolates of *F. oxysporum*, *F. solani*, and *F. roseum* pathogenic to white clover were recovered from larvae of CRC removed

from white clover roots (16). Leath and Hower (19) found that *F. o. medicaginis* (J.L. Weimer) W.C. Synder & H.N. Hans., causal fungus of Fusarium wilt, accounted for 63% of all isolates associated with CRC wound sites in a single alfalfa field in Pennsylvania. In greenhouse experiments, they found that CRC feeding activity caused an increase in Fusarium wilt (19). In their studies, pathogenic isolates of the fungus were recovered from the head capsules of field-collected CRC larvae (18,19). Hill et al (11) also reported predisposition of alfalfa to Fusarium wilt by CRC in greenhouse experiments. In Kentucky, alfalfa stand longevity was reduced up to 2 yr by the interactive stresses of CRC, alfalfa weevil (*Hypera postica* Gyllenhal), and soilborne fungi (7).

The current study was considered the first step in assessing the potential importance of crown and root rot pathogens and CRC to alfalfa production in New York, i.e., the need to develop management strategies. A field survey was utilized to assess the incidence, severity, and associated fungal flora of crown and root rots as well as the in-

cidence and severity of CRC injury and its possible association with root diseases in the diversity of soils, climates, and alfalfa stand ages encountered in the state. An additional objective was to determine the ability of *Fusarium* isolates from New York alfalfa to induce root cortical necrosis and/or Fusarium wilt.

#### MATERIALS AND METHODS

**Survey.** Between October 1989 and April 1991, 61 clear-seeded alfalfa fields were surveyed in six major, dairy-based agricultural areas representing four distinct physiographic (i.e., common soils and topography) regions of New York (Fig. 2). Regions and years sampled were: St. Lawrence/Champlain valleys, two areas, SC-A and SC-B, in 1990; Erie/Ontario/central lake plains, one area, LP, in 1990; southern plateau, two areas, SP-A in 1989 and SP-B in 1990; and Hudson/Mohawk valleys, one area, HM, in 1991. Counties and number of fields sampled by area were: SC-A, St. Lawrence County, nine fields; SC-B, Clinton County, 10 fields; LP, Genesee, Orleans, and Niagara counties, nine fields; SP-A, Erie, Cattaraugus, and

