Vascular occlusion associated with abiotic stress is a causal agent of silvertop in perennial grasses

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ARTICLE

Vascular occlusion associated with abiotic stress is a causal agent of silvertop in perennial grasses

Juliana J. Soroka, Garry L. Lees, and Bruce D. Gossen

Abstract: Silvertop (sterile heads) of perennial cool-season grasses is common in aging stands grown for seed production. It is associated with yield reduction, but the causal agent(s) has not been determined. A study was conducted to examine the cytology of grass tillers with and without silvertop. A series of cross sections of stems from field samples of Kentucky bluegrass and meadow bromegrass (where signs of insect injury or fungal infection were absent) revealed abnormalities in the tissues above the last node in tillers exhibiting silvertop, while cells in the stems of healthy tillers appeared normal. Most cells from stems exhibiting silvertop became irregular and distorted, and eventually lost their integrity and shape altogether. At the terminal nodes of these stems, the sieve plates of xylem vessels were occluded with unidentified material, preventing the passage of water to the seed head. The xylem and phloem cells in the leaf sheath and blade surrounding the silvertop stems appeared normal. Spherical bodies in x-section, believed to be fungal mycelium and spores, were occasionally found above the point of tissue necrosis above the last node. Very few spherical bodies were observed below the symptomatic tissue, and none were found in the terminal node. The blockages observed in the water-conducting vessels to the seed head appear to have been induced by the plant as a response to abiotic stress, rather than by external biotic factors such as insects or pathogens.

Key words: Silvertop, seed production, perennial grasses, microscopic analysis, cytology, occlusions.

Résumé : La coulure (stérilité de l'épi) des graminées vivaces de saison froide est un problème courant chez les peuplements vieillissants employés pour la production grainière. On l'associe à une baisse du rendement, mais on n'a pu établir en établir les causes. Les auteurs ont étudié la cytologie des talles de graminées avec et sans coulure. La coupe transversale des tiges de pâturin des prés et de brome des prés (ne présentant aucun signe d'attaque par des insectes ou d'infection par un cryptogame) prélevées sur le terrain révèle des anomalies dans le tissu au-dessus du dernier nœud de la talle atteinte de coulure, alors que les cellules dans la tige des talles saines semblaient normales. Dans les tiges affectées par la coulure, la plupart des cellules sont irrégulières et distordues, et finissent par perdre leur forme et leur intégrité. Dans le nœud terminal de ces tiges, les perforations des vaisseaux du xylème sont obturées par un matériau inconnu qui interdit à l'eau de parvenir à l'épi. Les cellules du xylème et du phloème dans la gaine de la feuille et la feuille entourant l'épi malade paraissent normales. Parfois la coupe transversale révèle des corps sphériques, peut-être du mycélium et des spores, au-dessus du tissu nécrosé supérieur au dernier nœud. Très peu de ces corps sphériques ont été relevés sous le tissu symptomatique et on n'en a trouvé aucun dans le nœud terminal. Le blocage des vaisseaux qui acheminent l'eau jusqu'à l'épi semble plus venir de la réaction de la plante à un stress abiotique que d'un facteur externe tels les insectes ou les agents pathogènes. [Traduit par la Rédaction]

Mots-clés : coulure, production grainière, graminées vivaces, analyse microscopique, cytologie, occlusions.

Introduction

Silvertop is an abnormality of perennial grasses that occurs on tillers above the terminal node (where the culm originates in the seed tiller), manifested by a damaged culm base and a seed inflorescence that turns a light-brown to silvery colour and fails to produce viable seed. Silvertop has been attributed to a number of causes, including arthropods and fungal agents (Osborn 1891; Starks and Thurston 1962; Arnott and Bergis 1967; Suski 1984), environment (Pohjakallio et al.

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1960; Wetzel 1971), and mechanical injury (Beckman 1971; Kozlowski 1976; Lamb et al. 1989).

Research studies have assessed various causal agents in attempts to induce silvertop (Peterson and Vea 1971; Soroka and Gossen 2021), and researchers have dissected tillers to examine where injuries occur in relation to silvertop (Pohjakallio et al. 1960; Wagner and Ehrhardt 1961; Kamm 1979). The majority of reports have associated silvertop with damage from insects or plant pathogens (e.g., Nedělník et al. 2015; Trněný and Nedělník 2018), but grass fields on the Canadian Prairies often develop silvertop without any consistent biotic association (Soroka and Gossen 2021, 2022).

