Low-Temperature Interactions in Fusarium Wilt and Root Rot of Alfalfa

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ABSTRACT

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Interactions of low temperature and two Fusarium diseases of alfalfa were investigated. Fusarium root rot and Fusarium wilt reduced survival of alfalfa after the freeze test. The effect of Fusarium wilt was greater, raising the frost hardiness level (LT_{50}) from −10.5 to −14.5 C. Freezing the plants before inoculation enhanced the development of the disease and reduced survival and yield.

RÉSUMÉ

Les interactions de deux maladies à *Fusarium* et du froid ont été étudiées chez la luzerne. La présence du pourridié fusarien et du flétrissement fusarien a réduit la survie des plantes lors de tests de congélation. L'effet du flétrissement fusarien a été plus marqué, élevant le seuil de résistance au froid (TL_{50}) de −10,5 à −14,5 C. La congélation des plantes avant leur inoculation a accentué le développement de la maladie. L'inoculation après la congélation a diminué la survie et le rendement des plantes.

Additional key words: *Medicago sativa*.

Alfalfa (*Medicago sativa* L.) is generally affected by Fusarium root rot in the northeastern United States and Canada. It also suffers greatly from winter killing in eastern Canada (12). There is increasing evidence that insects and diseases reduce cold resistance and winter survival of plants. *Curvularia trifolii* (Kauff.) Boed. and *Stemphylium sarcinaeforme* (Cav.) Wiltshire reduced survival rate of white and red clover after a freeze test (10). Cold hardiness of alfalfa was reduced by infestation of nematodes (18) and pea aphid (7). Hardiness of winter barley, as measured by development of new roots from crown meristems was depressed by inoculation with *Fusarium roseum* f. sp. *cerealis* 'Avenaceum' after controlled freeze tests (17). Winter barley, winter oats, and winter wheat, infected with barley yellow dwarf virus, showed a decrease in survival after ice encasement as compared with an uninfected control (13). Mildew attack before the onset of winter enhanced winter damage in barley (3). Recently, Tu (21) demonstrated with different concentrations of fungal inoculum that root rot predisposes alfalfa to winterkill in the field. Alfalfa mosaic virus inoculated to 12 alfalfa cultivars also reduced winter survival (22).

On the other hand, exposure of plants to low temperatures may also affect their susceptibility to disease. Jones (9) presented evidence that spring infection of alfalfa plants by *Corynebacterium insidiosum* (McCull) Jens. was due to the entrance of bacteria through wounds caused by winter injury. Diurnal freezing and thawing causing root injury was related to root rot incidence in alfalfa (15). Gagnon (4) concluded from a study in the field that winter conditions favored root rot of red clover.

This study was undertaken to investigate the effect of Fusarium infection on cold hardiness of alfalfa, to study the effects of freezing on the subsequent development of alfalfa root rot under controlled environmental conditions, and to determine to what extent pathogens introduced during the recovery phase modify survival following controlled freeze tests.

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MATERIALS AND METHODS

Plants, fungal pathogens and inoculation techniques. Two fusaria were used in this study: *F. acuminatum* Ellis & Everh., which is one of the organisms associated with Fusarium root rot in alfalfa (4), and *F. oxysporum* f. sp. *medicaginis* (Weimer) Snyd. & Hans., which causes Fusarium wilt of alfalfa (5).

Alfalfa plants of cultivar Saranac were grown in a growth room at 24 C with a 16-hr photoperiod and a light intensity of 320 to 345 $\mu E/sec/m^2$ at plant height. For the Fusarium root rot test, plants were grown for 12 wk in 10-cm pots (five seedlings per pot) containing a pasteurized garden soil-Vermiculite mixture (9:1, v/v) and then inoculated with *F. acuminatum*. The fungus was cultured on strips of polyester on the surface of V-8 juice agar. Roots were inoculated by removing the soil-root mass from the pot, cutting transversely through the sod 3 cm below the crown, placing a 1-wk-old strip of fungal inoculum against the cut end of the tap-root, and reassembling and repotting the sod (14).