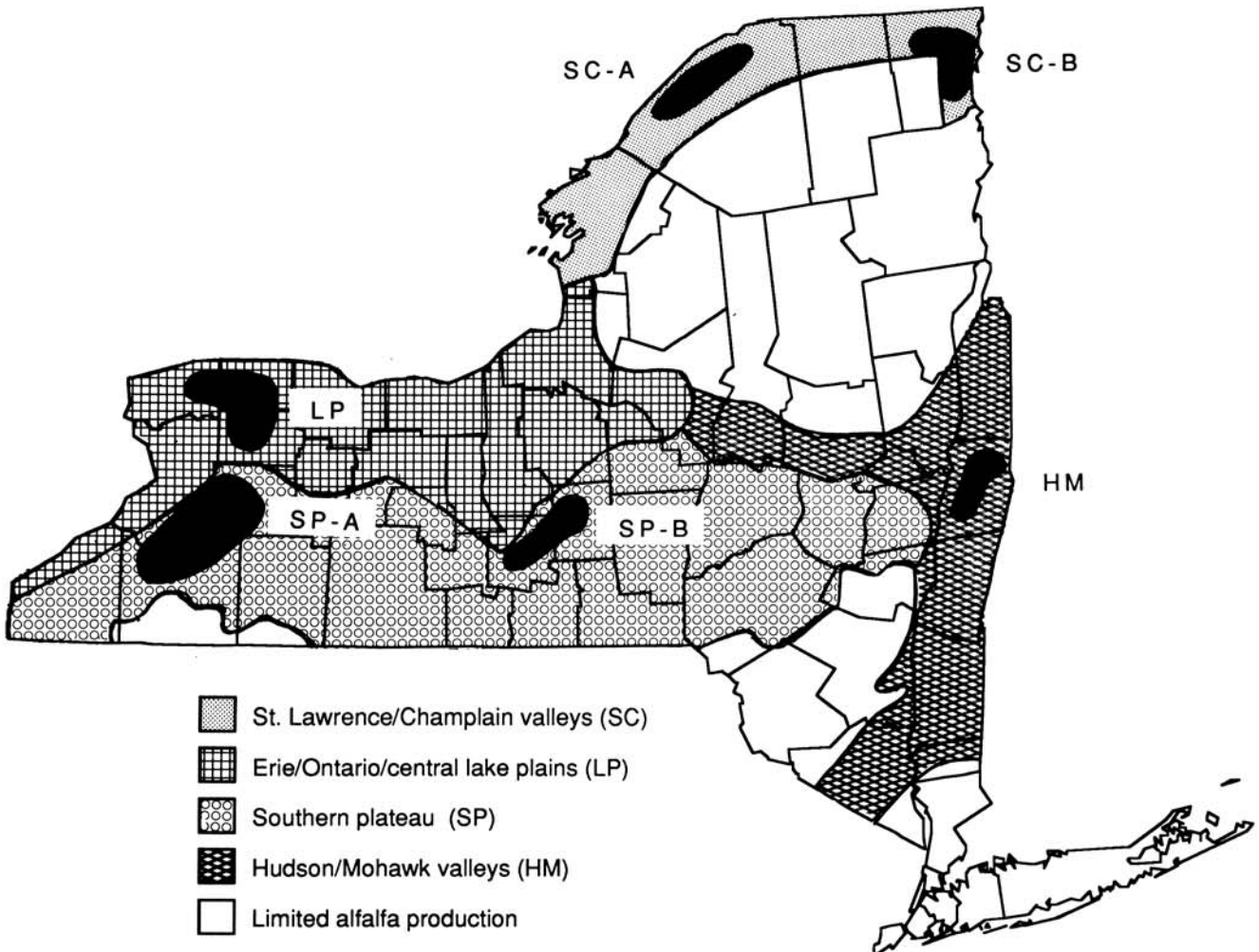


Fig. 2. Location of six areas (darkened) in New York, representative of four physiographic regions (legend), in which alfalfa was surveyed for crown and root rot and injury by clover root curculio during 1989 to 1991.

Wyoming counties, 12 fields; SP-B, Tompkins and Cortland counties, 12 fields; and HM, Rensselaer County, nine fields. A roughly equal number of 1-, 2-, and 3-yr-old fields (i.e., numbered production year following the seeding year) were chosen randomly in each sampling area for a total of 21 1-yr, 20 2-yr, and 20 3-yr fields. In each field, five random samples of four to nine plants were dug and removed with as much taproot as possible. Shoots were removed to reduce desiccation, and plants were placed in ice chests and returned to the laboratory. Roots were washed, and the number of deep feeding wounds (i.e., those that penetrated the root cortex into vascular tissues) on the taproot and main secondary roots was recorded. A pretransformed rating scale of 0–5 was used to make rough visual estimates of the percentage of the root epidermal area that was scarred by CRC larvae. The six increments of the scale represented the following percentages: 0 = 0%, 1 = 0.1–9.5%, 2 = 9.6–34.5%, 3 = 34.6–65.5%, 4 = 65.6–90.5%, and 5 = 90.6–100% (12,21). The same scale was used after splitting each root longitudinally to estimate the percentage of internal root and crown tissue that was necrotic. Data were collected on each root of each subsample, and subsample means were averaged for each field. The means of the ratings for crown and root rot (percentage of root/crown tissue necrotic per plant) were regressed on the mean number of deep feeding wounds per plant (28). The relationship between deep feeding wounds and epidermal scarring by CRC was also examined.