The objective of the current study was to examine the cytology of meadow bromegrass (*Bromus riparius* Rehm.) and Kentucky bluegrass (*Poa pratensis* L.) tillers with and without silvertop to investigate the reasons for the culm and seed panicle discoloration and withering. The focus of this study was silvertop-affected tillers without obvious insect or disease damage, i.e., the tillers with silvertop selected for detailed examination had characteristic symptoms but no clear biotic cause. These tillers were selected because other studies using application of insecticide and exclusion cages had provided a strong indication that external biotic factors were not the most important cause of silvertop on the Canadian Prairies (Soroka and Gossen 2005, 2021, 2022). In this study, the terminal node, where the culm originates in the seed tiller, is referred to as the 'node'. The research study was conducted many years ago, but almost no work has been conducted on silvertop since that time, so the authors believe that this information is still highly relevant.

Materials and Methods

Initial assessments

Tillers were collected from plots of meadow bromegrass cv. Regar and Kentucky bluegrass cv. Dormie at the Saskatoon Research and Development Centre farm (52°07'N, 106°35'W) at weekly intervals for 4 weeks during June and July of 1997. The samples consisted of 25 healthy tillers and 25 tillers with silvertop per plot. The plot of Dormie was seeded in 1993 and Regar was seeded in 1994 on a orthic, dark brown, chernozemic soil, and production of seed heads and subsequent seed yield declined substantially in 1997 from that in 1996 (Soroka and Gossen 2021).

Individual tillers with and without silvertop symptoms were visually assessed for the presence of insect eggs and larvae, puncture marks and other symptoms of insect feeding, general form and colour, and symptoms of pathogen infection such as discolouration and tissue breakdown. The tillers were then dissected to examine the interior structure of the node and sheath, and symptoms of insect and pathogen activity that might not have been apparent in the initial assessment. Tillers with obvious insect or pathogen damage were occasionally dissected and photographed but were not chosen for microscopic analysis.

Microscopic observations

Samples for microscopy were taken from the upper culm (inner stem within the sheath) near the panicle (seed inflorescence), just above the node where the culm originates, the node itself, and the sheath beneath the node. In addition, samples were taken above and below the separation point of the culm in silvertop-affected tillers.

Small (2–3 mm) pieces of node and internode tissue, including the sheath and culm, were removed from healthy and symptomatic (silvertop) tillers, then fixed and embedded in plastic resin for subsequent semi-thin sectioning. In some cases, tillers with panicle and culm with some discolouration were also assessed. Samples were fixed in two changes of 6% glutaraldehyde in buffer (0.1 M sodium phosphate) for 2 d, then washed in buffer until no smell of glutaraldehyde remained. The samples were further fixed for 4 h in 1% osmium tetroxide in buffer and rinsed with distilled water. Dehydration occurred in a graded series of aqueous acetone (10% steps, 30 min/step) followed by two 30 min periods in 100% acetone. Samples were embedded in resin (Araldite 502 - Jembed 812 - DDSA - DMP 30, 17.1:30.5:55.2:1.3 *w/w*) by transfer through three acetone-resin solutions with increasing resin content (2:1, 1:2, 0:3 parts acetone to resin, over 6, 16, and 30 h). Samples were placed on a 1- to 2-mm-thick layer of resin previously polymerized in shallow aluminum pans or neoprene forms. After careful spacing and orientation, the samples were covered with another 1–2 mm of embedding medium and polymerized for 48 h at 60 °C in an oven. Where necessary, pieces containing individual samples were cut from the polymerized resin for subsequent trimming and sectioning.

Semi-thin sections for light microscopy were obtained using a diamond knife in an LKB Ultratome III ultramicrotome. Sections were transferred to a glass slide, allowed to dry for 30 min on a slide warmer and stained for 12–15 min in 0.5% safranin O *w/v* in 50% ethanol for general observation. For differential staining, slides were stained with ruthenium red (0.1% in aqueous solution for 15 min), toluidine blue (1% in 70% ethanol for 20 min), and methylene blue (0.5 g in a mixture of 1 ml of 1% potassium hydroxide, 30 ml ethanol, and 100 mL distilled H_2O for 6 min). Mounted fresh sections were stained in situ using 1 g of phloroglucinol in 20% hydrochloric acid, 50% ethanol, and 30% water. Photomicrographs were obtained under bright-field conditions using a Nikon Optiphot compound microscope with an automated camera system.