For Fusarium wilt, seedlings were grown in plastic flats for 6 wk and inoculated by removing the seedlings from the flats, washing the soil from the roots, cutting them 4 cm below the crown, and dipping them immediately for 2 min into a mycelial and spore suspension of *F. oxysporum* f. sp. *medicaginis* ($14–28 \times 10^6$ propagules per milliliter). Seedlings were then replanted in pots. For both diseases, the uninoculated controls were treated the same as the inoculated plants except that inoculum of *F. acuminatum* was replaced by a sterile polyester strip and inoculum of *F. oxysporum* f. sp. *medicaginis* by a sterile culture medium. After inoculation, all the pots were randomly distributed in the growth room. Seedlings were then incubated for 4 wk.

All experiments were done twice with 10 replicates for each treatment (10 pots containing five seedlings each).

Hardening and freeze test. Plants were allowed to harden in a growth chamber for 3 wk at a constant temperature of 1 C, and 8-hr photoperiod, and a light intensity of 135 $\mu E/sec/m^2$. After hardening, plants were clipped 3 cm above the crown and the pots, previously assigned to each of the six test temperatures, were randomly distributed within each temperature treatment in a freezer (20) fitted with a temperature programmer (Data-Trak,

Model 5310, Research Inc., Minneapolis, MN). Pots of the unfrozen treatment were withdrawn at +1 C. Temperature was then lowered at a rate of 2 C/hr, maintained at −4 C for 12 hr and held for 2 hr after each 2 C decrease (6). When the time necessary to reach a test temperature had elapsed, pots preassigned to it were withdrawn at the end of the following 2 hr and the actual temperature of the freezer was recorded. Pots were then allowed to thaw for 24 hr at 4 C, after which they were returned to the initial growth conditions (24).