**Fungal isolations.** Two roots from two samples from each field (total of four roots per field) were selected randomly for determining the fungal flora of alfalfa roots. After the root had been split and rated for necrosis, pieces from different areas of the root and crown (primarily from the interface of necrotic and non-symptomatic tissues) were surface-disinfested with 0.05% sodium hypochlorite for 2 min, followed by a rinse in sterile distilled water. Smaller sections were then removed with a sterile scalpel and placed on water agar amended with 300 mg/L of streptomycin sulfate. Fungal mycelium growing from root and crown pieces was aseptically transferred to potato-dextrose agar or V8 juice agar. Cultures were maintained at 22 C under cool-white fluorescent lights with a 12-hr photoperiod. Single macroconidial subcultures were then made from cultures identified as *Fusarium* spp. Further identification of *Fusarium* to the appropriate species was made using the procedures outlined by Nelson et al (24).

**Pathogenicity tests.** Pathogenicity tests were limited to *Fusarium* spp. obtained from root/crown tissues. A procedure similar to that used by Salter (27) and Zeiders and Hill (33) was used in

which the taproot was severed to facilitate pathogen ingress. Fungal isolates were grown for 14 days in a sterilized mix of vermiculite saturated with V8 juice (1:1, v/v). Six 6-mm-diameter disks from cultures on agar plates were used to seed 250-ml flasks that contained 150 ml of vermiculite mix. Five 3-wk-old alfalfa seedlings, cv. Iroquois, were transplanted into 13-cm-diameter pots (750 ml) filled with coarse-grade vermiculite. Plants were fertilized with STEM micro-nutrients (soluble trace element mix, Peters Co., Allentown, PA) at transplanting, followed by weekly applications of soluble N-P-K at 200 mg/L each. Ten weeks after transplanting, shoots were trimmed to approximately 4 cm, the root mass was lifted, and the roots were cut 5 cm below the crown, with the bottom half of the root mass returned to the pot. Approximately 50 ml of inoculum was distributed evenly over the bottom portion of the root mass. The top portion of the root mass was then placed on top of the inoculum, and plants were allowed to regrow for 5 wk. At 18 wk of age, plants were harvested and evaluated. Roots were washed free of potting medium, split longitudinally, and rated for root/crown necrosis. The first experiment involved three replications of seven isolates of *F. oxysporum*, seven

isolates of *F. solani*, one reference isolate of *F. o. medicaginis*, and a noninoculated control. A second experiment utilized three replications of seven additional isolates of *F. oxysporum*, five additional isolates of *F. solani*, two reference isolates of *F. o. medicaginis*, and a non-inoculated control. There were 10 plants per pot, and plants were 15 wk old at inoculation and 25 wk old at evaluation. Data for both experiments were subjected to analysis of variance, and means were separated by Fisher's protected LSD test (28).

A root-dip inoculation method similar to that described by Frosheiser and Barnes (6) was utilized to determine the ability of *F. oxysporum* isolates to induce vascular wilt characteristic of Fusarium wilt caused by known isolates of *F. o. medicaginis*. Reference isolates of *F. o. medicaginis* were obtained from Ken Leath (USDA Pasture Laboratory, University Park, PA). Fungal isolates were grown in nutrient broth on a shaker (100 rpm) for 4–5 days. Liquid cultures were filtered through two layers of cheesecloth, and the concentration was adjusted to  $10^6$  spores per milliliter. Six-week-old seedlings of cvs. Oneida VR and Narragansett were removed from vermiculite growth medium, one-third of the root mass was trimmed with scissors,