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Table 1. Number of Kentucky bluegrass and meadow bromegrass plants sampled at weekly intervals over a 4 week period, the number discarded due to insect or disease damage, those used in microscopic analyses, and those with vascular bundle blockages at Saskatoon, Saskatchewan 1997.

Grass species	Collection date	No. of plants with silvertop sampled	No. of plants with insect or disease damage	No. of plants used in analyses	No. of plants with vessel blockages
Kentucky bluegrass	25 June	29	20	9	1
	02 July	25	20	5	4
	08 July	28	21	7	6
	15 July	25	21	4	4
Total		107	82	25	15
Meadow bromegrass	25 June	30	23	7	1
	02 July	25	20	5	4
	08 July	25	21	4	3
	15 July	25	20	5	4
Total		105	84	21	12
Overall total		212	166	46	27

Note: 59% (27/46) of plants analyzed had blockages of the vascular bundle.

Results

Initial assessments

Symptom-free tillers from both grass species had erect, green sheaths and leaves and culms ending in a panicle with green, immature seeds. Even if insect damage was apparent, the panicle and culm would not pull away from the sheath at the node, a characteristic common in silvertop-affected tillers. While tillers affected by silvertop also were erect and had green leaves and sheaths, they could easily be recognized by the silver-brown colour of the panicle and culm. Tillers with silvertop were statistically but not substantially shorter than their healthy counterparts (data not shown).

Of the 212 silvertop-affected tillers examined, 166 (82 Kentucky bluegrass and 84 meadow bromegrass) had symptoms of insect or pathogen activity (Table 1). Dissection of some of these tillers showed that the culm became atrophied around the insect damage. Insect eggs and sometimes larvae or adults were found within the sheath, but the insect pests involved were not identified. Fungal hyphae were found around insect wounds and also within the sheath, either locally with atrophied tissue or throughout the internode.

In silvertop-affected tillers with no apparent insect or pathogen damage, separation occurred with a gentle pull on the panicle in both grass species; however, culm breakage always occurred in the internode just above the node where the culm originated. In tillers with silvertop where no other damage was apparent, the tissue at the point of separation was occasionally still green but soft, indicating that tissue breakdown had started but was not yet advanced. Sometimes the node beneath the separation point had a dark brown band on the surface. In other stems, the sheath and node remained green, but the culm just above the node was necrotic at the separation point. The remainder of the culm, although seemingly intact, was light brown. The point of culm separation in a silvertop-affected tiller with no apparent insect damage or disease is illustrated in Fig. 1*a*.

When insect or pathogen damage was apparent, there was always one or two localized spots on the culm that were completely atrophied and the tissue was dark and shriveled. Above this point, the culm usually was light brown to silver-brown. Any seeds that had formed were small and discoloured. A gentle pull on the panicle always resulted in the separation of the culm from within the sheath at a point of atrophy. Figure 2*a* shows a typical point of culm separation in a culm with biotic damage.

In tillers with only partial discolouration of the culm, a gentle pull would not always separate the culm within the sheath. Where it did, the culm tissue was either green and soft, or light brown and atrophied.

Microscopic observations

Twenty-five Kentucky bluegrass tillers and 21 meadow bromegrass tillers that were free of visible insect damage and symptoms of disease were selected for microscopic analysis (Table 1). In healthy tillers of both grass species, tissue differentiation and individual cells within tissues appeared to be normal. Thin-walled parenchyma cells in the cortex were turgid, while regions of chlorophyll-containing cells near the epidermis clearly showed chloroplasts within the cells. Vascular bundles showed differentiation between unobstructed xylem and phloem conducting vessels, with large areas of thick-walled collenchyma tissue in the bundle sheath and inter-fascicular regions (Figs. 1*b* and 2*b*).