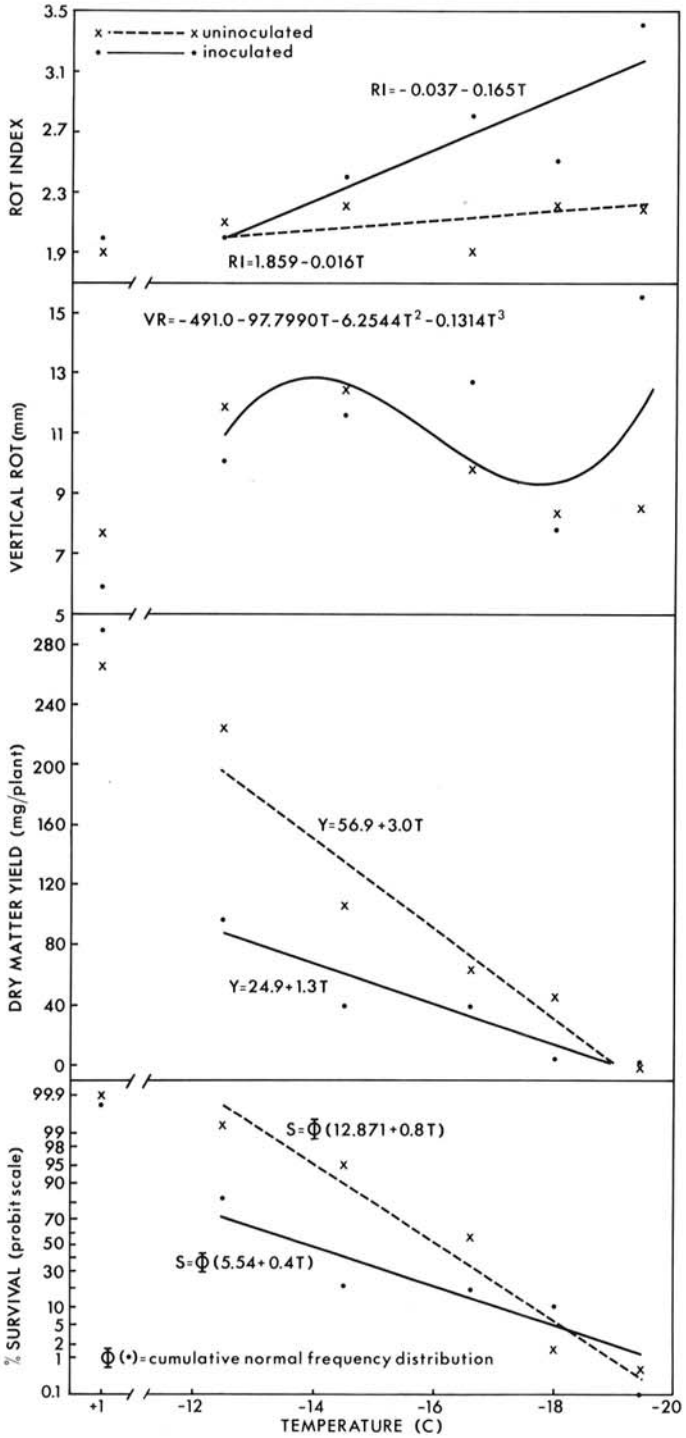

Fig. 1. Observed and estimated (polynomial regression) effect of temperature (T) on survival (S), dry matter yield (Y), root rot index (RI) and vertical root rot (VR) of alfalfa plants inoculated with *Fusarium acuminatum* before freeze test and of their uninoculated controls. Data points are the mean of 10 replicate pots containing five plants each. Vertical rot was measured upward from the cut end of the root. The root rot index is based on a 0 to 5 scale (0 = healthy tissue, 5 = 100% rotted).

The effects of disease on cold resistance were investigated by inoculating the plants with the pathogen before the hardening and freezing phase, whereas the effects of freezing on the subsequent disease development were studied by inoculating plants 1 wk after the freeze test.

Survival. Survival counts were made after 3 wk of regrowth and the top growth was recorded after drying the surviving seedlings at 80 C for 24 hr. Probit analysis was used to estimate the temperature at which 50% of the population was killed (LT_{50}).

Disease rating. At each step after inoculation (hardening, freezing), two pots (10 seedlings) each from the inoculated and uninoculated treatments were rated for root rot as a check. At the end of the experiments, all plants were evaluated for root rot on a scale of 0 to 5: 0 = healthy tissue; 1 = light browning, 0–10% of the tissue affected; 2 = browning, necrotic area, 10–50% of the tissue affected; 3 = general browning, large proportion of necrotic area, 50–90% of the tissue affected; 4 = general necrosis, 90–100% of the tissue affected; and 5 = 100% of the tissue affected. In the Fusarium root rot experiments, the vertical discoloration was also measured.

RESULTS

With either of the two pathogens, there was no evidence that interaction between temperature and inoculation had affected the rot index or vertical rot. The relationship between average rot