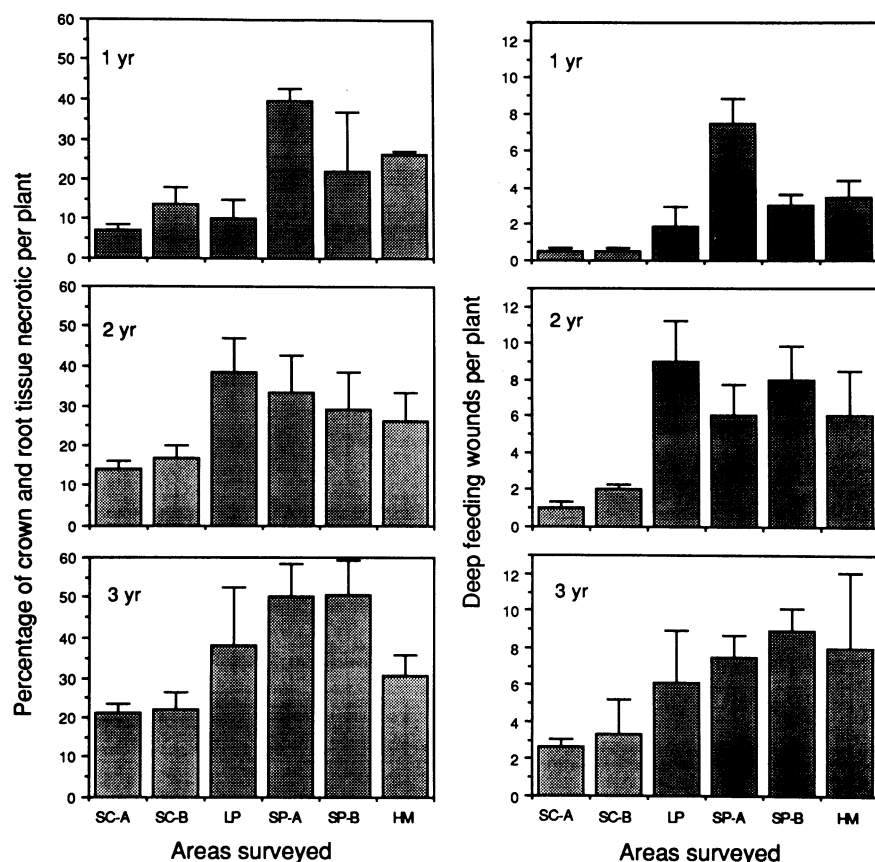


Fig. 3. Average severities of crown and root rot and of injury by clover root curculio in alfalfa plants from 1-, 2-, and 3-yr-old stands in six areas of New York (see Figure 2). Vertical bars indicate standard errors of the means. SC-A and SC-B = St. Lawrence/Champlain valleys, LP = Erie/Ontario/central lake plains, SP-A and SP-B = southern plateaus, and HM = Hudson/Mohawk valleys.

and the trimmed root mass was placed in the spore suspension for 30 min. Seedlings were transplanted singly into Cornell Peat-lite mix, a peat-vermiculite potting medium (1), in 8-cm-diameter pots (200 ml) with five replications of 23 treatments comprising 14 isolates of *F. oxysporum*, six isolates of *F. solani*, two reference isolates of *F. o. medicaginis*, and a noninoculated control for each cultivar. Isolates used in the root necrosis experiments were different from those used in the vascular wilt experiment. Plants were allowed to regrow for 6 wk, after which the plants were evaluated for symptoms of Fusarium wilt, i.e., shoot yellowing, wilting, death, and vascular necrosis.

## RESULTS AND DISCUSSION

Crown and root rot occurred in every field surveyed, with average tissue necrosis estimated at  $21 \pm 4\%$ ,  $27 \pm 3\%$ , and  $37 \pm 4\%$  in plants from 1-, 2-, and 3-yr-old stands, respectively (Fig. 3). The most severe disease was encountered in southern plateau sampling area A, where plants from 1-, 2-, and 3-yr-old stands had average tissue necrosis of  $40 \pm 3\%$ ,  $34 \pm 9\%$ , and  $50 \pm 8\%$ , respectively. The least severe disease occurred in St. Lawrence/Champlain valleys sampling area A, where plants from 1-, 2-, and 3-yr-old stands had average tissue necrosis of  $7 \pm 2\%$ ,  $14 \pm 2\%$ , and  $21 \pm 3\%$ , respectively. Levels of root disease found in each physiographic region of New York may be sufficient to contribute to premature decline of productive alfalfa stands.