In tillers from both species showing full silvertop symptoms, cross-sections of the culm well above the node

Fig. 1. Cross sections of silvertop-affected Kentucky bluegrass: (*a*) Dissected sheath at node of culm origination in Kentucky bluegrass affected with silvertop. The culm has not separated but has become misshapen and turned brown above the node. The brown area (arrow) will become the separation point (magnification ×6); (*b*) healthy culm showing well defined tissue regions and chloroplasts in chlorophyll-containing regions (arrow) (magnification ×180); (*c*) culm with silvertop sampled above the separation point, showing damaged chlorophyll-containing tissues (arrow) (magnification ×180); (*d*) culm with silvertop, sampled just above the separation point. Note the misshapen appearance and the damage to all tissues except the xylem elements in the vascular bundles (magnification ×358); (*e*) culm with silvertop, sampled just below the separation point. Damage is visible in most tissues except the vascular bundles, all of which have occluded xylem elements (arrow) (magnification ×180); (*f*) culm with silvertop, sampled within the node sheath. No tissue damage apparent, but all of xylem elements are occluded, (arrow) (magnification ×180); (*g*) sheath below the node. All tissues appear healthy except for some damage to the chlorophyll-containing tissues (arrow) (magnification ×180); and (*h*) discoloured culm sampled just below the separation point showing blockage of the xylem elements (arrow) (magnification ×180). The black size bar in Fig. 1*b* equals 0.3 mm for Figs. 1*b* to 1*h*.