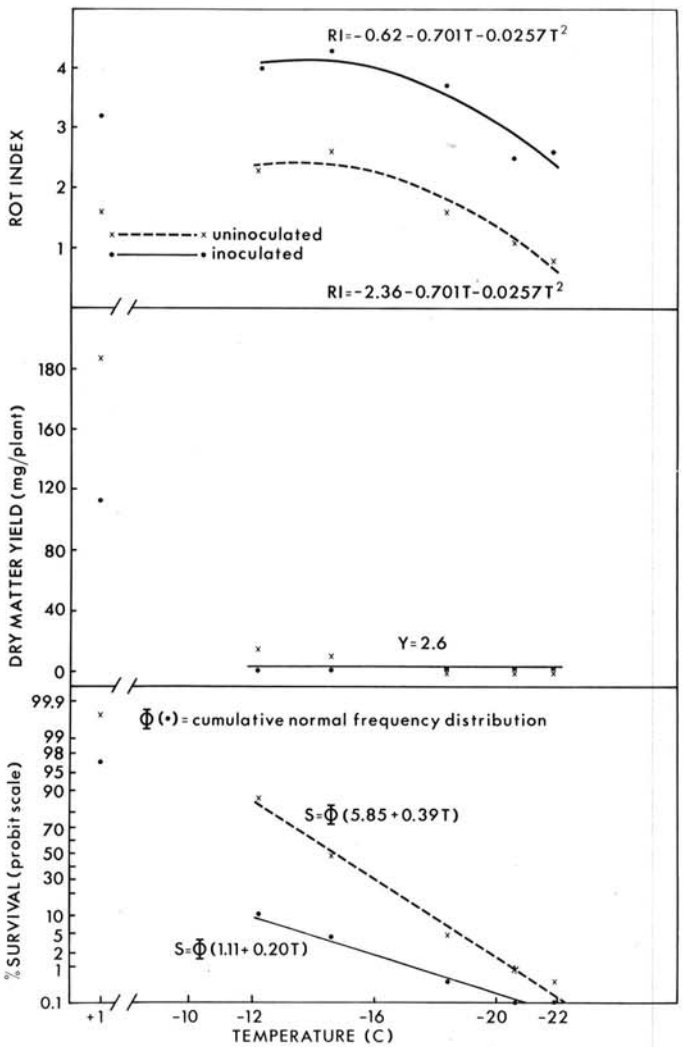

Fig. 2. Observed and estimated (polynomial regression) effect of temperature (T) on survival (S), dry matter yield (Y) and root rot index (RI) of alfalfa plants inoculated with *Fusarium oxysporum* f. sp. *medicaginis* before freeze test and of their uninoculated controls. Data points are the mean of 10 replicate pots containing five plants each. The root rot index is based on a 0 to 5 scale (0 = healthy tissue, 5 = 100% rotted).

index (vertical rot) and temperature for inoculated plants did not depart significantly from the corresponding relationship for uninoculated plants; but, since the relevant sum of squares in the analysis of variance nearly reached significance for the rot index in the root rot experiment ($P = 0.07$), two distinct slopes were estimated for the inoculated and uninoculated plants (Figs. 1 and 2). In both experiments, temperature produced appreciable differences in the average rot index ($P = 0.03$ for the rot experiment [Fig. 1] and $P < 0.00001$ for the wilt experiment [Fig. 2]) and in the average vertical rot ($P = 0.02$) for the rot experiment. In the latter, the average rot index increased with decreasing temperature at an average constant rate of 9.1% ($P = 0.01$, 16.5% for inoculated plants and 1.6% for uninoculated plants), for plants subjected to temperatures between −12.5 and −19.4 C. For the wilt experiment, the average rot index decreased at a variable rate with decreasing temperature (Fig. 2).

Survival rate and dry matter yield of freeze-tested alfalfa plants were generally reduced by inoculation with *Fusarium* spp. (Figs. 1 and 2). A significant effect of the interaction between temperature and inoculation on these two variables suggested separate analyses for inoculated and uninoculated plants, except for the dry matter yield in the wilt experiment where there was no interaction between the two factors (Figs. 1 and 2). *F. acuminatum* reduced cold resistance of the plants (LT_{50}) by 1.6 ± 0.34 C, whereas *F. oxysporum* f. sp. *medicaginis* reduced it by 3.5 C (Table 1).

