

# Effect of Spring Black Stem on Yield and Growth of Alfalfa in the Greenhouse

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## ABSTRACT

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The effect of spring black stem on the yield and growth of alfalfa was evaluated after artificial inoculation of *Phoma medicaginis* var. *medicaginis* in a greenhouse. Leaves and stems inoculated simultaneously produced 44% less dry forage than the uninoculated control. Inoculation of leaves or stems separately produced 32 and 21% less dry forage, respectively, than the uninoculated control. Inoculation of leaves and stems simultaneously, leaves separately, and stems separately caused reductions of 26, 9, and 17%, respectively, of alfalfa growth measured as stem elongation 15 days after inoculation.

Additional key words: *Medicago sativa*

Spring black stem of alfalfa (*Medicago sativa* L.), caused by *Phoma medicaginis* Malbr. & Roum var. *medicaginis* Boerema, is distributed worldwide and is one of the most destructive diseases of this crop. Although the fungus is capable of infecting most alfalfa tissues, leaf spotting and stem blackening are the most common symptoms in the field. The disease affects alfalfa at all stages of growth and causes extensive losses when severe (8). Losses are manifested as yield reduction due to defoliation (3), reduction in forage and seed quality (5,6,14), and death of plants (4).

Spring black stem develops most rapidly at low temperatures and in the presence of free moisture from dew or rain on plants (2,7,10,13). Thus the disease is more prevalent in spring and fall, during relatively cool and wet weather, although it may be present throughout the growing season (13).

The disease is considered a complex (10,11, E. W. Hanson and M. F. Kernkamp, unpublished) in which several fungi and bacteria may be associated with the syndrome. *P. medicaginis* var. *medicaginis*, however, appears to be the most common cause of stem blackening in alfalfa.

The amount of damage caused by the disease in the field can be estimated visually (9,12) or by comparing yields of unsprayed field plots and of plots sprayed with fungicides. Because chemicals also

control other pathogens, the precise loss caused by the spring black stem fungus alone is difficult to measure.

Richards (12) reported a 40–50% yield loss from a severe outbreak of spring black stem in Utah. Peterson and Melchers (9) reported a more than 15% loss of leaves in plots in Kansas and indicated that many shoots had been killed. Willis et al (14) reported that *P. medicaginis* var. *medicaginis* and *Leptosphaerulina briosiana* (Poll.) Graham and Luttrell were the major pathogens in the first and second cuttings. Control of these two diseases with a fungicide increased the yields of four alfalfa varieties from 4 to 19%. Banttari et al (1) reported reductions in leaf spotting and stem blackening of 45 and 5%, respectively, by heavy and frequent applications of fungicides.

In this study, stems and leaves were inoculated simultaneously and separately to determine the effect of each phase of the disease on the yield and growth rate of alfalfa in the greenhouse.

## MATERIALS AND METHODS

Isolates of *P. medicaginis* var. *medicaginis* were collected from six alfalfa fields in Minnesota. Inoculum was increased by growing each isolate separately on an autoclaved barley grain medium. After 3 wk at laboratory temperature (about 21 C), the infested grain was dried under a dust hood for 24 hr.

For inoculum preparation, the grain cultures were flooded with distilled water for 2–4 hr. The spore suspension was then filtered through cheesecloth, spores were counted in a hemocytometer, and the suspension was diluted to the desired concentration. A mixture of the six isolates was used in each experiment.

Forty-eight clay pots, 23 cm in diameter, each contained six Ranger

alfalfa plants. Plants were grown in a steamed sand, loam, peat, and manure mixture. When plants were 2 mo old, the pots were divided into three groups of 16 pots each. Four pots of each of four treatments comprised one replicate. The experiment was replicated six times.

Before inoculation, the stem length and number of stems per pot were recorded. The pots were assigned to each treatment so that the total stem length was the same for each treatment within a replicate.