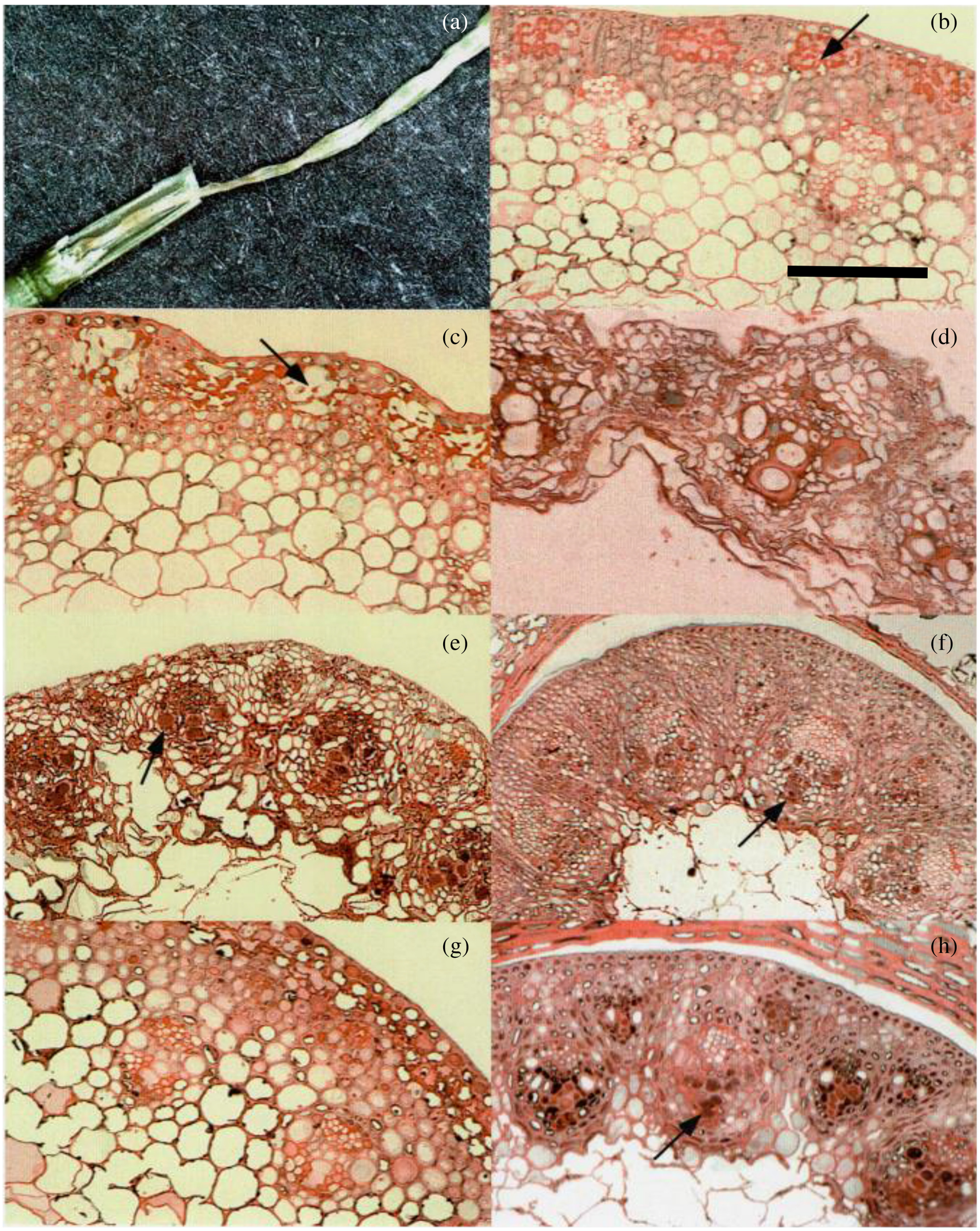
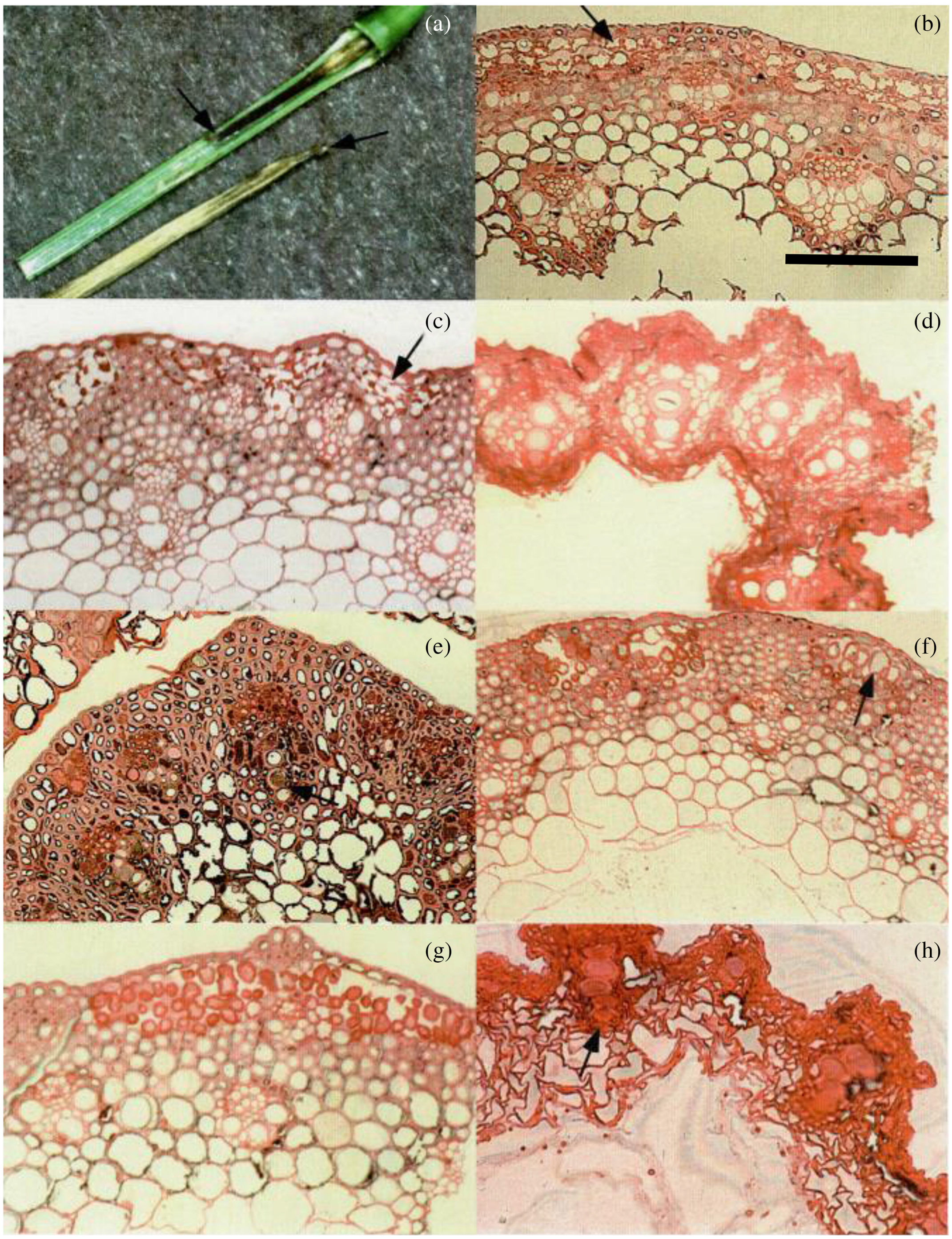