Subjecting seedlings to subfreezing temperature before inoculation with *F. acuminatum* or *F. oxysporum* f. sp. *medicaginis* enhanced the development of root rot compared with that in unfrozen inoculated controls (Figs. 3 and 4). When plants were inoculated with *F. acuminatum*, 50% of the between-temperature variance of the rot index was due to the difference between the check at +1 C and the frozen plants; when plants were not inoculated, this difference accounted for 39% of the corresponding variance. For temperatures between −7.6 and −13.9 C, the average rot index of plants inoculated with *F. acuminatum* increased with decreasing temperature at the constant rate of 21.5% ($P < 0.00001$); the corresponding rate for uninoculated plants in the same experiment was 49.3% ($P < 0.00001$). The average vertical rot decreased with increasing temperature at the constant rate of 109.9% ($P < 0.00001$) for both inoculated and uninoculated plants, over the range of −7.6 to −13.9 C. More seedlings were killed after the freeze test when they were inoculated with *F. oxysporum* f. sp. *medicaginis* than when uninoculated; the difference between the two survival rates varied from 14 to 36% among temperatures.

The relative yield of inoculated seedlings (percentage of uninoculated control) was established and showed yield losses due to the effect of frost on disease development caused by both *Fusarium* spp. (Table 2).

The differences in cold resistance and in disease rating between inoculated plants and controls would probably have been greater if the controls had been completely free of other fungi. We isolated *Chromelosporium fulva* (Link) McGinty, Hennebdrt & Korf from the soil of control pots. It is a common contaminant of sterilized soil and vermiculite in greenhouses (1). We also isolated *Alternaria alternata* (Fr.) Keissler, *Fusarium arthrosporioides* Sherb., *F. moniliforme* Sheld. var. *intermedium* Neish & Leggett, *F. oxysporum* Schlecht., *Mucor* sp., *M. Hiemalis* Wehm., *M. plumbens* Bon., and *Pythium ultimum* Trow from roots of uninoculated plants.

TABLE 1. Effect of Fusarium inoculation on frost resistance of hardened alfalfa plants

Fusarium species	LT_{50} (C)[a]	
	Inoculated plants	Uninoculated plants
F. acuminatum	−14.3 ± 0.3	−15.9 ± 0.2
F. oxysporum f. sp. *medicaginis*	−10.6[b]	−14.1 ± 0.3

[a] Temperature at which 50% of the population is killed ± standard deviation.
[b] Estimated by graphic extrapolation, only 22% of plants having survived at the highest temperature tested (−12.2 C).

DISCUSSION

Results clearly showed that the two pathogens inoculated into alfalfa plants reduced their cold resistance as measured by survival rate and yield.

Cold resistance of plants was affected more by Fusarium wilt than by Fusarium root rot. Wilt raised the LT_{50} by 3.5 C, whereas root rot raised it by only 1.6 C. This difference was expected because the effects of Fusarium wilt on alfalfa are much more