The majority of fungi isolated from necrotic alfalfa root tissues were *Fusarium* spp. (60% of total), with the two most frequently isolated species being *F. oxysporum* (25% of total) and *F. solani* (21% of total) (Table 1). In pathogenicity tests, most isolates of *F. oxysporum* and *F. solani* from alfalfa roots caused significantly more root cortical necrosis

(15–50%) than those from noninoculated plants (15–20%), but with no evidence of shoot/wilt symptoms (*data not shown*). Among isolates collected in the survey, those of *F. oxysporum* and those of *F. solani* did not differ significantly in ability to cause necrosis. However, two isolates of *F. o. medicaginis* from Pennsylvania not only caused more severe root necrosis (75–95%) than any New York isolate tested but, moreover, caused severe yellowing, shoot necrosis, and plant death. Fusarium wilt severity was greater in Narragansett than in Oneida VR alfalfa. No isolate of *F. oxysporum* recovered from plants in this survey induced rapid systemic symptoms typical of Fusarium wilt. This contrasts with the findings of Leath and Hower (19), who reported that 89% of *F. oxysporum* isolates from a single alfalfa field in Pennsylvania induced Fusarium wilt. On the basis of our results and a historical lack of field evidence of Fusarium wilt, we feel that, while this disease may occur, it is not a widespread problem in New York. The reason for this is not entirely clear but may include the fact that New York's climate is, in general, cooler than that of locations where this disease is known to be a serious problem. Surveys are warranted in New England states and Pennsylvania to determine if the New York findings are representative of al-

falfa production in the northeastern United States.

The third most frequently isolated fungus was *Phoma* (12% of total) (Table 1). Although the pathogenicity of *Phoma* isolates was not tested, they were assumed to be *P. medicaginis* Malbr. & Roum. in Roum., the causal agent of spring black stem and leaf spot of alfalfa, which has been shown to be a virulent crown and root pathogen (25,26) and may be an important component of the crown and root rot complex in New York.

Every field and 92% of all individual plants in the survey exhibited injury from CRC. Epidermal scarring and deep feeding wounds were highly correlated ( $r = 0.78$ , *data not shown*) as measures of CRC activity. In our survey, the number of wounds that breached the root cortex ranged from 0 to 45 per plant, and means ( $\pm$  standard error) were  $3.0 \pm 0.6$ ,  $5.5 \pm 0.9$ , and  $6.3 \pm 0.9$  for 1-, 2-, and 3-yr-old alfalfa stands, respectively (Fig. 3). Of the sampled plants that escaped CRC injury, 80% were from 1-yr-old fields. In southern plateau sampling area A, 1-yr-old fields were impacted by a single generation of CRC producing an average of  $7.5 \pm 1.3$  deep feeding wounds per plant (Fig. 3). On the basis of insect injury alone, multiple deep injuries, especially on 1-yr-old roots, may weaken these plants enough to be an important factor in stand decline. There is a possibility that alfalfa planted in early spring could grow enough to attract egg-laying CRC adults, with larval emergence and feeding injury occurring in the seeding year. This aspect of the insect's life cycle has not been examined and warrants further study.

Although individual fields in each physiographic region showed CRC injury levels that potentially could result in economical reductions in yield, the two areas surveyed in the St. Lawrence/Champlain valleys had significantly less CRC injury, regardless of stand age. Soils in the St. Lawrence/Champlain valleys tend to be more poorly drained and have a mean annual temperature approximately 4 C lower than soils in the other regions. This region also tends to have less protective snow cover. Each of these factors may increase CRC mortality during the winter and, therefore, decrease egg laying in the spring.