Fig. 2. Cross sections of meadow bromegrass with silvertop: (*a*) dissected sheath at node of culm origination in meadow bromegrass affected with silvertop. The separated culm is lying beside the dissected sheath. The sheath and node were green, but the culm had turned brown above and below the black necrotic area where separation had occurred (arrows) (magnification ×6); (*b*) healthy culm showing well defined tissue regions and chloroplasts in chlorophyll-containing regions (arrow) (magnification ×180); (*c*) silvertop culm well above the separation point showing damaged chlorophyll-containing tissues (arrow) (magnification ×180); (*d*) culm with silvertop, sampled just above the separation point. Note the misshapen appearance and the damage to all tissues except the xylem elements in the vascular bundles (arrow) (magnification ×180); (*e*) culm with silvertop, sampled just below the separation point. Damage has occurred to all tissues except the vascular bundles, some of which have occluded xylem elements and phloem cells (arrow) (magnification ×180); (*f*) culm with silvertop, sampled within the node sheath. No tissue damage is apparent (arrow) (magnification ×180); (*g*) sheath with silvertop, sampled below the node. All tissues appear healthy; and (*h*) discoloured culm, sampled just below the separation point showing blockage occurring in the xylem elements (arrow) (magnification ×180). The black size bar in Fig. 2*b* equals 0.3 mm for Figs. 2*b* to 2*h*.



where separation occurred showed that the culm retained its shape and most tissue areas appeared normal. However, either chloroplast deterioration or a complete breakdown of the chlorophyll-containing cells was apparent (Figs. 1*c* and 2*c*), accounting for the brown discolouration of the culm. None of the samples examined had symptoms that indicated pathogen infestation. Examination of the area just above the separation point revealed extensive tissue and cellular damage within the culm, which was consistent with its atrophied appearance. The culm had a flattened shape and there were no identifiable tissue regions except for the xylem and phloem tissues in the vascular bundle (Figs. 1*d* and 2*d*).

The culm below the separation point and just above the node did not suffer as much damage as at the separation point. However, in addition to atrophy of the areas that contained chlorophyll, some or all of the xylem parenchyma, xylem elements, and phloem cells in 15 of the Kentucky bluegrass and 12 of the meadow bromegrass samples were occluded (Table 1, Figs. 1*e* and 2*e*). Also, there was some damage to cells in the node sheath that contained chlorophyll, while the culm within the node had more extensive tissue damage, ranging from distortion of the overall shape of the culm to total cell deterioration.

The xylem elements were occluded in some or all of the vascular bundles within the culm within visually healthy nodes of Kentucky bluegrass (Fig. 1*f*). There may have been some blockage of the culm within the node in meadow bromegrass, but no blockage was apparent (Fig. 2*f*) in the only complete node containing both the sheath and culm that was available for examination (the culms from the other samples were destroyed during the fixation and embedding process). The sheath below the node showed no evidence of any damage in either grass species (Figs. 1g and 2g), which was expected as the culm remained healthy below the damaged node. Of the 25 Kentucky bluegrass tillers examined, 15 showed blockages in the vascular bundles of the culm just above or in the node, and 12 out of 21 meadow bromegrass tillers had blockages between the node and the separation point (Figs. 1*h* and 2*h*). Only 2 of 16 tillers sampled in June had blockages in the vascular tissue, but blockages were observed in the majority of tillers sampled in July.

Fungal hyphae were observed in cross-sections from eight samples (four from each species) where there were no external signs of fungal damage during dissection. Blockages of xylem tissue were seen in only two of eight samples infected with fungi. In one case, the hyphae were present below the node of a diseased culm.

In tillers with discolouration of culms, the chlorophyll-containing parenchyma tissues in the area of the culm just above the point of separation were damaged, while the remaining tissues appeared to be healthy. Just below the break, the cells in chlorophyll-containing tissues were either crushed or absent. All other tissues appeared to be healthy. Within the node, the sheath had some chlorophyll damage, but was otherwise healthy in appearance. In the culm, some xylem elements were blocked in the vascular bundles (Fig. 1*h*). In the sheath below the node, there was some deterioration of chlorophyll-containing cells, but otherwise the tissues appeared healthy.

Safranin O, used as a general stain to differentiate the tissues, stained the occlusions, giving them a dark red appearance. Also, osmium tetroxide fixation stained the blockages a light brown. Ruthenium red stained the pectinaceous middle lamellae between cells but did not stain the blockages. Toluidine blue stained cell walls a medium blue, with the pectinaceous middle lamella showing a darker blue. Lignified tissues in xylem and thick-walled collenchyma tissues stained an aqua colour. Toluidine blue staining of the blockages in the xylem and phloem tissue gave a dark, purplish blue, while methylene blue gave much the same reaction as toluidine blue, but the colours were not as dark.