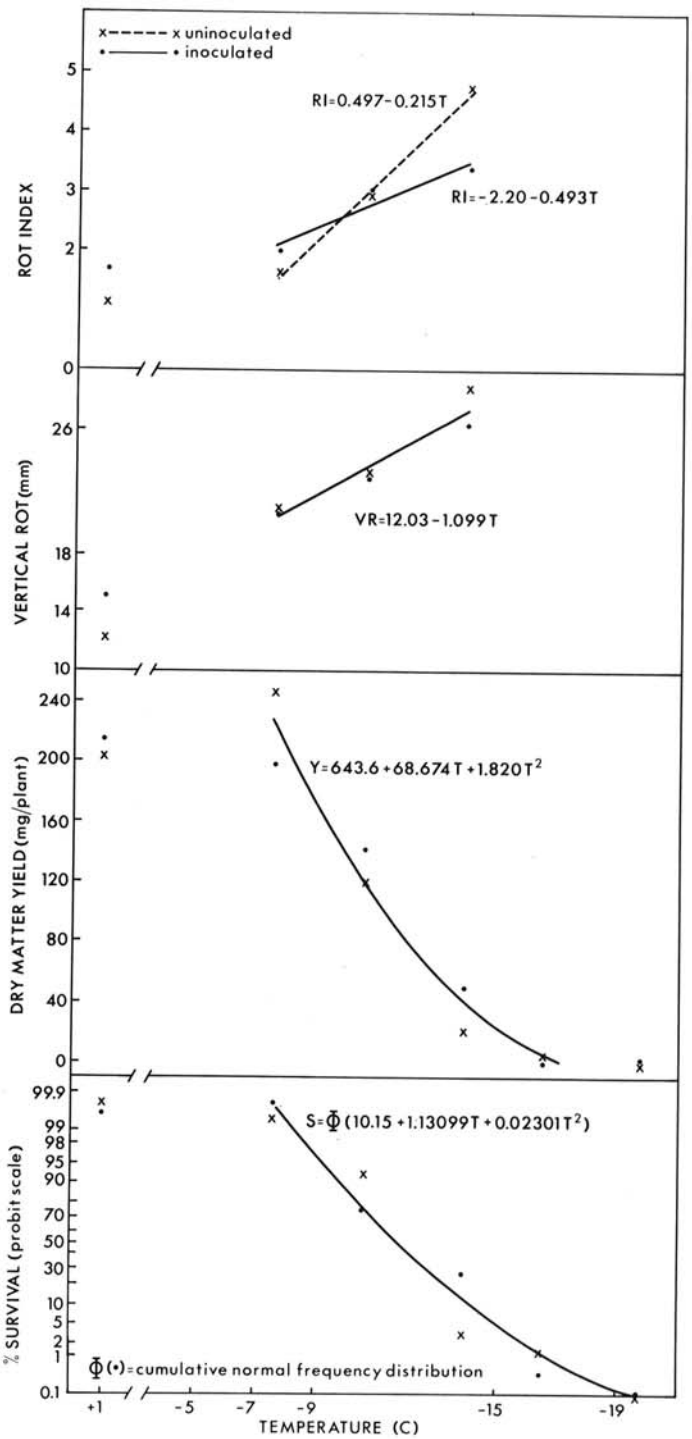

Fig. 3. Observed and estimated (polynomial regression) effect of temperature (T) on survival (S), dry matter yield (Y), root rot index (RI) and vertical root rot (VR) of alfalfa plants inoculated with *Fusarium acuminatum* after freeze test and of their uninoculated controls. Data points are the mean of 10 replicate pots containing five plants each. Vertical rot was measured from the cut end of the root upwards. The root rot index is based on a 0 to 5 scale (0 = healthy tissue, 5 = 100% rotted).

severe than those of Fusarium root rot.

Infection with *Fusarium* spp. probably affects physiological processes that normally lead to hardening. *Fusarium tricinctum* (Cda) Snyd. & Hans. was found to reduce carbohydrates in clipped alfalfa plants compared with uninoculated controls (11). According to Talboys (19), root storage tissue is invaded and water transport is disrupted by fungal wilts. Pea aphid-infested alfalfa seedlings contained fewer carbohydrates than uninfested ones and were less cold-hardy (7). Suzuki and Willis (18) explained the loss in cold tolerance of nematode-infested alfalfa plants by their predisposition to desiccation under freezing temperatures. Reduced root mass and nodulation made alfalfa plants affected by root rot more susceptible to winterkilling (21).

In investigating the influence of disease on cold resistance, we noted that the highest disease ratings were associated with the lowest temperatures. This led us to investigate the influence of freezing on the predisposition of plants to disease. Plants stressed by freezing before inoculation were affected more by inoculation than unstressed ones. Survival of alfalfa was lower, especially in plants inoculated *F. oxysporum* f. sp. *medicaginis*. Disease ratings increased with decreasing temperatures. Moreover, relative yield of inoculated plants which had been frozen before inoculation with *F. acuminatum* or with *F. oxysporum* f. sp. *medicaginis* was generally lower than that of unfrozen plants.

A few studies have been conducted on the effects of low temperature stress on disease susceptibility of tree branches, fruits (16) and agronomic crops (25). According to Jones (8,9) and Weimer (23) and as related by Elliot et al (2) and Graham et al (5), frost injury provide sites of entrance for pathogens. The results of these studies suggested that freezing damage either allows the fungus to enter the plant or predisposes it to colonization.