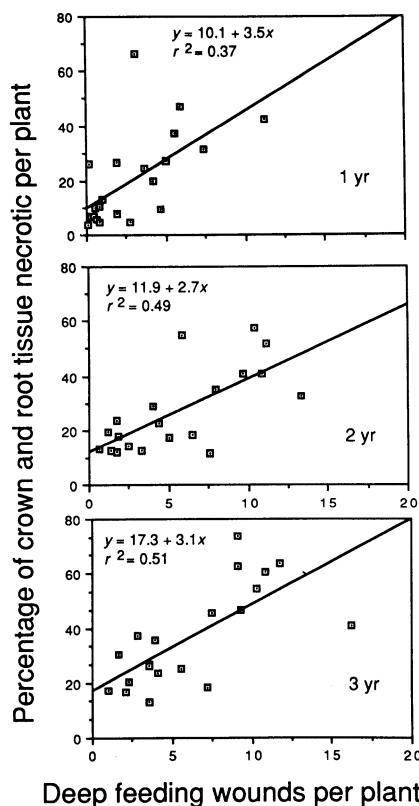
There were positive linear correlations between average number of deep wounds and average percentage of crown and root tissue necrotic for fields of each age (Fig. 4). The  $r^2$  for 1-, 2-, and 3-yr-old fields surveyed was 0.37 ( $P < 0.004$ ), 0.49 ( $P < 0.0006$ ), and 0.51 ( $P < 0.0005$ ), respectively. This suggests that CRC feeding injury quantitatively predisposed alfalfa plants to more severe crown and root rot. Similar suggestions were made by Dickason et al (4) and Godfrey et al (7–9).

**Table 1.** Fungi isolated from necrotic alfalfa roots and crowns from six areas of New York

Fungi	No.	Percent of total
<i>Fusarium</i>		
<i>oxysporum</i>	58	25
<i>solani</i>	48	21
<i>avenaceum</i>	9	4
Other identified species <sup>a</sup>	6	3
Unidentified species	17	7
<i>Phoma</i>	27	12
<i>Pythium/Phytophthora</i>	5	2
<i>Thielaviopsis</i>	5	2
<i>Stemphylium</i>	5	2
Presumed saprophytic genera <sup>b</sup>	34	15
Unknown	17	7

<sup>a</sup> *F. acuminatum*, *sambucinum*, and *semitectum*.

<sup>b</sup> Species of *Trichoderma*, *Penicillium*, *Chaetomium*, *Helminthosporium*, *Acremonium*, *Rhizopus*, and others.



**Fig. 4.** Association of crown and root rot severity in alfalfa with injury by clover root curculio in 61 alfalfa fields surveyed in New York during 1989 to 1991. Separate linear regressions were performed on data from 1-, 2-, and 3-yr-old stands.

Although CRC injury and associated crown and root rot have been shown to be prevalent and severe in New York alfalfa fields, we do not know the effects of this pest complex on yield and quality or the potential benefits, if any, of pest management. It is difficult to exclude CRC adults from alfalfa plots, because adults can lay eggs from August to May whenever temperatures are conducive. Hower and Leath (13) were unable to reduce adequately CRC adults by placing cages over plots, and we (Kalb et al, *unpublished*) achieved poor control with applications of methyl parathion (Penn-cap-M) at 9.5 L/ha) at 10-day intervals from late August to mid-October and again in April and May. Soil-applied insecticides are not useful for yield assessment experiments because of growth regulator effects on alfalfa and effects on nontarget organisms (e.g., nematodes). The impact of this pest complex on alfalfa production may not be known until control measures are available.

The greatest promise for management of the CRC/crown and root rot complex lies with advances in alfalfa breeding and biological control. Progress in breeding cultivars that resist or tolerate *Fusarium* fungi and/or CRC is expected to be slow and incremental. Exciting potential biological controls for CRC include the establishment of persistent, cold-tolerant entomopathogenic nematodes in CRC-infested soils (14), establishment of parasitoids such as *Microctonus aethiopoides* Loan. (29), and inoculation of alfalfa with *Rhizobium meliloti* Dang. strains that have been transformed with an appropriate *Bacillus thuringiensis* Berliner (Bt) endotoxin gene. Early instar CRC larvae may die after feeding on root nodules in which Bt toxin is present. Successful management of this pest problem will rely on concomitant control of CRC and fungi that cause crown and root rot.

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