Discussion

In the current study, only 2 of 16 tillers sampled in June had blockages in the vascular tissue, but the majority of tillers sampled in July had blockages in the vascular tissue. Precipitation in June and July 1997 was well below the long-term average, resulting in abiotic stress and subsequent reduction in seed yield in the mature grass stands that were assessed. A previous report had hypothesized that factors such as xylem blockage caused by abiotic stress could be responsible for the host response that produced silvertop symptoms (Starks and Thurston 1962). Defense responses such as tyloses and gum formation that result in xylem blockages could be also induced by physical and (or) mechanical damage (Beckman 1971; Vandermolen et al. 1977; Lamb et al. 1989).

Occlusions of xylem and phloem cells occur when tyloses, gels, or gums are produced by the plant or plant cellular material are degraded by microorganisms (Subramanian and Saraswathi-Devi 1959; Beckman 1964, 1971; Talboys 1968; Koslowski 1976; Vandermolen et al. 1977; Mace 1978; Ride 1983; Lamb et al. 1989). Blockages are often a primary defense response to invading pathogens (Beckman 1971; Ride 1983). Although essential for plant survival, these blockages often result in wilting or eventual death of the affected part of the plant (Talboys 1968). With silvertop in grasses, it is the upper culm and seed panicle of the tiller that succumbs. Vascular blockages may have also been present in the insect-damaged or diseased tillers, which were not analyzed in this study; many reports indicate that blockages produced by the host plant can be associate with insect damage or microorganism infestation (Koslowski 1976, VanderMolen et al. 1977, Mace 1978).

In the current study, the tissues were embedded in epoxy resin, so the potential for histochemical testing of the substances in the occlusions was limited. Safranin O stained the occlusions dark red, which indicated the presence of tannin (O'Brien and McCully 1981) and osmium tetroxide stained the blockages light brown, which indicated the presence of phenolics. This supported the diagnosis of these occlusions as tyloses, gels, and vessel plugs, which are frequently strengthened by polymerization of phenolic compounds (Beckman 1964; Ride 1983). Ruthenium red did not stain the blockages, which indicated that they did not contain pectin (Rawlins 1933). Toluidine blue stained the blockages a dark, purplish blue, while methylene blue gave much the same reaction as toluidine blue, but the colours were not as dark. This indicated that there is no pectin or lignin in the occlusions, and that there are probably cellulosic and phenolic components present (Smart and O'Brien 1979; Mausch and Shoene 1989).

Fungal hyphae were observed in cross-sections from eight samples (four from each species), but blockages of xylem tissue were apparent in only two of the eight samples. It is not known when the infection occurred, or whether these samples represent infection by a pathogen or an endophyte; however, the low incidence of fungal mycelium in the tillers indicated that fungal invasion was not a critical factor inducing the development of silvertop in either grass species.

Previous studies have attempted to introduce silvertop in controlled experiments, but the results have often been negative or inconclusive (Arnott and Bergis 1967; Kamm 1971, 1979; Peterson and Vea 1971; Soroka and Gossen 2021). The current study demonstrated that at least some of the silvertop symptoms in both grass species resulted from restriction or cessation of water and nutrients flow to the culm and seed head caused by blockages in the vascular tissue near the node of affected seed tillers. The reasons for this blockage are not clear, but drought stress from lower than normal precipitation in late June and July in 1997 was associated with a substantial increase in silvertop incidence in the current study. Clearly, silvertop need not be caused by insect damage or microorganisms, even though obvious signs of both factors were present in the majority of tillers assessed. Instead, our study indicated that grasses respond to stress by shutting down water and nutrient transport to the seed head. This may also occur in response to a biotic stress, isolating the threat away from the rest of the plant. This would be a more effective trade-off for a perennial species than for an annual species, which may explain why few annual grasses exhibit silvertop. Even without external stress, perennial plants that depend on underground food reserves for survival and reproduction in the next year often begin allocating photosynthate to the roots prior to the current year's flowering (Mooney and Billings 1960). The plant may cut off resource flows to some tillers to maintain flows to other important resource sinks.

To our knowledge, this is the first reports of vascular occlusion as a cause of silvertop symptoms. The methodology that underlies this study is still reliable and the actual causal agent of silvertop has not been identified in the more than 20 yr since this study was conducted, so the results are still relevant.

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