Our experiments show that diseased plants become less productive and less able to harden. Consequently, they are more susceptible to winterkilling. On the other hand, plants injured by freezing are more susceptible to pathogens.

TABLE 2. Effect of frost and Fusarium diseases on yield of alfalfa plants inoculated after freezing

Fusarium acuminatum				*Fusarium oxysporum* f. sp. *medicaginis*			
Temp.	Dry matter yield (g per pot)			Temp.	Dry matter yield (g per pot)		
(C)	U[a]	I[a]	% of U	(C)	U[a]	I[a]	% of U
+ 1.0	0.98	1.04	106.0	+ 1.0	0.85	0.43	50.6
− 7.6	1.17	0.97	82.9	− 7.5	1.00	0.29	29.0
−10.6	0.61	0.41	67.2	−10.4	0.77	0.27	35.0
−13.9	0.07	0.24	343.0[b]	−13.3	0.44	0.10	22.7

[a] I = inoculated plants, U = uninoculated controls.

[b] The high relative yield obtained at −13.9 C is related to the very low survival of the uninoculated controls.

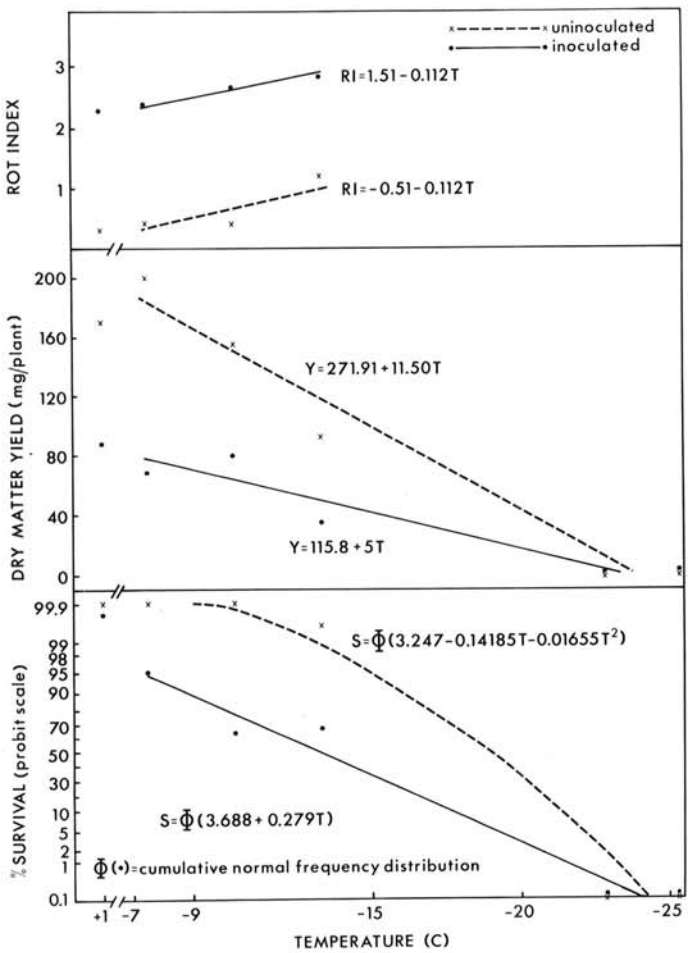

Fig. 4. Observed and estimated (polynomial regression) effect of temperature (T) on survival (S), dry matter yield (Y) and root rot index (RI) of alfalfa plants inoculated with *Fusarium oxysporum* f. sp. *medicaginis* after freeze test and of their uninoculated controls. Data points are the mean of 10 replicate pots containing five plants each. The root rot index is based on a 0 to 5 scale (0 = healthy tissue, 5 = 100% rotted).

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