In one treatment, stems only were inoculated by manually rubbing each stem lengthwise, using silicon carbide, grit 600, as an abrasive. A suspension of  $2.5 \times 10^7$  spores per milliliter plus 1% glucose and two drops of Tween 20 in 50 ml of water was immediately brushed onto the stems. In another treatment, the leaves only were inoculated by atomizing the plants with a suspension of the same additives and  $2.5 \times 10^5$  spores per milliliter. This spore concentration produces heavy leaf infection but minimal stem infection. In a third treatment, the stems were inoculated first and then the leaves, with the same spore concentrations as in the other two treatments. Stems and leaves of control plants were brushed and atomized with distilled water and the same additives.

Inoculated and control plants were maintained in a moist chamber for 72 hr with a 12-hr day length of artificial light provided by a 400 W metal-halide lamp suspended above the chamber, then transferred to a bench in the greenhouse at  $21 \pm 3$  C.

The plants were harvested 15 days after inoculation. Disease severity was estimated by relative area covered with lesions. A 1–10 scale was used in which 1 = no infection and 10 = 100% infection. Dry forage weight per pot and total stem length per pot were also recorded.

## RESULTS

Infection was adequate in all the inoculated treatments (Table 1). In treatments to inoculate leaves only, some stem infection resulted because inoculation of stems could not be completely prevented when leaves were atomized. In inoculation of stems only, a few leaves were accidentally infected by droplets of inoculum. Some very young inoculated stems died.

Yield reductions were highly significant for all inoculated treatments. Simultaneous inoculations of leaves and stems resulted in about 44% less dry matter than

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**Table 1.** Effect of inoculation with *Phoma medicaginis* var. *medicaginis* on Ranger alfalfa plants in the greenhouse

Treatment	Disease <sup>a</sup> severity		Dry weight		Stem elongation	
	Stems	Leaves	g/pot	% of control	cm	% of control
Control	1.0 <sup>b</sup>	1.0	8.2	100	21.5	100
Leaves	2.0	8.3	5.6	68.4	19.5	90.7
Stems	6.7	3.8	6.5	78.6	17.8	82.8
Leaves and stems	6.4	8.3	4.6	56.0	15.9	73.9
LSD (5%)	1.4	1.0	0.9		3.0	
LSD (1%)	2.0	1.3	1.3		4.2	
C.V. (%)		13.5	12.4		13.2	

<sup>a</sup> Disease severity was rated on a scale of 1-10; 1 = no infection, 10 = 100% infection.

<sup>b</sup> Each value represents the average of six replicates.

the control treatment. Leaf infection alone resulted in 32% less dry matter than the control, whereas stem infection caused a 21% yield reduction. Leaf infection was the same, 8.3, when leaves or stems and leaves were inoculated. If we assume that leaf infection had the same effect in either case, then the difference in dry weight (5.6 - 4.6 = 1.0) was due to the stem infection (2.0 vs. 6.4). Stem infection was practically the same when stems or leaves and stems were inoculated. The difference in dry weight (6.5 - 4.6 = 1.9) can be assumed to be due to the difference in leaf infection severity (8.3 vs. 3.8).

During these experiments, the stems were measured before and 15 days after inoculation to determine if this disease caused reduction in the growth rate of infected plants. When stems and leaves were simultaneously inoculated, the stems elongated significantly less (26%) than the stems in the control group. Differences were also significant between this treatment and inoculated leaves alone. Inoculated stems elongated 17% less than the control and this difference was significant. Differences between treatments were not significant, however. Leaf infection alone caused a 9% stem

growth reduction compared with the control, but the difference was not significant.

### DISCUSSION

*P. medicaginis* var. *medicaginis* caused reduction in forage yield due to both leaf and stem infection. The greater yield reduction caused by leaf infection was due largely to leaf drop. Many heavily infected leaves had dropped by harvest time.

Infection of the leaves alone did not affect stem elongation of the plant, but elongation was reduced when the stems were infected. The reduction in total stem elongation can be explained by the premature death of some very young, severely infected stems.

The results of these experiments show that *P. medicaginis* var. *medicaginis* has the potential to produce significant forage losses in alfalfa. However, these results are based on studies done in a greenhouse environment, which may differ from field conditions. Levels of infection were more severe than those usually found in the field, and therefore the yield losses due to spring black stem in the field may be less than the greenhouse

data indicate. Field studies are needed to relate these greenhouse results with field damage estimates.

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